Schedule and Abstracts

Annual Meeting 2009
Simon Fraser University

12-15.06-2009

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Welcome

Welcome to the 51st annual meeting of the Canadian Society of Plant Physiologists and to Simon Fraser University situated atop Burnaby Mountain in the scenic lower mainland of British Columbia. This year's meeting follows on the heel's of last year's highly successful 50th annual meeting and, while that meeting reflected on the many achievements of plant physiology, the theme of this year's meeting is the future or "next generation" of plant physiology research in Canada. Accordingly we have a suite of exciting scientific talks and posters assembled, which will be presented, in many instances, by students and post-docs. This year's meeting captures the amazing breadth of plant physiology and we have four major themes: performance, environment, cell biology and development, and technology with dedicated plenary and concurrent sessions. In addition we have a professional development session that includes advice on networking and delivering effective oral presentations. We finish the meeting with a field trip to the UBC Botanical garden.

We hope that you enjoy this meeting and that you leave us better informed and refreshed! If there is anything we can do to assist you, please do not hesitate to let us know.

Jim Mattsson, Sherryl Bisgrove, Aine Plant
Co-chairs, organizing committee

President's Welcome:

I am pleased to add words of welcome to those of the Co-chairs of the organizing committee to all participants of the 51st meeting of the CSPP in Burnaby this year. It promises to be a wonderful meeting, focusing on the "next generation" of plant physiologists. I am looking forward to listening and seeing the exciting work that will be presented in the plenary talks, contributed papers and posters in the theme areas including: performance, environment, cell and development and technology. The breadth of the topics emphasizes the rich diversity of talent we have in the membership of the CSPP and the relevance of the scientific work they do.

I would like to thank the entire organizing committee, including: Sherryl Bisgrove, Jim Mattsson, and Aine Plant from Simon Fraser University and Jin-Gui Chen, Soheil S. Mahmoud, Lacey Samuels, and Geoff Wasteneys, from the University of British Columbia for all of the work they have done to organize what promises to be an excellent meeting in such a lovely venue.

Sincerely

K. Peter Pauls

Acknowledgments

2009 Executive committee

President **Peter Pauls**	(U Guelph)	Vice-president **Carl Douglas**	(U British Columbia)
Secretary **Line Lapointe**	(U Laval)	Treasurer **Harold G Weger**	(U Regina)
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Science policy director **Barbara Moffatt**	(U Waterloo)	Senior director **Priti Krishna**	(U Western Ontario)
Student & PDF Representative **Alexandra Reid**	(AAFC-*AAC*)		

2009 Conference organizing committee

Co-chairs		Members	
Sherryl Bisgrove	(Simon Fraser U)	**Jin-Gui Chen**	(U British Columbia)
Jim Mattsson	(Simon Fraser U)	**Lacey Samuels**	(U British Columbia)
Aine Plant	(Simon Fraser U)	**Soheil Mahmoud**	(U British Columbia)
		Geoff Wasteneys	(U British Columbia)

Volunteers

Muhammad Arshad
Adam Foster
Laura Gleeson
Dua'a Riyal
Mathias Schuetz
Shannon Squires
Carol Wenzel
Robin Young

Supporters

SFU, conference fund
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Special thanks for sharing information from the organization of the 2008 CSPP meeting
Brian Miki
John T Arnason
Douglas A Johnson

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General Information

Poster & exhibit hours

Participants may set-up their posters in the ASSC hallway as of 3 pm on Friday, June 12 and be posted for viewing until Monday, 9 am at which time all posters must be removed.

Poster session I: Friday 7:00 - 10:00 PM, participants with odd-numbered posters are presenting

Poster session II: Saturday 4:30 - 7:00 PM, participants with even-numbered posters are presenting

Exhibitors' booths will be available over the duration of the conference in ASSC hallway.

General and executive meetings

Saturday, June 13 12:00 - 1:30 PM	CSPP Outgoing Executive Meeting, ASSC 10061
Sunday, June 14 4:30 PM	Annual Business Meeting, ASSC 10051
Monday, June 15 11:40 - 1:30 PM	CSPP Incoming Executive Meeting, ASSC 10061

Meals and special events

Continental breakfasts and lunches will be held in the ASSC atrium and coffee breaks in the ASSC hallway. These meals are included in the registration.

Friday, June 12 7:00-10:00 PM	**Poster session and mixer** (ASSC hallway) Appetizers and a cash bar will be available during the poster session
Saturday, June 13 4:30 - 7:00 PM	**Poster session and mixer** (ASSC hallway) Appetizers and a cash bar will be available during the poster session
Sunday, June 14 5:30 - 10:00 PM	**Dinner BBQ social**, SFU pub, Maggie Benson Centre The conference banquet will include a CSPP awards ceremony Tickets for the social must be purchased separately.
Monday, June 15 1:30 ~ 5 PM	**Field trip**, bus at bus loop, going to UBC Botanical Garden and UBC Museum of Anthropology Return trip leaves at **4:30 PM**

Supporters


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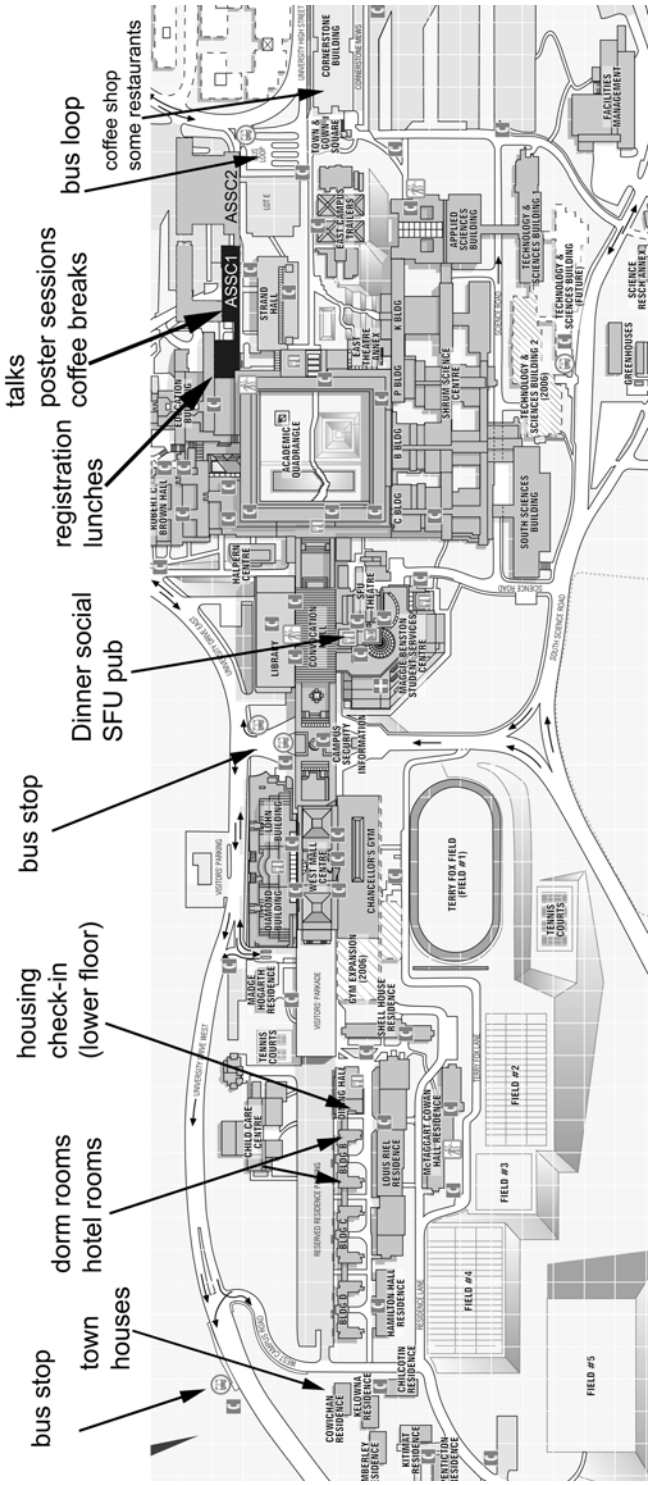
Map, Housing and Events



Friday, June 12

3:00 - 9:00 PM	Registration, ASSC atrium, north-east corner of Academic Quadrangle,
5:00 - 5:20 PM	Opening Ceremonies, ASSC 10081
	Chairperson: Peter Pauls, President of the CSPP
	Welcome from Dr. Mario Pinto, Vice-President Research, Simon Fraser University

Friday Plenary Session
ASSC 10081
Chairperson: **Peter Constabel**, University of Victoria

5:20 - 6:00 PM	**Plenary 1-1, Dr. William Plaxton,** Queen's University Metabolic biochemistry helps to close the gap in plant functional genomics
6:10 - 6:50 PM	**Plenary 1-2, Dr. Linda Walling,** University of California, Riverside The art of host deception: the Arabidopsis-whitefly interaction

7:00 - 10:00 PM	Reception and poster session I, ASSC hallway Participants with odd-numbered posters are presenting

Saturday, June 13

7:30 AM	Registration, ASSC atrium
7:30 - 8:30 AM	Continental Breakfast, ASSC atrium

Saturday Plenary Session
ASSC 10081

8:30 - 8:40 AM	Chairperson: **Dr. Pierre Meulien**, Chief Scientific Officer, Genome British Columbia
8:40 - 9:20 AM	**Plenary 2-1, Dr. Jennifer Nemhauser**, University of Washington It isn't easy being green: signal integration during seedling development
9:30 - 10:10 AM	**Plenary 2-2, Dr. Gane Ka-Shu Wong**, University of Alberta Sequencing 1000 Plants (1KP)

10:20 - 10:50 AM Coffee break, ASSC hallway

Concurrent Session, **PERFORMANCE I**
ASSC 10081

Chairperson: **Dr. Barbara Hawkins**, University of Victoria

10:50 - 11:10 AM	**Brendan O'Leary**, Queen's University, T1 Bacterial-type Phosphoenolpyruvate Carboxylase Functions as a Catalytic and Regulatory Subunit of the Novel Hetero-octameric Class-2 PEPC Complex of Vascular Plants
11:10 - 11:30 AM	**Jeffrey C. Waller**, University of Florida, T2 Investigation of the Role of a Folate-Dependent Protein in the Metabolism of Iron-Sulfur Clusters
11:30 - 11:50 AM	**Fushan Liu,** University of Guelph, T7 Protein-Protein Interactions between Starch Biosynthetic Enzymes in Normal and Mutant Maize Amyloplasts

Concurrent Session, **CELL AND DEVELOPMENT I**
ASSC 10041
Chairperson: **Dr. Sherryl Bisgrove**, Simon Fraser University

10:50 - 11:10 AM	**Anja Geitmann**, University of Montreal, T17 Mechanical modeling of cytoskeletal dynamics and vesicle transport in growing pollen tubes
11:10 - 11:30 AM	**Chris Ambrose**, University of British Columbia, T20 Cellular roles of the microtubule associated protein CLASP
11:30 - 11:50	**Wesley Farquharson,** Wilfrid Laurier University, T31 Protein interactions between members of the chloroplast import machinery in *Arabidopsis thaliana*: A study using fluorescence spectroscopy

12:00 - 1:30 PM	Lunch, ASSC atrium
12:00 - 1:30 PM	CSPP Outgoing Executive Meeting, ASSC 10061

Concurrent Session, **PERFORMANCE II**
ASSC 10081
Chairperson: **Dr. Soheil Mahmoud**, University of British Columbia, Okanagan

1:30 - 1:50 PM	**Tariq Akhtar**, University of Florida, T4 Control of Flavonoid Biosynthesis in *Lemna Gibba* by the Redox State of the Plastoquinone Pool: Evidence for Retrograde Signaling via an ROS-independent Mechanism
1:50 - 2:10 PM	**Adam Foster,** Simon Fraser University, T16 The expression of a Western redcedar *(Thuja plicata)* sabinene synthase is localized to the epithelium of foliar resin glands and correlate with natural genetic variation in thujone content
2:10 - 2:30 PM	**Dawn Hall,** University of British Columbia, T10 Comprehensive analysis of the 3-carene synthase gene family of Sitka spruce
2:30 - 2:50 PM	**Jonathan Neilson,** University of New Brunswick, T6 Evolution of Light-Harvesting-Like Proteins in Photosynthetic Eukaryotes

Concurrent Session, **CELL AND DEVELOPMENT II**
ASSC 10041
Chairperson: **Dr. Fred Sack**, University of British Columbia

1:30 - 1:50 PM	**Jin-Gui Chen,** University of British Columbia, T22 Regulation of Post-embryonic Cotyledon Development by a Subset of Arabidopsis Ovate Family Proteins
1:50 - 2:10 PM	**Mathias Schuetz**, Simon Fraser University, T25 The Auxin Response Factors *ETTIN/ARF3* and *NPH4/ARF7* redundantly function with *MP/ARF5* to promote leaf initiation in *Arabidopsis thaliana*
2:10 - 2:30 PM	**Shucai Wang,** University of British Columbia, T24 Regulation of Epidermal Cell Patterning by Single-Repeat R3 MYB Transcription Factors in Arabidopsis
2:30 - 2:50 PM	**Jürgen Ehlting**, University of Victoria, T26 Retroposition and positive selection led to the evolution of a novel phenolic pathway needed for pollen development

2:50 - 3:20 PM Coffee in ASSC hallway

Concurrent Session, **ENVIRONMENT I**
ASSC 10081
Chairperson: **Dr. Neil Emery**, Trent University

3:20 - 3:40 PM	**Allison R. Hayward,** Trent University, T8 Phytochelatins and their involvement in the multiple heavy metal tolerances of *Deschampsia cespitosa* from Canadian mine sites
3:40 - 4:00 PM	**Dennis Maxwell,** University of Western Ontario, T9 Plasticity of a psychrophilic green alga to short-term heat stress
4:00 - 4:20 PM	**Rainer Bode,** University of Western Ontario, T5 Redox state of photosynthetic intersystem electron transport controls leaf sectoring in variegation mutants of *Arabidopsis thaliana*

Concurrent Session, **CELL AND DEVELOPMENT III**
ASSC 10041
Chairperson: **Dr. Jin-Gui Chen**, University of British Columbia

3:20 - 3:40 PM	**Donna M. Yee,** University of Toronto, T18 The increasing diversity of plant U-box E3 ubiquitin ligases in Arabidopsis: from hormone to abiotic stress responses
3:40 - 4:00 PM	**Hongxia Liu,** Dalhousie University, T28 Abscisic acid increases ABI5 protein levels by promoting KEG self-ubiquitination and degradation by the 26S proteasome
4:00 - 4:20 PM	**Santosh Kumar,** University of Manitoba, T27 The barley flowering time protein, FCA, is ABA inducible and may be involved in processes related to seed germination and dormancy

4:30 - 7:00 PM	Mixer and Poster session II, ASSC hallway Owners of even-numbered posters are presenting

Sunday, June 14

7:30 - 8:30 AM Continental Breakfast in ASSC atrium

Sunday Plenary Session
ASSC 10081
Chairperson: **Dr. Norman Huner**, University of Western Ontario

8:30 - 9:10 AM **Plenary 3-1, Dr. Uwe Hacke**, University of Alberta
The resistance of xylem to cavitation in boreal conifers and hybrid poplar

9:20 - 10:00 AM **Plenary 3-2, Dr. Keiko Yoshioka,** University of Toronto
Environmental effects on pathogen resistance in Arabidopsis

10:10 - 10:50 AM Coffee break, ASSC hallway

Concurrent Session, **ENVIRONMENT II**
ASSC 10081
Chairperson: **Dr. Gordon Gray**, University of Saskatchewan

11:00 - 11:20 AM **Nuria De Diego,** T11
Physiological characterization of six different *Pinus radiata* origins in response to water stress,

11:20 - 11:40 AM **Anthony Anyia,** Alberta Research Council and the University of Alberta, T14
Water use efficiency and crop productivity on the Canadian Prairies

11:40 - 12:00 **Michael Shane,** University of Western Australia, T12
Functional Diversity in Root Systems that are Critical for Water and Nutrient Acquisition by Australian Native Plants

Concurrent Session, **CELL AND DEVELOPMENT IV**
ASSC 10041
Chairperson: **Dr Wayne Snedden**, Queen's University

11:00 - 11:20 AM	**George Templeton,** University of Calgary, T19 A potential link between lipid signaling and protein phosphatase activity in Arabidopsis
11:20 - 11:40 AM	**Thomas DeFalco,** Queen's University, T33 Characterization of GmCaMK, a novel calmodulin kinase from soybean root nodules
11:40 - 12:00	**Monika Rewers,** University of Technology and Life Sciences (Poland), T23 Endoreduplication in the seeds and young seedlings of Fabaceae

12:00 - 1:30 PM Lunch, ASSC atrium

Concurrent Session, **TECHNOLOGY**
ASSC 10081
Chairperson: **Dr. Deep Saini**, University of Waterloo

1:30 - 1:50 PM	**Xu He**, Simon Fraser University, T34 Production of active human glucocerebrosidase in Arabidopsis *cgl* (*complex-glycan-deficient*) seeds
1:50 - 2:10 PM	**Jaswinder Singh,** McGill University, T35 Dissecting QTLs with *Ac/Ds* transposons
2:10 - 2:30 PM	**Hargurdeep Saini,** University of Waterloo, T36 A novel approach to enhancing salinity tolerance by engineering a chloride volatilizing enzyme in plants
2:30 - 2:50 PM	**Lee Kalcsits,** Agriculture and Agri-Food Canada, University of Saskatchewan, and University of British Columbia, T37 Magnetic Resonance Micro-Imaging (MRMI) indicates water mobility is correlated with axillary bud dormancy induction in hybrid poplar (*Populus* spp.)

Concurrent Session, **CELL AND DEVELOPMENT V**
ASSC 10041
Chairperson: **Dr. Jim Mattsson**, Simon Fraser University

1:30 - 1:50 PM	**Allan DeBono,** University of British Columbia, T21 Arabidopsis LTPG is Required for Export of Lipids to the Plant Surface
1:50 - 2:10 PM	**Teagen Quilichini,** University of British Columbia, T29 Examining the role of ABCG26 in *Arabidopsis thaliana* pollen wall formation: A putative sporopollenin monomer transporter
2:10 - 2:30 PM	**Heather McFarlane,** University of British Columbia, T30 Export of the waxy cuticle by ABCG transporters**.**
2:30 - 2:50 PM	**Katrina Haasen,** University of Toronto, T32 An investigation of the Exocyst Complex and its role in compatible pollen-pistil interactions in Arabidopsis

2:50 - 3:20 PM Coffee in ASSC hallway

Concurrent Session, **ENVIRONMENT III**
ASSC 10081
Chairperson: **Dr. Sophia Stone**, Dalhousie University

3:20 - 3:40 PM	**Sophia Stone**, Dalhousie University, T3 The RING E3 ligase mutant, rak2, overproduces ethylene which contributes to it's reduce lateral root phenotype
3:40 - 4:00 PM	**Tawhidur Rahman,** University of Western Ontario, T13 Brassinosteroid and Stress Tolerance: New Players in the game
4:00 - 4:20 PM	**Sarah Klatt,** University of Saskatchewan, T15 TsDHN-2, A Unique Dehydrin Protein from *Thellungiella* and its Role in Salt Tolerance

Concurrent Session, **PROFESSIONAL DEVELOPMENT**
ASSC 10041
Chairperson: **Dr. Lacey Samuels**, University of British Columbia

3:20 - 3:40 PM	**Dr. Allison McDonald,** University of Western Ontario Networking Tips for Early Career Scientists
3:40 - 4:00 PM	**Dr. Lacey Samuels**, University of British Columbia How to Give a Talk
4:00 - 4:20 PM	**Dr. Mario Lamarca,** NSERC NSERC update

4:30 PM	Annual Business Meeting ASSC 10051
5:30 - 10:00 PM	Dinner BBQ social, SFU pub, Maggie Benson Centre

Monday, June 15

7:30 - 8:30 AM	Continental Breakfast in ASSC atrium
8:00 - 9:00 AM	Judges Meeting for Student Poster Awards, ASSC 10061
8:00 - 9:00 AM	Judges Meeting for Student Oral Presentation Awards, ASC 10051

Monday Plenary Session
ASSC 10081
Chairperson: **Dr. Brian Ellis**, University of British Columbia

9:00 - 9:40 AM	**Plenary 4-1, Dr. Eliot Herman,** Donald Danforth Plant Science Center Insoluble Problems - Biology and Biotechnology of ER Bodies In Seeds
9:50 - 10:30 AM	**Plenary 4-2, Dr. Ljerka Kunst,** University of British Columbia Plant Cuticular Wax Deposition: Molecular and Biochemical Studies in *Arabidopsis thaliana.*

10:40 – 11:10 AM	Coffee break, ASSC hallway
11:10 – 11:40 AM	Announcement of best poster and best oral presentation Farewell and Thanks, Peter Pauls, CSPP President
11:40 - 1:30 PM	Lunch, on your own at various SFU avenues
11:40 - 1:30 PM	CSPP Incoming Executive Meeting, ASSC 10061
1:30 PM	Field trip, bus at bus loop, going to UBC Botanical Garden

Abstracts

Plenary Session 1

1-1

Metabolic biochemistry helps to close the gap in plant functional genomics

William C. Plaxton
Departments of Biology and Biochemistry, Queen's University, Kingston, Ontario, Canada
plaxton@queensu.ca

Remarkable insights into plant metabolism are being provided by the ever-growing collections of plant gene and bioinformatic databases, as well as the implementation of high-throughput transcriptomic and mutant/transgenic studies. Although genomics provides an essential blueprint and a host of powerful tools for systematic metabolic studies, it also reveals that the organization and control of plant metabolism is very complicated and poorly understood. A significant proportion of plant genes encode unknown enzymes, whereas many annotated genes encode multiple isozymes having poorly defined properties and roles. Thus, genomics needs to be well integrated with parallel studies of the corresponding proteome, metabolome, *in vivo* metabolic fluxes, and membrane transporters/metabolic compartmentation, together with detailed biochemical and structural analyses of purified native enzymes. Although largely overlooked in current plant sciences research, native enzyme characterization helps to establish: 1) gene and isozyme function; 2) enzyme physical, immunological, and kinetic/regulatory properties; 3) protein:protein interactions that may prevail *in vivo*; 4) enzyme transit peptides and targeting; & 5) pivotal post-translational enzyme modifications such as phosphorylation, ubiquitination, or glycosylation. The many roles that metabolic biochemistry can play in helping to 'close the gap' in functional genomics will be illustrated using a few examples from our recent research. This concerns several unexpected discoveries that pertain to novel phosphorylated or monoubiquitinated isozymes of the key anaplerotic enzyme phosphoenolpyruvate carboxylase in developing *versus* germinated castor oil seeds.

1-2

The art of host deception: the Arabidopsis-whitefly interaction

Linda L. Walling, Department of Botany and Plant Sciences, Center for Plant Cell Biology, University of California, Riverside, CA, USA, **Linda.walling@ucr.edu**

In response to herbivore feeding, plant perceive signals generated in response to tissue damage and insect oral secretions to activate and/or suppress defense-signaling pathways. The genetic and genomics resources from *Arabidopsis thaliana* were used to identify the defense pathway that controls basal resistance to the silverleaf whitefly (SLWF; *Bemisia tabaci* type B). Sentinel defense-gene RNAs were quantitated after SLWF infestation revealing that responses to SLWFs were biphasic; the responses to adults and nymphs were distinct. During the first 18 hrs of SLWF adult feeding, the jasmonic acid (JA)- and ethylene (ET)-responsive *PDF1.2* RNA levels increased. In contrast, salicylic acid (SA)-responsive RNAs were repressed relative to non-infested plants. After 24 hr of feeding, a shift in defense-signaling pathways occurred. At these later times after adult feeding and during nymph feeding (7 to 24 days), SA-regulated gene RNAs and free and glucose-conjugated SA accumulated locally and systemically. In accordance with the cross-talk between the JA and SA signaling pathways, JA- and ET-responsive gene RNA levels declined or were not modulated in SLWF-infested leaves. To determine if the SA- and JA-regulated defense pathways influenced SLWF-Arabidopsis interactions, insect choice and no-choice studies with mutants/transgenic lines that activated or impaired SA-regulated defenses (*npr1,* NahG*, cim10*) or JA-regulated defenses (*coi1, cev1, jar1*) were performed. These studies showed that adult choice (as measured by egg deposition) was not influenced by SA- or JA- regulated defenses. In contrast, nymph development was accelerated in the mutants that activated SA-regulated or impaired JA-regulated defenses (*cim10* and *coi1,* respectively). Reciprocally, lines that activated JA-regulated (*cev1*) or impaired SA-regulated (*npr1,* NahG) defense gene expression slowed SLWF nymph development. Finally, when *npr1* plants, which do not activate SA-regulated defenses, were treated with MeJA, a dramatic delay in nymph development was observed. Unlike the aphids, *SLWF* performance on wild-type, *ein2* and *pad4* mutants was similar. Collectively these results showed that SLWF-repressed, JA-regulated defenses deter SLWF nymph development.

Plenary Session 2

2-1

It isn't easy being green: signal integration during seedling development

Jennifer Nemhauser, Department of biology, University of Washington, Seattle, WA, USA, **jn7@u.washington.edu**

Plant growth is shaped by the dynamic integration of environmental, developmental, and metabolic cues. Information from many of these input pathways feeds into the highly connected network of small molecule phytohormones. Our studies focus on two interlocking questions: how does light shape seedling development and how do different hormone response pathways coordinately regulate growth. To begin to unravel the complex growth network, we are

focusing on the molecular mechanism contributing to the interplay between the light cue and two hormones, brassinosteroids and auxins. Recently, we have identified repressor-type Auxin Response Factors as a potential hub for interactions.

2-2
Sequencing 1000 Plants (1KP)
Gane Ka-Shu Wong
University of Alberta and Beijing Genomics Institute in Shenzhen**, gane@ualberta.ca**

1KP is a new initiative to sequence the transcriptomes of 1000 plant species. We will sample broadly across evolutionary phyla, from land plants to algae. At least one gigabase of data will be generated for each species with next generation sequencing technology and these fragments will be computationally assembled. Preliminary data benchmarked against rice indicate that full length transcripts are recovered for the 2000 most abundant genes, with progressively less recovered for the next 10000 genes, but still better than traditional 3' ESTs that tend to favor 3' UTRs instead of coding sequences. The project is lead from the University of Alberta but all sequencing is done at the Beijing Genomics Institute in Shenzhen. The species are being chosen by an international consortium of plant experts to answer multiple scientific questions of broad interest to the community, from the evolution of land plants to applications in medicine and other bioproducts. All of the data will be freely released to GenBank.

Plenary Session 3

3-1
The resistance of xylem to cavitation in boreal conifers and hybrid poplar
Uwe Hacke
University of Alberta, AB, **uwe.hacke@ales.ualberta.ca**

Plants frequently face xylem tensions that may cause cavitation and embolism. Cavitation-induced losses in hydraulic conductance limit gas exchange and productivity. The resistance of xylem to cavitation influences species distribution and the ability of plants to cope with abiotic stresses like drought and freeze-thaw cycles. The question of what structural features underlie differences in resistance to xylem cavitation is an area of active research. We investigated this question by studying tracheid and pit structure in three boreal conifer species. Using a combination of scanning and transmission electron microscopy, light microscopy, and hydraulic measurements, we evaluated variation of torus-margo pit structure and linked it with differences in cavitation resistance. Conifer pit membranes combined a high degree of safety from cavitation with minimal flow resistance. While tracheid size explained at least 90% of the variation in specific conductivity for stem and root samples, the strongest correlations with cavitation resistance occurred at the pit level. Both torus thickness and depth of the pit chamber showed a linear increase with greater vulnerability to cavitation. A thinner torus may be more flexible and better able to seal the pit aperture. The pit chamber depth is proportional to the distance that the margo needs to deflect for pit aspiration. I will also report on a second study which focused on hydraulic adjustments to nitrogen fertilization in hybrid poplar. While nitrogen fertilization often leads to increased shoot growth, our data suggests that in hybrid poplar, it may also result in greater vulnerability to cavitation. This finding, combined with the data from the conifer study, suggests that increased transport efficiency is linked with increased vulnerability to cavitation.

3-2
Environmental effects on pathogen resistance in Arabidopsis
Keiko Yoshioka
Department of Cell and Systems Biology, University of Toronto, **keiko.yoshioka@utoronto.ca**

It has been reported that under particular climate conditions, some pathogens cause severe disease outbreaks in agriculture. Generally, this has been reasoned to be due to the preference of microorganisms for particular environmental conditions. On the other hand, it has been known for a long time that the hypersensitive response (HR), which is one of the important defense responses, can be completely suppressed in tobacco plants carrying the *N* gene when plants are kept at more than 28°C. Similarly, resistance responses induced by the interaction of the *Cladosporum fulvum* avirulence factors Avr2, Avr4 and Avr9 and their cognate tomato R proteins Cf-2, Cf-4 and Cf-9, are suppressed by high humidity. These findings suggested that there is a significant influence of environmental effects, especially temperature and humidity on defenseresponses. Recently, it has been reported that a number of *Arabidopsis* lesion mimic mutants show alterations in abiotic stress responses as well as pathogen resistance. Two such *Arabidopsis* mutants, *cpr22* and *ssi4* are typical lesion mimic mutants; they exhibit elevated levels of salicylic acid, HR-like spontaneous cell death, constitutive expression of defense-related genes, and enhanced resistance to various pathogens (Shirano et al., 2002; Yoshioka et al., 2006). Despite the fact that the mutations are in different genes, their defense phenotypes are similarly suppressed by relatively high humidity and temperature and on the other hand enhanced by relatively lower humidity and temperature (Yoshioka et al., 2001; Zhou et al., 2004; Mosher et al., submitted). To investigate the environmental effects on these mutants, we have conducted genome wide transcriptome analysis using these two defense mutants under different humidity conditions. Interestingly, expression of a number of genes that are related to abiotic stress signaling was significantly altered in both mutants compared to wild type plants. Environmental effects on defense responsesas well as an alteration in abiotic stress responses in these *Arabidopsis* defense mutants will be discussed.

Plenary Session 4

4-1

Insoluble Problems - Biology and Biotechnology of ER Bodies In Seeds

Eliot Herman
Donald Danforth Plant Science Center, St. Louis, MO, USA,
EHerman@danforthcenter.org

Plant cells produce and accumulate insoluble triglycerides, proteins and rubber that are assembled into inert ER-derived organelles broadly termed ER-bodies and more specifically protein bodies and oil bodies. ER-bodies appear to originate from tubular ER domains that are maintained by cytoskeletal interactions and integral ER proteins. ER-bodies sequestering insoluble substances usually are either transferred to the vacuole or remain as cytoplasmic organelles. In addition some otherwise soluble ER-synthesized proteins are converted to insoluble aggregates to produce ER-bodies for transfer to the vacuole. This process constitutes an alternate secretory system to assemble and traffic transport-incompetent insoluble materials. How insoluble materials are produced in the ER, accreted into the core matrix of ER-bodies without triggering ER-stress response and then used by plant cells as transient storage and transport platforms presents novel cell biology questions many of which are specific to plants. This laboratory has been perturbing the formation and accumulation of both protein bodies and oil bodies in maturing soybean seeds to investigate the biological processes involved and to use this information for biotechnology goals. Transgenic soybean seeds will accumulate small quantities of foreign proteins in protein bodies. We have shown that large increases in foreign proteins accumulated in protein bodies can be achieved by trading with the capacity to accumulate intrinsic storage proteins in protein bodies. Seeds down-regulate the accumulation of intrinsic proteins in favor of the massive accumulation of the foreign protein showing the ER-assembly of protein bodies is protein-species specific. In other experiments the assembly of oil bodies have been perturbed by silencing the oil body protein oleosin. This results in disruption of the normal process of oil body assembly of the ER producing a massive oil body/ER complex. Altering the formation and/or abundance of either protein bodies or oil bodies does not appear to impact the accumulation of either seed oil or protein content indicating the processes for assembling these organelles and the allocation of nutrient reserves for their storage substance's synthesis are distinct parallel processes. Although the assembly of both protein bodies and oil bodies is ER-mediated the cellular processes involved are not tightly cross-regulated. This shows that maturing seed cells have the capability to simultaneously produce protein bodies and oil bodies using distinct domains of the ER without the production of either organelle affecting the capacity to assemble the other organelle. Our investigations of the biological processes that control protein body and oil body assembly can be used to design new biotechnological approaches to alter seed composition as a means to produce improved food and feed as well as to construct a biofactory platform for the inexpensive production of foreign proteins.

4-2

Plant Cuticular Wax Deposition: Molecular and Biochemical Studies in *Arabidopsis thaliana*

Ljerka Kunst
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The outer epidermal cell walls of plant shoots are covered by a cuticle, a thin lipid structure that protects plants against desiccation, UV-light, pathogens, and insects. The cuticle is composed of a cutin polyester matrix embedded in and covered with waxes. Even though major pathways for cuticular wax biosynthesis have been proposed, our knowledge of the enzymes involved, as well as proteins involved in wax transport to the cuticle, and regulation of wax deposition is incomplete. We are taking advantage of wax-deficient *eceriferum (cer)* mutants in Arabidopsis to identify and functionally characterize gene products involved in these processes. We have also carried out transcriptional profiling in the epidermis of rapidly expanding Arabidopsis inflorescence stems using Affymetrix ATH1 arrays. Candidate genes up-regulated in the epidermis during active wax synthesis with expression patterns similar to known wax biosynthetic genes were selected for further evaluation using reverse genetic approaches. Our recent work on the dissection of cuticular wax biosynthetic pathways will be presented.

Performance, Concurrent Session Abstracts

T1

Bacterial-type Phosphoenolpyruvate Carboxylase Functions as a Catalytic and Regulatory Subunit of the Novel Hetero octameric Class-2 PEPC Complex of Vascular Plants

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Phosphoenolpyruvate carboxylase (PEPC) is a tightly controlled cytosolic enzyme situated at a major branch point in plant glycolytic C-metabolism. All plant genomes contain several closely related plant-type PEPC (PTPC) genes, as well as a distantly related/enigmatic bacterial-type PEPC (BTPC) gene. PEPC is highly expressed in developing castor oil seed (COS) endosperm where it is believed to play a role in controlling photosynthate partitioning to storage lipids vs. protein synthesis. Two native PEPCs sharing the same 107-kD PTPC subunit (p107) were discovered in developing COS. The association of p107 with a 118-kD BTPC polypeptide (p118) results in pronounced differences between the Class-1 PEPC homotetramer and the novel Class-2 PEPC p107/p118 hetero-octamer. COS BTPC was expressed from its cDNA in *E. coli* as an active PEPC displaying unusual properties including a high *Km*(PEP), insensitivity to metabolic effectors and an enhanced thermal stability. A 900-kD Class-2 PEPC hetero-octamer was isolated from *E. coli* lysates containing heterologously expressed COS BTPC and an Arabidopsis Class-1 PEPC. Purified chimeric Class-2 PEPC was relatively insensitive to

metabolite effectors and demonstrated biphasic PEP saturation kinetics with high and low affinity active sites attributed to its PTPC and BTPC subunits, respectively. The BTPC subunits: i) catalyzed the bulk of Class-2 PEPC's *Vmax* especially at low physiological pH, and ii) function as Class-2 PEPC regulatory subunits by modulating PEP and allosteric effector binding, and pH sensitivity of the PTPC subunits. BTPC appears to allow Class-2 PEPC's to maintain high flux from PEP under *in vivo* conditions that would largely inhibit a Class-1 PEPC. COS Class-2 PEPC is hypothesized to catalyze a significant PEP flux to malate which is imported into the leucoplast in support of fatty acid and hence storage lipid synthesis.

T2

Investigation of the Role of a Folate-Dependent Protein in the Metabolism of Iron-Sulfur Clusters

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Folates are known principally as cofactors in one-carbon (C1) transfer reactions. The widely-occurring COG0354 protein is therefore intriguing. It is structurally similar totetrahydrofolate-dependent proteins that capture formaldehyde moieties (e.g., the glycine cleavage complex T protein and dimethylglycine oxidase) and has a folate binding site. Yet it has been implicated in the assembly and regeneration of iron-sulfur (Fe-S) clusters, processes that are apparently unrelated to C1 transfer. Comparative analysis of prokaryote genomes strongly implies the existence of functional connections between COG0354, folate, and Fe-S cluster metabolism: in archaea, only folate-containing organisms have COG0354 genes, and in bacteria COG0354 genes are clustered with genes specifying various Fe-S proteins. A mutational approach in *Escherichia coli* was used to probe the role of folate in COG0354. Deleting the COG0354 gene (*ygfZ*) greatly reduced the activities of several Fe-S enzymes, including the tRNA modification enzyme MiaB. A similar reduction in MiaB activity was seen in a *folE* (GTP cyclohydrolase I) deletant, which lacks folates. In contrast, an increase in MiaB activity was seen in a *gcvP glyA* (Gly decarboxylase and Ser hydroxymethyltransferase) deletant, which accumulates unsubsitituted folates that can accept formaldehyde fragments. Deletion of *ygfZ* also caused hypersensitivity to oxidative stress and growth defects on glycerol or acetate as carbon source. Plasmid-borne COG0354 genes of archaeal, fungal, protistan, or animal origin, but not cyanobacterial origin, complemented both of these growth phenotypes. Mutation of the only conserved region of YgfZ gave indications that YgfZ's functions may be to accept formaldehyde fragments. Both the Arabidopsis mitochondrial YgfZ homologue, AtYgfZ1, and the plastidial homologue, AtYgfZ2, could complement the *E. coli ygfZ* deletant. Furthermore, the *atygfz1* mutation was found to be a recessive embryo-lethal. Collectively, these results indicate that COG0354 has an ancient unction that requires folate.

T3

The RING E3 ligase mutant, rak2, overproduces ethylene which contributes to its reduced lateral root phenotype

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Ubiquitination plays a major role in regulating various signal transduction pathways. RING (Really Interesting New Gene) E3 ligases play an essential role in the ubiquitination pathway as the substrate recruiting component. Analysis of the *Arabidopsis* proteome identified over 450 potential RING E3 ligases. Here we discuss some of our results which show that a member of the RING E3 family, RAK2, has a potential role in regulating ethylene biosynthesis. *rak2* mutants display a number of phenotypes including reduced lateral root production. Overproduction of ethylene in *rak2* seedlings, almost double the amount produced by wild-type seedlings, contributes to its reduced lateral root phenotype. The *rak2* lateral root defect could be rescued by inhibition of ethylene perception and synthesis. Unlike wild-type roots, treatment of *rak2* roots with abscisic acid (ABA) had a stimulatory effect on the lateral root production, possibly by blocking ethylene signaling in *rak2* seedlings to increase lateral root production. Exogenous auxin only partially rescue lateral root production, however the number of lateral roots produced by *rak2* mutant was comparable to that of wild- type when auxin treatment was coupled with abscisic acid. The *rak2* seedlings did not display altered responses to ABA, for example germination rates, and primary root growth in the presence of ABA were similar to that of wild-type. However, the *rak2* seedlings display increased sensitivity to salt stress. Further studies are aimed at identifying the component(s) of the ethylene biosynthesis pathway that are possible targets for RAK2 E3 ligase activity.

T4

Control of Flavonoid Biosynthesis in *Lemna Gibba* by the Redox State of the Plastoquinone Pool: Evidence for Retrograde Signaling via an ROS-independent Mechanism

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Plants accumulate flavonoids in response to a myriad of environmental challenges, predominantly when exposed to ultraviolet (UV) radiation or situations causing oxidative stress. However, the origin and nature of the signal that triggers their accumulation remains obscure. In this study, a

group of flavonoids belonging to the flavone class were identified from *Lemna gibba* (duckweed). These flavones accumulated upon exposure to UV radiation, low temperature, copper, and the photosynthetic electron transport inhibitors 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB) and 1,2-dihydroxyanthraquinone (DHATQ). All of these stressors were also shown to promote plastoquinone (PQ) pool reduction, however, in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) or a light regime that oxidized the PQ pool, flavonoid accumulation ceased. Chloroplast derived reactive oxygen species (ROS) were not associated with all the stress conditions that promoted both PQ pool reduction and flavonoid synthesis, indicating that ROS were not a strict requisite for flavonoid accumulation. Transcripts for the flavonoid biosynthetic genes, chalcone synthase and chalcone isomerase, were similarly responsive to the redox state of the PQ pool, as were a panel of transcripts uncovered by differential display PCR. Collectively, these results suggest that reduction or oxidation of the PQ pool signals the up- or down-regulation of flavonoid biosynthesis, respectively. This now places flavonoid biosynthesis under the emerging theme of retrograde control.

T5

Redox state of photosynthetic intersystem electron transport controls leaf sectoring in variegation mutants of *Arabidopsis thaliana*

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Since variegation in the *Arabidopsis thaliana immutans* mutant can be completely suppressed by growth under low light, we hypothesized that excitation pressure (EP) governs the extent of variegation in *immutans*. To test this, we developed an imaging technique to quantify variegation *in vivo*. The plants were grown at either 25ºC or 12ºC with increasing irradiance (50, 150 and 450 µmol photons $m^{-2}s^{-1}$). As growth irradiance increased, the extent of variegation increased in *immutans*, as well as in several other variegated mutants (*var1*, *var2*, *atd2*). *immutans* grown at 12ºC exhibited greater variegation than *immutans* grown at 25ºC at all light intensities. The extent of variegation was positively correlated (r2 = 0.759) with an increase in EP. Structural and functional analyses indicated that and thylakoid membrane biogenesis and assembly were inhibited under high EP in *immutans*. WT plants displayed a significantly lower EP compared to *immutans* during early stages of chloroplast development. These results support the thesis that the variegated phenotype is controlled by cellular energy imbalances. We conclude that the lack of IMMUTANS is necessary but not sufficient to account for the extent of variegation in several mutants of Arabidopsis. Rather, it is the EP experienced during early chloroplast development that governs the patterns of variegation.

T6

Evolution of Light-Harvesting-Like Proteins in Photosynthetic Eukaryotes

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Light-harvesting-like (LIL) proteins are low-molecular mass, pigment binding proteins related to light-harvesting complex (LHC) proteins. LILs are found in both photosynthetic eukaryotes and cyanobacteria, and likely involved in photoprotection. To analyze the LIL protein family we mined a number of publicly available databases as well as our own EST data to identify LIL proteins in a broad range of photosynthetic eukaryotes. LIL proteins are diverse having one to four predicted transmembrane helices. One and two-helix LIL proteins were found in all the major photosynthetic eukaryote lineages (glaucophytes, red, and green), and are particularly well conserved in the green algae and land plants. In most cases, however, these proteins are not conserved between major lineages, and in some cases, as with the two-helix LILs, appear to have evolved independently. Three-helix LILs are only found in the green lineage. These proteins are well conserved within the gymnosperms and angiosperms, but are much more divergent, and have been duplicated multiple times, in the green algae and bryophytes. We also identified an LIL protein in two *Micromonas* strains that has a fourth hydrophobic region like PsbS, though these are more related to the three-helix LILs, and not homologous to the plant PsbS proteins. This analysis identifies conserved members of the LIL protein family signifying their importance to photosynthetic eukaryotes. It also indicates that classification of these proteins based on structural characteristics alone inadequately reflects the evolutionary history, and potential functional conservation observed in this complex protein family.

T7

Protein-Protein Interactions between Starch Biosynthetic Enzymes in Normal and Mutant Maize Amyloplasts

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Starch is widely used in food and non-food sectors. Its biosynthesis is known to involve at least four groups of committed enzymes: ADP-glucose pyrophosphorylase, starch synthases (SS), starch branching enzymes (SBE) and debranching enzymes. Significant proportions of the starch biosynthetic enzymes, particularly SSI, SSIIa, and SBEIIb are bound to starch granules in maize. Recent studies have shown protein-protein interactions between SSI, SSIIa, and SBEIIb in maize amyloplasts. The present study therefore investigated the effect of loss of SBEIIb activity on the interactions between enzymes of starch biosynthesis in the maize *amylose extender* (*ae*-) mutant. The phenotype of which has reduced amylopectin branch point frequency, and longer glucan chains. Distinct patterns of protein-protein interactions were observed in amyloplasts of *ae*- mutants compared with wild type suggesting functional

complementation for the loss of SBEIIb in protein complexes by SBEI, SBEIIa and starch phosphorylase (SP). Co-immunoprecipitation and affinity chromatography using recombinant proteins showed that, in amyloplasts from normal maize endosperm, protein-protein interactions involving SSI, SSIIa, and SBEIIb could be detected. By contrast, in *ae*- amyloplasts, SSI and SSIIa were shown to interact with SBEI, SBEIIa and SP. All interactions in normal maize were strongly enhanced by ATP, and reduced by the addition of alkaline phosphatase, indicating a role for protein phosphorylation in assembly. We propose that during amylopectin biosynthesis in amyloplasts SSI and SSIIa form the core of a phosphorylation-dependent glucan-synthesizing protein complex which, in normal endosperm, recruits SBEIIb, but when SBEIIb is absent (*ae*-), recruits SBEI, SBEIIa and SP. These differences in protein complexes, including similar study on *sugar2* (*su2*-) mutant lacking SSIIa activity, are mirrored in the complement of starch synthesizing enzymes detected in starch granules of each genotype, strongly suggesting a functional role in starch biosynthesis

Environment, Concurrent Session Abstracts

T8

Phytochelatins and their involvement in the multiple heavy metal tolerances of *Deschampsia cespitosa* from Canadian mine sites

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Heavy metal contamination in the environment can occur naturally or be the result of anthropogenic processes. In either case, certain species or ecotypes of plants have adapted to these harsh conditions through a number of physiological mechanisms. One common mechanism in metal tolerant ecotypes is the production of chelators, such as phytochelatins, organic acids, or amino acids. Phytochelatins have been given considerable attention in recent years because of their apparent involvement in cadmium tolerance. However, detection and quantification of phytochelatins has been difficult because of their low concentrations and tendency to easily oxidize. In our study, a method was developed to analyze phytochelatins using a High Performance Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometer (LC-(ESI)MS/MS). It allows for the detection of phytochelatins without derivatization, thus increases the precision of the results. *Deschampsia cespitosa* is a tufted hair grass found throughout Canada.

Preliminary results using heavy metal tolerant ecotypes of this grass indicate that though phytochelatin are induce in the presence of cadmium, phytochelatins are not induced when challenged by toxic levels of nickel. Based partly on this result, we have developed a model to explain involvement of multiple metal chelators and potential for metal co- tolerance depending on the type of chelators produced and their specificity for the metals in excess.

T9

Plasticity of a psychrophilic green alga to short-term heat stress

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The green alga *Chlamydomonas raudensis* UWO241 is endemic to Antarctica and is a psychrophile – its optimum growth temperature is approximately 10°C and it cannot grow above 20°C. Using flow cytometry, it was found that after 12h at the non-permissive temperature of 24°C approximately 6% of the cells died. This increased to 50% by 34h and by 65h all cells were dead. The rate and extent of cell death was found to be light independent. Chlorophyll *a* fluorescence analysis was employed to examine changes in photosystem II function. After 24 h at the non-permissive temperature photochemical efficiency (Fv/Fm) decreased by 35% and by 60 h at 24°C there was an absence of fluorescence induction indicating a total loss of photosystem II photochemistry. We assessed the plasticity of changes brought about by exposure to non-permissive temperature by exposing UWO241 to a stress-recovery regime that consisted of shifting 10°C-grown cells to 24°C for 12h followed by recovery back at 10°C for up to 54h. Changes in chlorophyll *a* fluorescence parameters due to the 12h stress treatment at 24°C were reversed within 54h of recovery at 10°C. As well, using RNA blot analysis it was shown that the abundance of a number of transcripts encoding components of the photosynthetic apparatus decreased rapidly in response to the non-permissive temperature, before recovering to near control values after 54 h at 10°C. This included transcripts encoding proteins of the light harvesting complex of photosystem II and the electron transport component ferredoxin. In contrast, the abundance of the mRNA encoding the small subunit of rubisco (*rbcS*) showed only minor changes during the stress-recovery regime. Although the non-permissive temperature was only 24°C this resulted in a canonical heat shock response with rapid induction of transcripts encoding hsp90a and hsp22a. While 24°C represents a temperature that does not permit growth of this Antarctic alga, and results in significant alterations to cell structure and function, cellsdo fully recover from a 12h heat stress. The long-term goal of this research is to further our understanding of the temperature stress response as well as the elucidation of the underlying basis of psychrophily in *C. raudensis* UWO241.

T10

Comprehensive Analysis of the 3-carene synthase gene family of Sitka spruce

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Conifer trees are constantly challenged by pathogens and herbivores and have evolved complex terpenoid chemical defense mechanisms to combat these threats. Hundreds of structurally diverse terpenoids are formed in a single conifer tree, the biosynthesis of which is dependent on the tree's genotype and the environment. The basis of this diversity is

related to the size of the terpene synthase (TPS) gene family, and the single- and multi-product profiles generated by these enzymes. Recent investigation into the biosynthesis of a particular monoterpene, (+)-3-carene, in pest-resistant and susceptible genotypes of Sitka Spruce identified a small subfamily of TPS genes encoding (+)-3-carene synthases and closely related (85-90% amino acid identity) sabinene synthases. Enzyme assays with native Sitka spruce protein reveal that the resistant chemotype is capable of producing high levels of (+)-3-carene, whereas only low levels of (+)- 3-carene synthase activity are detected in the susceptible chemotype. Preliminary kinetic analyses and a comparison of the product profiles of the recombinant enzymes suggest possible mechanisms underlying the absence of (+)-3-carene in susceptible Sitka spruce.

T11

Physiological characterization of six different *Pinus radiata* origins in response to water stress

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Drought is considered to be the most important factor limiting production, growth, development and distribution of plants. Water stress in vegetables is a result of a complex combination among soil, plant and climate. All these factors interact to determine water absorption and water loss rate in plants. Forest tree species are not an exception and water stress can affect the outcome of conifer seedling reforestation programmes. Physiological characterization (measuring water potential, net photosynthesis, transpiration, stomata conductance, cytokinins (Ck) and abscisic acid (ABA) concentrations, among others) is a good tool to study water stress plant response in order to select the best genotypes to introduce in for breeding programmes. Moreover, phytohormones analysis could give information about a possible premature marker to select plants in juvenile stages of their development. *Pinus radiata* is one of the most important conifers in the Basque Country. Considering the importance of woody production of this pine species around the world and the global climate changes during the last years, it is important to carry out studies to increase the knowledge about the response to conifer species to drought stress. There are 5 original provenances of *Pinus radiata* but due to sanitary problems it is impossible to work with them. In order to investigate differences between 6 origins (New Zeland, Australia, Baja California (Cedros and Guadalupe Island), Oihanberri (Basque Country), and *Pinus radiata* x *attenuata* hybrid) in water relation, we placed seeds in pots containing peat and perlite (7:3 v/v) in the greenhouse in controlled conditions. When plants were 4 months old, a random selection of 36 plants from each origin were put in extreme drought conditions. When water stress was morphologically evident for each group (more than 50% of plants presented apical curvature), morphological, hydric and gas exchange parameters were determined. Furthermore, cytokinins and abscisic acid were quantified.

Different drought tolerance in *Pinus radiata* origins was observed. Cedros Island and *Pinus radiata* x *attenuata* plants differed from the others and showed more resistance to water deficit conditions. Cedros origin showed more water use efficiency and seemed to present a plastic behaviour under drought conditions.

T12

Functional Diversity in Root Systems that are Critical for Water and Nutrient Acquisition by Australian Native Plants

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The ancient, weathered and heavily leached landscapes of Mediterranean environments of the Australian southwest, with their great varieties of soil types, topographies and regolith structures and compositions, offer a veritable smorgasbord of opportunities for studying responses of native plant taxa to exacting environments. Across this region are severe constraints on the availability of key nutrients such as phosphorus, seasonal temperature stress and an extreme range in seasonal access to water. A relatively large proportion of the native vegetation from the extremely phosphorus deficient soils do not produce a symbiotic association with a mycorrhizal fungus, instead utilizing highly specialised ephemeral root structures. Species may develop dense clusters of longitudinal rows of hairy rootlets termed 'proteoid' or 'capillaroid' roots (e.g., in woody Proteaceae or herbaceous Restionaceae), whereas herbaceous perennial sedges (e.g., Cyperaceae) develop dense clusters of root hairs on carrot-shaped branch root axes termed 'dauciform' roots. These root structures release organic acids which mobilise phosphorus and micronutrients. Root functional diversity is illustrated also by the roots of perennial herbaceous monocotyledons which have strong seasonal patterns of growth and dormancy with associated effects on xylem maturation that enable plants to utilise winter rain, while also sustaining them through summer heat and drought. Perennial roots that become dormant before the onset of summer extremes resume growth each winter eventually reaching depths of 2 to 4 m. Before reaching moisture at these depths these roots maintain full hydration through summer by specialised sand-sheaths, osmotic adjustment, and presumably, hydraulic redistribution from deeper roots and rhizome.

T13

Brassinosteroid and stress tolerance: new players in the game

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Brassinosteroids (BRs) are a group of steroidal plant hormones that are essential for proper plant development. Without BRs, plants are dwarfs and infertile. In addition to their pivotal role in plant development, BRs also promote stress tolerance in plants. We have previously demonstrated that treatment with 24-epibrassinolide (EBR) increases tolerance of *Arabidopsis thaliana* and *Brassica napus* seedlings to a range of abiotic stresses such as high and low temperatures, high salt and drought. To understand the molecular mechanism of BR-mediated stress tolerance, we

analyzed global gene expression in EBR-treated and untreated seedlings under non-stress and stress conditions by using ATH1 arrays. Results of the study revealed that the majority of the genes up-regulated by EBR are associated with abiotic and biotic biosynthesis, and genes responsive to ABA. We addressed the ABA-deficient and ABA-insensitive mutants of *A. thaliana*. The positive effect of EBR on stress tolerance was significantly greater in the ABA-deficient *aba1-1* mutant as compared to wild type, indicating that ABA masks BR effects in plantstress responses. To explore further the role of BR in stress tolerance, we characterized T-DNA insertion mutants for select genes identified in the microarray screen and evaluated stress tolerance of the true knockout mutants. Our studies have led to the identification of new genes involved in stress tolerance, as well as uncovered some negative regulators of the stress response. Ongoing studies are addressing the specific functions of these genes in relation to BR, ABA and abiotic stress signaling in *A. thaliana.*

T14

Water Use efficiency and crop productivity on the Canadian Prairies

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Seasonal and temporal variation in soil moisture is a major limitation of crop yield on the Canadian prairies. Although plant breeders in Canada have made good progress in yield improvement, average yields have remained almost static in the last two decades. High water use efficiency (WUE) is a desirable trait for increasing grain yield under low moisture environments. Although WUE is often difficult and expensive to measure in breeding programs, empirical research suggests that carbon isotope discrimination (Δ13C) provides an integrated measurement of WUE of C3 crop species. While selection for low leaf Δ13C was used successfully in Australia to breed new wheat cultivars with improved WUE and yield advantage in several low moisture environments, experience has shown that improvements in WUE may not always lead to higher performance or yield in all environments. In the short growing season of the Canadian Prairies, crop production relies heavily on soil stored moisture as the amount of precipitation during the growing season in not sufficient to meet crop demand. Although moisture is often not limiting early in the season, it may become limiting in mid or late season thereby causing severe reduction in yield. In this type of environment, early crop establishment coupled with high WUE may improve yield and yield stability. This presentation will describe on-going research to study genetic diversity and stability of leaf Δ13C as a measure of WUE and productivity of barley and canola on the Canadian prairies. Since measurement of Δ13C is an expensive tool to use in breeding programs, our efforts at
identifying molecular markers diagnostic of quantitative trait loci (QTLs) linked with leaf Δ13C in barley will be discussed.

T15

TsDHN-2, A Unique Dehydrin Protein from *Thellungiella* and its Role in Salt Tolerance

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Dehydrins (D-11 subgroup of late embryogenesis abundant (LEA) proteins) are intrinsically unstructured proteins that accumulate in all photosynthetic organisms exposed to dehydrating conditions such as salinity, drought, or low temperatures. Dehydrins are highly hydrophilic and are characterized by three conserved sequences designated K-, S-, and Y-segments. While their exact function is unknown, they are proposed to act as chaperones or emulsifiers in the cells by protecting membranes against unfavourable structural changes caused by dehydrating conditions.
Recent studies have isolated a unique dehydrin protein whose cDNA accumulates in response to drought and salinity in *Thellungiella salsuginea*. This protein is a basic Y2SK2 dehydrin and has been designated dehydrin-2 (TsDHN-2). In silico predictions found TsDHN-2 to be highly disordered, characteristic of dehydrin proteins. *Thellungiella* was transformed to create individual RNAi lines with reduced TsDHN-2 expression and these were used in order to elucidate the role of TsDHN-2 during salinity stress. When germinated in the presence of salt (up to 500 mM NaCl) the RNAi lines demonstrated decreased germination in comparison to wild-type. A potential role for TsDHN-2 was revealed during salinity stress experiments which treated the RNAi lines and wild-type with 300 mM NaCl for 1 week.
Greater phenotypic damage was observed in all of the RNAi lines in comparison to wild-type. Further experimentation is underway to further characterize the role of TsDHN-2.

T16

The expression of a Western redcedar *(Thuja plicata)* sabinene synthase is localized to the epithelium of foliar resin glands and correlate with natural genetic variation in thujone content

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Western redcedar (*Thuja plicata* Donn ex D. Don) is an economically and culturally important tree species in British Columbia. Wood from this tree is light, dimensionally stable, and prized for its natural durability; however, reforestation with *T. plicata* is severely hampered by extensive ungulate browsing of plantlets. High foliar monoterpenoid content correlates with reduced browsing, providing a potential target for resistance breeding. The most abundant terpenoids in *T. plicata* foliage are the monoterpenes a and b-thujone, both of

which strongly deter ungulate browsing. The genetic basis of monoterpenoid biosynthesis in *T. plicata* is unknown; however it is suspected that the monoterpene sabinene is the precursor of thujone biosynthesis. Metabolite profiling of *T. plicata* foliage indicates that monoterpenes are exclusively stored in foliage resin glands. Based on this information, we identified a putative monoterpene synthase gene which is highly expressed in the epithelium of foliar resin glands, and whose gene expression is positively correlated with the natural genetic variation in foliar thujone content in the *T. plicata* breeding populations. *In vitro* enzyme assays confirm that the partially purified recombinant protein converts geranyl pyrophosphate into sabinene and several minor monoterpene products. The use of this gene as a first generation genetic marker for marker-assisted-selection of *T. plicata* monoterpene content will be discussed.

Cell and Development, Concurrent Session Abstracts

T17

Mechanical modeling of cytoskeletal dynamics and vesicle transport in growing pollen tubes

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Plant cell growth requires the transport of cell wall matrix material to the growing regions of the cell surface. The addition of cell wall material is crucial to prevent thinning of the expanding cell wall that would otherwise lead to rupture. In tip growing plant cells, surface expansion occurs exclusively at a very small region of the cell - the apical end of the elongating tube. In pollen tubes this spatially confined growth activity requires enormous amounts of pectic cell material to be delivered to the growth site. Because of the extremely rapid growth rates typical for this cell type, the delivery process must occur at high rates and with precise targeting. The spatio-temporal movement patterns of exocytotic vesicles in growing pollen tubes are controlled by the actin cytoskeleton. Remarkably, the target region at the apical pole of the cell does not contain much filamentous actin. We use high temporal resolution confocal laser imaging to monitor the rapid movements of vesicles within the pollen tube cytoplasm. To understand how the characteristic movement patterns are generated even in cellular regions without filamentous actin, we model actin dynamics and actin-myosin-mediated vesicular trafficking. Vesicle movement in the actin-free zone is assumed to depend on diffusion processes. Boundary conditions include the expanding cell wall and the actin array forming the subapical actin fringe. Dynamic advancement of the fringe in the model was obtained by imposing a steady shape and constant polymerization rate of the actin filaments. Letting vesicle flux into and out of the apical, actin-free region be determined by the orientation of the bordering actin microfilaments and by exocytosis was sufficient to generate a flow that corresponds in magnitude and orientation to that observed experimentally. This model is able to explain how the cytoplasmic streaming pattern in the apical region of the pollen tube can be generated without the presence of actin microfilaments. The predictive value of the model is confirmed when applying it to different types of pollen (angiosperm and gymnosperm) in which the orientation of actin filaments and hence the spatio-temporal patterns of cytoplasmic streaming differ significantly.

T18

The increasing diversity of plant U-box E3 ubiquitin ligases in Arabidopsis: from hormone to abiotic stress responses

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The ability of plants to sense and respond to environmental and endogenous signals is essential to their growth and development. And as part of the diverse cellular functions during growth and development, ubiquitin-mediated proteolysis has emerged to be an integral process. Of the three enzymes involved in linking ubiquitin to protein targets, the E3 ubiquitin ligases are of particular interest as they confer substrate specificity during this ubiquitination. In plants, the U-box family has undergone a large gene expansion that may be attributable to the regulation of biological processes unique to the plant life cycle. For example, in Arabidopsis, there are 64 predicted plant U-box (PUB) proteins, and the biological roles of many of these have yet to be determined. The focus of this research is on ARM repeat-containing PUB E3 ligases, for which a 41-member family exists in Arabidopsis. A number of single mutation T-DNA insertion lines for PUB-ARM genes were screened for altered plant growth and development, and no readily discernable changes were observed. With closer inspection of selected insertion lines, however, interesting phenotypes emerged. Insertion lines for *PUB44* showed altered growth during seed germination and seedling growth. Insertion lines for the closely related *PUB43* did not show the same poor seedling growth exhibited by *pub44* seedlings, but did show similar altered germination effects in the presence of ABA as seeds from *pub44/+* plants. Meanwhile, expression profiling has also been used as a starting point to elucidate PUB-ARM function. *PUB19* and closely related *PUB18* are notable for their increased expression during abiotic stresses, but, on the basis of their consistent clade formation from phylogenies based on sequence similarity of several domains, are thought to operate redundantly. Preliminary condition-directed growth assays showed a slight increase in salt sensitivity for *pub19* seedlings, and we are currently investigating whether recently generated *pub18 pub19* double insertion lines will show an enhanced sensitivity to salt and/or to other abiotic stresses noted from the microarray expression profiling. Thus, while *PUB44* appears to have a role in regulating cell death and ABA responses, *PUB19* and *PUB18* appear to have a role during adaptation to abiotic stresses.

T19

A potential link between lipid signaling and protein phosphatase activity in Arabidopsis

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Phosphorylation of proteins, a reversible reaction controlled by opposing protein kinases and phosphatases, is intimately involved with almost all aspects of cell biology. It has been estimated that one third of all eukaryotic proteins are regulated by phosphorylation. Protein phosphatases, the enzymes that remove the phosphate, are classified into four major families. The PPP family, which is one of the most highly conserved families of proteins known, includes protein phosphatases 1 and 2a (PP1 and PP2a). The activity of PP1 is controlled by an array of regulatory proteins, which function to localize PP1 to the appropriate substrate and modify its substrate specificity. Most PP1 binding proteins contain an amino acid motif defined as RVXF/W, which is responsible for their interaction with PP1. In a screen for PP1 binding proteins in Arabidopsis thaliana, one candidate was identified which contains an RVXF/W motif and a GRAM domain (Glucosyltransferases, Rab-like GTPase Activators and Myotubularins). This domain is known to bind members of the signaling lipid family of phosphatidyl-inositol phosphates, and is similar to the Pleckstrin Homology (PH) domain in fold. Our candidate protein was confirmed to bind PP1, dependent on its RVXF motif, and the phosphatidyl-inositol phosphates PI(3,5)P2 and PI(3,4,5)P3. This combination of phosphatase and signalling lipid binding is unique to the over 100 known PP1 regulatory proteins.

T20

Cellular roles of the microtubule associated protein CLASP

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The microtubule cytoskeleton assumes diverse arrangements that drive cell division and expansion. The formation and functioning of these microtubule arrays requires the activity of numerous microtubule associated proteins (MAPs). CLASPs (Clip Associated Proteins) are conserved MAPs known to function in the attachment of microtubule plus ends to chromosomes and the cell cortex in animals. Here we show that the Arabidopsis CLASP protein is a MAP with roles in organizing both interphase and mitotic arrays. This organizing activity is accomplished in part via stabilization of microtubules, and in part to CLASPs ability to anchor microtubules along their lengths to the cell cortex. Despite relatively normal microtubule arrays, plants lacking CLASP protein are severely dwarf and exhibit marked defects in cell expansion and division. CLASP function is investigated here in terms of protein localization, microtubule organizational changes, and the observed cellular defects upon knockout.

T21

Arabidopsis LTPG is Required for Export of Lipids to the Plant Surface

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Aerial surfaces of terrestrial plants are sealed and protected by the lipids of the plant cuticle. Lipid transfer proteins (LTPs) have long been hypothesized to act as shuttles of cuticular lipids from epidermal cells to the plant surface. We tested this hypothesis by searching for LTP genes that were highly expressed in the epidermis during cuticle deposition using an Arabidopsis microarray made from epidermis tissue of rapidly expanding regions of primary inflorescence stems. This approach identified several candidate LTP genes, whose function was tested using T-DNA insertional mutants and gas chromatography. Most of these single gene mutant lines had wax loads identical to the wild-type plants with the exception of *ltpg,* a gene encoding a glycosylphosphatidylinositol-anchored lipid transfer protein. *ltpg-1* mutants had reduced wax load on the stem surface as a result of decreased nonacosane. This result showed that LTPG is involved either directly or indirectly in cuticular lipid deposition. Using a recombinant protein for *in vitro* assays, we showed that the mature, processed LTPG has lipid binding capacity. LTPG was primarily localized to the plasma membrane on all faces of stem epidermal cells during cuticle secretion. Taken together, these results indicate that LTPG is a new component of the cuticular lipid export machinery

T22

Regulation of Post-embryonic Cotyledon Development by a Subset of Arabidopsis Ovate Family Proteins

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There are a total of 18 genes in the Arabidopsis genome that are predicted to encode proteins containing an OVATE domain, designated as Arabidopsis Ovate Family Proteins (AtOFPs). Previously, we demonstrated that one member of the AtOFPs, AtOFP1, functions as a transcriptional repressor that suppresses cell elongation. AtOFP1 acts, in part, by suppressing the expression of *AtGA20ox1*, a gene encoding a key enzyme in gibberellin biosynthesis. However, little is known about the functions of other AtOFPs, or their relationship to AtOFP1. Furthermore, nothing is known about other genes whose expression might be regulated by AtOFP1. We generated and compared transgenic lines over-expressing each of the 18 *AtOFP* genes in Arabidopsis and found that among these, overexpression of *AtOFP1*, *AtOFP2*, *AtOFP4*, *AtOFP5* and *AtOFP7*, but not of any other *AtOFP* genes, resulted in characteristic kidney- shaped cotyledons in young seedlings. This small subset of *AtOFP* genes, designated as class I *AtOFPs*, have both overlapping and distinct expression patterns in various tissues and organs. Every class I AtOFP protein was found to function as a transcriptional repressor in Arabidopsis protoplast transfection assays, implying that they may represent a previously unrecognized family of transcription factors. We

demonstrated that AtOFP1 functions in the nucleus and that cotyledon development is controlled by AtOFP1 in a post-embryonic-specific manner. Microarray analysis revealed a list of genes whose expression is down-regulated by AtOFP1. Functional characterization of the *AtOFP* gene family thus identifies a subset of *AtOFP* genes as important regulators of post-embryonic cotyledon development, and reveals the global gene expression profile controlled by *AtOFP1*.

T23

Endoreduplication in the seeds and young seedlings of Fabaceae

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Endoreduplication is an alternative form of the cell cycle in somatic tissues, in which the repeated rounds of nuclear DNA replication occurs without subsequent mitosis. The significance of endoreduplication is still not clear, but it seems to be related to the systematic position, genome size, life cycle type, type and age of the tissue/organ. The present study aimed at the comparison of the level of endoreduplication in the seeds and young seedlings in the species from the Fabaceae family. The proportions of the cells with different DNA content was estimated in various organs of 27 species of different genera, genome size, ploidy level, and life cycle using flow cytometry. The nuclei were isolated by chopping in the isolation buffer, stained with DAPI and analyzed using Partec CCA (Partec GmbH, Münster, Germany) flow cytometer. After histograms evaluation the mean C-value was established. Different endoreduplication level was found depending on the species and organ. The highest endopolyploidization was observed in the cotyledons of the dry seed of the genus *Phaseolus*, where the DNA content reached 128C, after undergo four or five endocycles. In contrast, in cotyledons of the species belonging to genera *Medicago*, *Cassia*, and *Parkinsonia* no endoreduplicated nuclei occurred. In the embryo/seedling axis, depending on the species, nuclei up to only 16C were detected. In this part, the proportion of endopolyploid nuclei increased upon imbibition. The relationship between the species characteristics and endoreduplication level in their organs will be discussed.

T24

Regulation of Epidermal Cell Patterning by Single-Repeat R3 MYB Transcription Factors in Arabidopsis.

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The patterning of epidermal cell types in Arabidopsis is an excellent model for studying the molecular basis of cell specification in plants. The distribution of different cell types in the Arabidopsis epidermis is regulated by several different types of transcription factors. It has been proposed that during trichome and root hair development, an R2R3 MYB-type transcription factor (GL1 or WER), a bHLH transcription factor (GL3 or EGL3), and a WD-repeat protein (TTG1) form a transcriptional complex to induce the expression of both *GL2*, which encodes a homeodomain protein, and single-repeat R3 MYB genes, including *TRY*, *CPC*, *TCL1*, *ETC1*, *ETC2* and *ETC3*. By using Arabidopsis protoplast transient expression assays as well as using transgenic plants expressing GL1-GL3 fusion protein, we confirmed that a complex between GL1 or WER and GL3 or EGL3 is required and sufficient to induce the expression of *GL2* and a subset of single-repeat R3 MYB genes. However, because GL2 and single-repeat R3 MYBs have opposite roles in epidermal cell patterning, it remains unclear how GL2 and single-repeat R3 MYBs are coordinated to regulate epidermal cell patterning. By analyzing the double and higher order mutants between *gl2* and single-repeat R3 MYB mutants, we found that in all mutants between *gl2* and single-repeat R3 MYB mutants, trichome number was dramatically reduced and trichome morphology changed, compared with single-repeat R3 MYB mutants. On the other hand, mutants between *gl2* and single-repeat R3 MYB gene mutants phenocopied the *gl2* single mutants in root hair patterning and mucilage formation. These findings revealed distinct mechanisms between GL2 and single-repeat R3 MYBs in the regulation of trichome and root hair patterning in Arabidopsis.

T25

The Auxin Response Factors *ETTIN/ARF3* and *NPH4/ARF7* redundantly function with *MP/ARF5* to promote leaf initiation in *Arabidopsis thaliana*

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The plant hormone auxin has been implicated in many diverse processes in plant development including the formation of leaves from the shoot apical meristem and subsequent leaf vascular tissue patterning. Genetic evidence from *Arabidopsis thaliana,* links both processes to the Auxin Response Factor (ARF) gene *MONOPTEROS (MP)*, involved in auxin signaling and the *PIN-FORMED1 (PIN1)* gene, involved in auxin transport. We have previously shown that *MP* and *PIN1* play essential roles in leaf vascular patterning and that leaf formation is disrupted in *mp pin1* double mutants (Wenzel et al., 2007; Schuetz et al., 2008).
While it is clear that *MP* is an important player in both processes of leaf initiation and vascular patterning, loss of function mutations in *MP* have a relatively limited phenotypic impact. By analyzing the temporal and spatial expression profiles of ARF genes phylogentically most similar to *MP,* we identified the *ETTIN / ARF3* and *NPH4 / ARF7* genes as candidates for putative overlapping function with *MP*. Subsequent analysis of *mp arf3* and *mp arf7* double mutants confirmed novel functional roles for *ETTIN / ARF3* and *NPH4 / ARF7* in leaf initiation. The results presented provide a framework for how local auxin gradients lead to differential gene regulation and add an important component to the established mechanism of auxin signal transduction.

T26

Retroposition and positive selection led to the evolution of a novel phenolic pathway needed for pollen development

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Natural product diversity is an essential feature of plant adaptation. The underlying need for metabolic plasticity largely relies on the creation of new genes, and has led to evolution of large gene families. A typical example is provided by the diversification of cytochrome P450 monooxygenases in plants. We describe here a retroposition, neofunctionalization and duplication sequence that via selective and local amino acid replacement led to the evolution of a novel phenolic pathway in *Brassicaceae*. The parental gene (CYP98A3) encodes coumaroyl-shikimate 3'-hydroxylase catalyzing the *meta*-hydroxylation of the phenylpropanoid pathway leading to lignin biosynthesis among many other natural products. The retro-copies (CYP98A8 and A9) gained novel regulatory elements and are expressed specifically in the tapetum of the anthers. They have lost the parental activity with the shikimate ester completely, but maintained *meta*-hydroxylase activity of a phenolic moiety. The novel substrate contains three phenylpropanoid moietys coupled to spermidine. The novel pathway involves a cascade of 6 successive hydroxylations catalyzed by CYP98A8 and A9 (in partial redundancy) leading to the formation of N^1,N^5-di(hydroxyferuloyl)-N^{10}-sinapoyl spermidine, a major pollen constituent and so far overlooked player in phenylpropanoid metabolism. This example shows how positive Darwinian selection can favor structured clusters of non-synonymous substitutions needed for transition to new function.

T27

The barley flowering time protei, FCA, is ABA inducible and may be involved in processes related to seed germination and dormancy

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The RNA binding protein Flowering Control Locus A (FCA) has been shown to regulate flowering in rice and Arabidopsis. FCA interacts with FY to auto-regulate its own transcripts as well as to control flowering by downregulating FLC. Recently, FCA has been shown to be involved in RNA mediated chromatin silencing of various single and low copy gene loci in the Arabidopsis genome. In addition, the expression of the poplar homologue of *FCA* was shown to be up-regulated when apical dormancy is triggered in poplar. Here we report the cloning and characterization of the gamma (γ) isoform of *FCA* from barley. HvFCA protein showed higher sequence similarity to wheat and rice than to Arabidopsis FCA. It contains two RNA recognition motifs (RRMs), a glycine rich region at the N-terminal end, a polyglutamine region immediately downstream of a WW domain. Contrary to the findings in poplar, *FCA* transcript levels did not change in developing barley embryos and could be detected from dough to desiccated stage of embryos. *FCA* levels declined as germination progressed in barley. This decline in FCA was inhibited when germination was stopped by the application of ABA or by cold (40C) treatments. *FCA* also showed up-regulation by ABA and abiotic stress in germinating barley seeds and seedlings.

T28

Abscisic acid increases ABI5 protein levels by promoting KEG self-ubiquitination and degradation by the 26S proteasome.

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The E3 ubiquitin ligase, KEep on Going (KEG), negatively regulates of Abscisic acid (ABA) signaling by maintaining low levels of ABI5 in the absence of stress. KEG encodes a multi-domain protein that includes a RING domain, a kinase domain, ankyrin repeats, and HERC2-like repeats. keg mutant seedlings undergo growth arrest immediately after germination and accumulate high level of ABI5 protein. Here we demonstrate that the RING domain is required for the rescue of growth arrest and decrease of ABI5 protein level in the keg mutant seedlings. We have also found that overexpression of KEG leads to ABA insensitivity which correlates with the KEG protein levels in transgenic plants. These results further confirm that KEG promotes ABI5 turnover in the absences of ABA. In the presence of ABA, ABI5 levels increase drastically via increase in mRNA levels as well as decrease in 26S proteasome mediated turnover. ABA may promote the accumulation of ABI5 via ABA blocking KEG interaction with ABI5 or ABA inhibiting the E3 ligase activity of KEG, thus preventing ubiquitin mediated degradation of ABI5. We have found that ABA regulates KEG protein level by inducing ubiquitination and subsequent degradation of KEG through the 26S proteasome pathway. A functional RING domain is required for the ABA induced turn-over of KEG, suggesting that the degradation is due to self-ubiquitination. We have also found that kinase inhibitors can block the degradation of KEG induced by ABA indicating that phosphorylation, possibly self-phosphorylation, is involved in the ABA regulation of KEG protein levels. We discuss a possible model for how ABA may regulate KEG protein levels leading to accumulation of ABI5 and cellular responses.

T29

Examining the role of ABCG26 in Arabidopsis thaliana pollen wall formation: A putative sporopollenin monomer transporter

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Sporopollenin is the major constituent of the outer walls of spores and pollen, and provides protection from inherent terrestrial stresses including desiccation and UV radiation. Without it, land plants could not reproduce. Despite the importance of this biopolymer in plant life, our understanding of the monomeric components, structure, biosynthesis, transport and polymerization of sporopollenin remains largely incomplete. From the data available, sporopollenin is a co-polymer of specific fatty acids and oxygenated aromatic compounds chemically linked to form a structurally robust biopolymer. Uncharacterized sporopollenin monomers appear to be secreted by anther tapetum cells into anther locules, where polymerization on developing pollen grain walls occurs. Analyses of male sterile mutants defective in pollen wall formation, primarily in the model plant Arabidopsis, have revealed genes required for sporopollenin biosynthesis and deposition, including *MS2, DEX1, DEX2, ACOS5* and *DFRL1*. These mutants have provided clues regarding the mechanism of sporopollenin biosynthesis. In particular, the discovery of acyl-CoA synthetase5 (ACOS5), an enzyme required for sporopollenin synthesis, facilitated co-expression analyses revealing additional genes with similar expression patterns, potentially encoding enzymes and proteins with roles in sporopollenin biosynthesis and deposition. Among the genes identified was a gene encoding a membrane transport protein from the ATP-binding cassette (ABC) transporter superfamily (At3g13220 or *ABCG26*). This gene is of particular interest due to its potential role in the transport of sporopollenin components from their site of synthesis in tapetal cells to developing pollen grains, similar to ABC transporters involved in wax secretion from epidermal cells. A T-DNA insertion mutant in *ABCG26* exhibits partial male sterility, defective pollen formation, and *ABCG26* is specifically expressed in tapetal cells supporting such a role. This project aims to characterize the roles of this protein in sporopollenin monomer secretion, which will lead to a more comprehensive understanding of sporopollenin biosynthesis and deposition.

T30

Export of the waxy cuticle by ABCG transporters

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All plant growth, including agricultural and forestry production, requires cell wall synthesis. Despite its critical importance, there is a major gap in our understanding of cell wall biosynthesis: how are cell wall components, which are made inside the cell, exported to the outside of the cell to build a functional wall? Our approach is to integrate biochemistry with cell structure using advanced microscopy techniques such as high-pressure freezing/freeze substitution, cryo-electron microscopy and live cell imaging. The plant cell wall contains specialized functional domains where the polysaccharides are impregnated with other macromolecules, such as lignin in wood, or the waxy cuticle coating the plant surface. For export of lipids to the cuticle, Arabidopsis mutant analysis has identified the first components of the wax export system, two ATP-binding cassette (ABC) transporters of the ABCG subclass that are required for wax export from the epidermis to the cell wall.

T31

Protein interactions between members of the chloroplast import machinery in *Arabidopsis thaliana*: A study using fluorescence spectroscopy

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Protein interactions are an important and critical part of cellular function. Understanding interaction dynamics between proteins and ligands, as well as other proteins are necessary for system modeling. Protein import into chloroplasts is one such system where the understanding of interaction dynamics between members of the translocation complex is limited. The translocon at the outer envelope membrane of chloroplasts consists of three core components (TOC75, TOC159, and TOC34). Interactions between members of the TOC159 and TOC34 gene families in *Arabidopsis thaliana* have been shown to produce structurally and functionally distinct complexes. More specifically, there is evidence that complexes containing atTOC159 and atTOC33 import photosynthetic proteins, whereas complexes containing atTOC132/120 and atTOC34 seem to preferentially import non-photosynthetic proteins. Also, the interactions between TOC159 and TOC33 or TOC132 and TOC34 are mediated by their GTP-binding domains. The current project uses recombinant GTP-binding domains to investigate the specificities of these interactions in more detail using biophysical approaches. The putative binding domain(s) and the molecular properties that confer specificity for interaction(s) between family members will be determined using techniques such as fluorescence spectroscopy, which is a powerful tool for studying protein-protein interactions. By manipulating the location of tryptophan residues within the proteins, the intrinsic fluorescent properties of interaction events can be assessed based on quenching and activation. The current presentation will focus on the generation of mutant proteins required for testing the interactions, current data, as well as future directions related to this project.

T32

An investigation of the Exocyst Complex and its role in compatible pollen-pistil interactions in *Arabidopsis*

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Exocytosis is a highly ordered vesicle trafficking process where vesicles are delivered to the plasma membrane and secreted to the cells exterior. The exocyst is a large eight subunit multimeric complex that is primarily involved in polarized or regulated exocytosis in eukaryotic cells where it functions to tether exocytic vesicles to the plasma membrane. Originally identified in *Saccharomyces cerevisiae*, the exocyst is composed of eight subunits;

Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p and Exo84p, and putative homologs have been identified in both animal and plant genomes. In *Arabidopsis*, the majority of the exocyst subunit genes exist as single copies or duplicates while there has been an expansion of the Exo70 gene to produce a 23-member gene family. Our research has recently implicated one of the Exo70 family members, Exo70A1, in compatible pollen-pistil interactions in *Brassica* and *Arabidopsis*. Exo70A1 was isolated in a search for targets of the *Brassica* self-incompatibility response. This signalling pathway rejects 'self' pollen through a receptor kinase-based signaling cascade in the stigma. Exo70A1 appears to act at the intersection of two cellular pathways whereby it is required in the stigma for acceptance of compatible pollen in both *Brassica* and *Arabidopsis* and is negatively regulated by *Brassica* self-incompatibility. This discovery is quite significant as the cellular signalling pathway allowing for the recognition of compatible pollen grains by the stigma is largely unknown. My current research goals are to study the role and localization of the exocyst, specifically the Exo70A1 subunit, in the stigma during compatible pollen-pistil interactions in *Arabidopsis*.

T33

Characterization of GmCaMK, a novel calmodulin kinase from soybean root nodules

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Ca2+ ions are key second messengers in many eukaryotic signal transduction pathways, often mediated by the ubiquitous Ca2+ sensor protein, calmodulin (CaM). CaM binds Ca2+ and modulates the activity of numerous target proteins, including protein kinases. While Ca2+/CaM-dependent protein kinases (CaMKs) play critical and well understood roles in animals, few CaMKs have been found or studied in plants. Here we present the isolation of GmCaMK1, a novel CaMK from soybean (*Glycine max* c. Boyer) root nodules. GmCaMK1 was identified by screening a nodule cDNA expression library with 35S-radiolabeled CaM. GmCaMK1 is a 47kDa protein, with an internal conserved ser/thr kinase domain, and appears to be part of a two member subfamily in soybean. A transmembrane domain is highly predicted at the amino-terminus of GmCaMK1, and we have delineated the CaM-binding domain (CaMBD) to a 20 amino acid motif near the carboxy-terminus. The CaMBD is currently being analyzed via isothermal titration calorimetry, while initial evidence indicates that the Kd of this CaMBD is <40 nM. Furthermore, this CaMBD interacts with CaM in a Ca2+- dependent manner, which is consistent for CaMKs previously described. Preliminary ssays using recombinant enzyme indicate that CaM is not required for GmCaMK1 autophosphorylation. As such, we are currently testing the effect of Ca2+/CaM on the *in vitro* phosphorylation of a variety of substrates. RT-PCR expression analysis shows that GmCaMK1 is primarily expressed in roots, with some expression in developing nodules. In light of this expression pattern, we are using RNAi lines to examine a possible role for GmCaMK1 in nodule development and root morphology. GFP-fusion transgenics and westerns are being used to determine if GmCaMK is membrane associated. As this CaMK appears to be highly conserved amongst plants, we cloned the *Arabidopsis* homologue of GmCaMK (74% a.a. identity) from leaf tissue, and demonstrate that it is also a CaM-binding protein.

Technology, Concurrent Session Abstracts

T34

Production of active human glucocerebrosidase in Arabidopsis *cgl* (*complex-glycan-deficient*) seeds

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We are developing various plant-based systems to produce enzymes for the treatment of lysosomal storage disorders.One such system is seeds of the *Arabidopsis thaliana cgl* mutant, which are deficient in the activity of *N*acetylglucosaminyl transferase I (EC 2.4.1.101), and thus produce recombinant proteins containing glycans primarily in the high-mannose form. The terminal mannose residues are critical for the biological uptake by macrophage mannose receptors. Gaucher's disease is the most prevalent lysosomal storage disorder caused by mutations in the gene encoding glucocerebrosidase (GBA). Using the construct containing arcelin (*arc 5-I*) gene promoter, exchange of the 5′-UTR and signal peptide sequences of the GBA gene with those of the arcelin gene, and the arcelin gene 3′ end, we were able to obtain one transgenic line which produced high level of human GBA in *cgl* seeds. Immunolocalization data showed the GBA was mainly accumulated in the cell wall. The GBA protein was purified to apparent electrophoretic homogeneity using Con A and hydrophobic interaction chromatography. The specific activity of *cgl*-produced GBA was similar to that of Cerezyme® produced in CHO cells which is currently used for enzyme replacement.

T35

Dissecting QTLs with *Ac/Ds* transposons

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The maize *Ac/Ds* transposon system is an effective approach for gene identification and cloning in heterologous species. Using this system, single-copy *Ds* insertion lines (TNPs) were generated in barley to identify, tag, and determine genes and their function. Our recent successful demonstration in barley of *Ds* transposition at significant frequencies over multiple generations in addition to the preference of *Ds* to re-insert near the original site of excision and into genic regions facilitates saturation mutagenesis. Plants with single *Ds* insertions (TNPs), mapping near genes of interest, are important vehicles for gene identification

through re-activation and transposition of *Ds*. Mapping and bioinformatics analysis *Ds* flanking sequences indicate that the vast majority of *Ds* insertions (88%) are in genic regions. The bias of *Ds* towards genes is highly valuable for large genome cereals and *Ac/Ds* can be used as gene search tool. Data on multigenerational *Ds* re-activation is critical for localized saturation mutagenesis efforts, including the re-activation needed for "transposon walking", the sequential re-activation of *Ds* that can be used to identify QTLs and members of clustered gene families. One major QTL complex, QTL2 mapped on the short arm of chromosome 4H, affects several malting quality parameters. We are saturating malting-quality QTL regions with maize *Ds* elements to facilitate identification and characterization of genes associated with malting in the QTL2. TNP lines mapped on chromosome 4H, *Ds* elements were re-activated by crossing them with *Ac*TPase-expressing plants. New *Ds* transpositions have been identified by southern blotting and *Ds* tagged genes are being cloned using inverse PCR. This effort of saturation mutagenesis with *Ds* transposons will lead to a better understanding of malting quality traits and candidate genes that display quantitative variation.

T36

A novel approach to enhancing salinity tolerance by engineering a chloride-volatilizing enzyme in plants

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Several organisms possess enzymes that can catalyze one-step methylation of Cl ions to chloromethane gas using *S*-adenosyl-L-methionine as methyl donor. Presence of this enzyme in organisms that live in saline habitats has been interpreted as a mechanism for Cl– detoxification via its volatilization (Science **249:** 160-162), but this possibility has never been experimentally tested. While searching for chloride-methylating enzymes in plants, we identified a thiol methyltransferase (TMT) in cabbage that, aside from its natural role in the methylation of thiol compounds produced upon glucosinolate hydrolysis, was also able to methylate Cl– ions with greater efficiency than any other similar enzyme reported (J Biol Chem **270:** 9250-9257; Plant Cell Environ. **23:** 165-174). We cloned the gene encoding this TMT (Plant Mol. Biol. **50:** 511-521), and engineered it under the control of CaMV 35S promoter into tobacco, which otherwise lacks the ability to methylate Cl–. Transgenic tobacco plants acquired the ability to efficiently transform Cl– to chloromethane over extended periods, parallel with a dramatic enhancement in their salinity tolerance. Whereas both wild type and transgenic plants grew normally in 50 NaCl, transgenic plants grew significantly better at higher concentrations of the salt. The latter were able to complete their life cycle and produce viable seed at 200 mM NaCl, which was lethal to the wild-type plants. The results convincingly demonstrate that volatilization of Cl– is a detoxification event that can contribute to the plant's ability to withstand salinity stress. This ability can, therefore, be used to engineer crop species with enhanced salt tolerance.

T37

Magnetic Resonance Micro-Imaging (MRMI) indicates water mobility is correlated with axillary bud dormancy induction in hybrid poplar (*Populus* spp.)

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Biophysical changes in water are known to be associated with dormancy induction in woody plants. Magnetic Resonance Microimaging (MRMI) was used as a non-destructive method to measure T1 relaxation times and the apparent diffusion coefficient (ADC) in three regions (vascular tissue, vascular bud trace and axillary bud) during dormancy induction in hybrid poplar. T1 relaxation times are an indicator of water content and/or binding of hydrophilic molecules and ADC is an indicator of water mobility within plant tissue. Two poplar clones ('Okanese'—temperature-insensitive dormancy, 'Walker'—temperature-sensitive dormancy) were subjected to temperature regimes, 18.5/3.5 ºC and 18.5/13.5 ºC (day/night), that resulted in differences in dormancy development under short photoperiod for 'Walker' but not 'Okanese'. Apparent Diffusion Coefficient (ADC) correlated with depth of dormancy in the axillary bud and vascular bud trace regions. In contrast, T1 relaxation time did not correlate with dormancy induction in any of the regions examined. Although MRMI studies on T1 relaxation have dominated the phytological field, our work indicates water mobility normal to the axial plane is also an important consideration in studies examining water changes during dormancy induction in the critical tissues of woody plants.

Performance, Poster Abstracts

P1

The Antarctic psychrophile, *Chlamydomonas raudensis* UWO 241, phosphorylates a 17kDa subunit of photosystem I

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Chlamydomonas raudensis UWO 241 is a photosynthetic green alga isolated from a permanently ice-covered lake in Antarctica, where it has adapted to an extremely stable environment of low temperatures, low irradiance and high salt concentrations. Previous studies have shown that *C. raudensis* UWO 241 is the first natural variant deficient in state transitions and is locked in state I. Furthermore, UWO 241 exhibits a unique phosphorylation profile with the apparent absence of phosphorylation of PSII light harvesting (LHCII) polypeptides. Instead, UWO 241 shows phosphorylation of several high molecular mass polypeptides (70-150kDa). In order to identify these phospho-proteins, 2D Blue Native PAGE was used. Immunoblot analysis of the

second dimension with phospho-threonine antibodies indicated that these phosphorylated polypeptides are closely associated with the PSI complex. To confirm this, fractionation of thylakoid membrane complexes was performed using sucrose density gradients. Purification of the resultant fractions revealed the presence of two distinct forms of photosystem I complexes in the thylakoid membranes of UWO 241, which differed in overall abundance. The 'minor' PSI complex, present in low concentrations, exhibited phosphorylation of a 150kDa complex and a 17kDa protein which co-migrated with PsaD, whereas, the 'major' PSI complex did not exhibit any phospho-proteins. Immunoblot analysis confirmed the presence of photosystem I core complex proteins (including PsaA/B, PsaD, PsaL and PsaC) in both PSI fractions. However, these PSI complexes differed in the composition of LCHI chlorophyll a/b binding proteins that constitute the peripheral antenna of PSI. The 'minor' phosphorylated PSI complex exhibited a substantial increase in the accumulation of LHCI proteins, which is consistent with the observed decreased Chl a/b ratio of 2.2 compared to 5.6 in the 'major' complex. The formation of the 'minor' complex was inhibited by the kinase inhibitor, staurosporine, which indicates that the phosphorylation of the 17kDa subunit of this PSI complex affects the structure and/or stability of PSI.

P2

Characterization of allantoinase in soybean (Glycine max)

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Symbiotic biological nitrogen fixation allows plants to form symbioses with specific bacterial partners to allow conversion of atmospheric dinitogen (N2) to ammonium. In legumes assimilation of ammonium into organic compounds occurs in specialized root structures called nodules where it is either utilized or exported to other tissues. Soybean, and other closely related legumes, transport fixed nitrogen as ureides, primarily allantoin and allantoate. These can be exported to the leaves or other destination tissues and degraded through a series of enzyme-mediated steps to release ammonium for re-assimilation. The enzyme allantoinase catalyzes the conversion of allantoin to allantoate, the first step in ureide catabolism. Despite their importance in nitrogen mobilization, plant allantoinases have not been well characterized. This study aims to identify allantoinase genes and gene expression patterns in soybean. We identified GmALN1 and GmALN2, two putative allantoinases. Functional complementation using a *Saccharomyces cerevisiae dal1* mutant strain lacking a functional allantoinase demonstrated that either GmALN1 or GmALN2 can confer the ability to utilize allantoin as the sole nitrogen source. More recently, the release of the soybean genome sequence allowed identification of two additional closely linked allantoinase genes which we name GmALN3 and GmALN4. All four genes are transcribed and can be identified in germinated seedlings. Allantoinase enzyme activity and protein levels are also measurable in seedlings. These will be used to provide tools to give a comprehensive picture of allantoinase regulation in nitrogen-fixing soybeans. Ureide accumulation has been implicated in inhibiting biological nitrogen fixation in soybean during water limiting conditions. Investigating regulation of ureide catabolism will provide insight into how this process is related to regulation of nitrogen fixation.

P3

Genetic mapping of genes responsible for dehydrodiferulic acid mediated cross-linking in maize cell walls and their association with QTL for resistance to *Fusarium graminearum* (Schwabe)

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The fungus *Fusarium graminearum* Schwabe [sexual state: *Gibberella zeae* (Schweinitz) Petch] causes *Gibberella* ear rot in maize and *Fusarium* head blight in wheat. The disease can cause significant yield losses, but the most devastating effect is the deposition of mycotoxins in the grain. Cultural practices are not effective in epidemic years, and thus genetic resistance is the favored solution. Due primarily to the polygenic nature of resistance and significant genotype by environment interaction, a quantitative trait locus (QTL) analysis was conducted to identify major alleles that contribute to resistance. It was later shown that there was a significant negative correlation between the amount of dehydrodiferulic acid (DFA) in the pericarp and the severity of disease in the mapping population. These data were used to map DFA QTL, and many of the significant chromosomal regions corresponded to previously identified resistance QTL. Since DFA is a derivative of the well established phenylpropanoid pathway, we have adopted a candidate gene approach to identify the genes that are responsible for the resistance/DFA QTL. Candidate genes were roughly mapped *in silico* using maize genetics resources prior to molecular analysis. Polymorphisms have been discovered in putative phenylalanine ammonia lyase, caffeoyl-coenzyme A 3-O-methyltransferase, and hydroxycinnamoyl-coenzyme A shikimate/quinate hydroxycinnamoyltransferase genes. Notably, a polymorphism in the 3-O-methyltransferase has been previously associated with cell wall digestibility in silage maize. These polymorphisms have been converted to molecular markers, and their effects will be examined using analysis of variance and QTL analysis. This research should provide a foundation for marker assisted resistance breeding in elite maize adapted to the northern United States and Canadian corn growing areas.

P4

Physiological relationship of seed vigor to seedling vigor in barley

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Early seedling vigor contributes to better utilization of soil moisture in an environment where high soil moisture often prevails at the time of seeding while in mid and late season; moisture deficit is the limiting factor of crop yield.
Research has emphasized the physiological traits associated

with seed and seedling vigor, but information relating seed vigor to seedling vigor and plant establishment is scare. The physiology of stressed seed germination of barley (*Hordeum vulgare*) cultivars was studied under conditions of controlled deterioration (45oC/72 h/20% seed moisture content), accelerated aging (41oC/72 h/95% relative humidity), cold stress (5 oC/12 days in the continuous dark), and unstressed germination (5 oC/3 days followed by 20 oC/7days). Cultivars were evaluated in two environments for seedling characteristics including leaf area (LA), specific leaf area (SLA), and leaf area index (LAI). Results demonstrated that the accelerated aging and controlled deterioration were the most valuable tests in discriminating cultivars for seed vigor. The unstressed germination and cold stress tests did not significantly affect cultivars ranking. Greenhouse-grown plants produced significantly ($P < 0.01$) higher SLA and LA than field-grown plants. Specific leaf area ranged from 428 to 514 cm2 g-1 and from 189 and 249 cm2 g-1, respectively for greenhouse- and field-grown plants. The cultivars were grouped by multidimensional cluster analysis (CA) and canonical discriminate analysis (CDA). The CDA revealed that cultivars were genetically diverse. Pairwise *D*2 estimates ranged from 0.69 to 0.99 with the variety 'CDC Rattan' being the most genetically dissimilar. The influence of seed vigor did not persist during seedling establishment. The use of high vigor seed lots may not be sufficient to predict seedling vigor and adequate plant establishment. Our results suggest that cultivar genetic differences influence seedling characteristics contributing to variation in early vigor.

P5

5-Aminolevulinic acid improves salt tolerance of barley seedlings

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The content of chlorophyll (Chl) in green barley seedlings grown for 7 days on 100 and 150 mM NaCl decreased twice as compared to the control untreated plants. The ratio of carotenoids to Chl under salinity increased, in average, 1.8 times confirming their protective role in chloroplast. The level of free proline and capacity to synthesise ALA started to increase simultaneously beginning from 50 mM NaCl. The maximum amount of ALA was accumulated at 100 mM NaCl and was twice higher as compared to the control. The proline level also showed 2.8 times increase as compared to its amount in the control plants. Under the subsequent increase of salt concentration the rate of ALA accumulation was strongly reduced reaching the control values at 150 mM NaCl with the following decrease of rate at 200 mM of salt. Inhibition of the capacity to synthesise ALA correlated with increase of the rate to accumulate proline the level of which was 5 times higher than in the control. Increasing rates of ALA and proline accumulation under low NaCl concentrations could result from activation of metabolism of the glutamic acid, the common precursor of ALA and proline. Proline essentially accumulates in plants in response to salinity, chilling, drought and other stresses. It is known that exogenous ALA confers to plants chilling tolerance and improves salt tolerance of shoots. The role of exogenous ALA as an anti-stress agent could be accounted for by its participation in biosynthesis of Chl and heme preserving thereby photosynthesis and transpiration, its functioning as a plant growth regulator, enhancer of endogenous cytokinin level and promotor of the antioxidant enzymes. At low NaCl concentration endogenous ALA could act as an anti-stress agent like proline. Apparently, the subsequent increase of salt concentration results in inhibition of activity of the ALA-synthesizing enzymes, switching the glutamic acid metabolism from ALA route to proline formation and stimulating biosynthesis of the latter. Indeed, growing plants on 150 mM NaCl + 10 or 60 mg/l ALA increased the level of proline 1.8 and 4.2 times accordingly over its level at 150 mM NaCl alone and restored plant growth up to the control level.

P6

Functional Analysis of the Shikimate/Quinate Cycle

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The shikimate pathway connects primary metabolism with the synthesis of essential aromatic amino acids, i.e. phenylalanine, tyrosine and tryptophan. Both the endproducts and intermediates are also precursors of a wide array of pivotal plant natural products including alkaloids and phenylpropanoids, which play important adaptive functions in plant development and defense. Branching out from the main trunk of the shikimate pathway, quinate is being formed in a single step reaction. It is thought to be a precursor of defense-related chemicals in plants, and may act as an intermediate in lignin biosynthesis. It is evident that quinate can be synthesized from both shikimate and dehydroquinate (precursor of shikimate), and enzymes involved in those two processes are quinate hydrolyase (QH) and quinate dehydrogenase (QD). However, genes encoding those two enzymes have not been identified to date. The reaction mechanisms of QD and DH resemble that of shikimate dehydrogenase (SDH) and dehydroquinate dehydrogenase (DHQD) respectively. DHQD/SDH is abifuntional enzyme acting in the shikimate pathway in plants. The DHQD/SDH gene family comprises 5 members in poplar and we hypothesize that QD and QH are actually encoded by some of the five DHQD family members. The five DHQD/SDH genes have diverged into two phylogenetic groups, one of which does not include Arabidopsis homologues. Since quinate derivatives are not known to accumulate in Arabidopsis; it is plausible that the absent group of genes instead encode QD and QH. The main focus of our project is to characterize the DHQD/SDH gene family in poplar and to identify the genes involved in quinate biosynthesis employing a functional genomic approach that includes bioinformatics-based candidate gene identification and reverse biochemical characterization of the corresponding candidate genes.

P7

Dark Relaxation of Excitation Pressure in *Chlorella vulgaris*

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Excitation pressure refers to an imbalance in energy flow which occurs whenever the rate of energy absorption exceeds the capacity to either consume or dissipate this energy resulting in a tendency to over-reduce the plastoquinone (PQ) pool. Environmental conditions including high light and low temperature exacerbate the potential for imbalances in energy flow. Acclimation of *Chlorella vulgaris* to continuous light at either low excitation pressure (LEP) or high excitation pressure (HEP) has been previously demonstrated to result in a dark-green LEP phenotype or yellow-green HEP phenotype, respectively. This HEP phenotype reflects reductions in the capacity to absorb light energy as well as increases in the capacity to dissipate excess light energy as heat. Photobioreactors are cultivation vessels capable of dynamically modulating the light and temperature regime in a programmable manner while simultaneously measuring changes in growth kinetics and chlorophyll a fluorescence parameters. Exposure of HEP cells to darkness should relax EP; however, what length of time in the darkness is required to relax EP such that the LEP phenotype is recovered? Cultures were grown at either 28^{o}C / 150 µmol photons m^{-2} sec^{-1} (28/150) – a LEP growth regime – or 28^{o}C / 2000 µmol photons m^{-2} sec^{-1} (28/2000) – a HEP growth regime – with decreasing light periods in a 24 hour (h) cycle. Cultures of *C. vulgaris* developed a LEP phenotype at 28/150 regardless of the introduction of a dark period. At 28/2000, cultures grown under continuous light developed a HEP phenotype. The introduction of a 6h dark period at 28/2000 was not sufficient to relax EP and alleviate the HEP phenotype, whereas the introduction of a 12h dark period was sufficient to relax EP and recover a LEP phenotype. Cultures exhibiting a HEP phenotype exhibited higher EP, higher chlorophyll a/b ratio and concomitant lower light-harvesting complex polypeptide accumulation as well as higher non-photochemical quenching then those exhibiting a LEP phenotype. Preliminary data indicate that EP relaxes in the darkness more slowly than anticipated. Thus, we propose that this slow relaxation of EP may represent some metabolic "memory" of previous HEP conditions.

P8

Characterization of Nitrite Transport in *Arabidopsis thaliana*

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Nitrite (NO2-) is an intermediate in the nitrate assimilation pathway. NO2- is reduced in plastids to NH4+. It is a potential source of N for plants in soil. It can accumulate in aerated soil when pH exceeds 7.5 and at lower pH values in water- logged, poorly aerated soil. High concentrations of NO2- in nutrient solution can also have adverse effects on plant growth. Its toxicity is, however, more pronounced at low pH, possibly as a result of free nitrous acid permeation. NO2- can be taken up as an alternative N source under certain environmental conditions. The uptake may occur through bispecific nitrate–nitrite transporters or via specific NO2- transporters as reported recently in *C. reinhardtii, E. coli, H. polymorpha* and *A. nidulans.* Those organisms can survive on NO2- as a sole source of nitrogen. Nevertheless, NO2- transport in plants has not been characterized. It is, however, widely presumed that NO2- enters plant cells through bispecific nitrate/nitrite transporters.

We used *Arabidopsis thaliana* wild type (WT) (var. Columbia) and knock-out mutants of well described nitrate porter genes *Nrt2.1*, *Nrt2.2* and *Nrt3.1* to examine NO2- uptake. Plants were grown in hydroponics for 5 weeks before short uptake and influx studies were conducted. Results of high-affinity nitrite net-uptake and sensitive 13NO2- tracer influx studies indicate the possible existence of another NO2- transporter. However, Measurement of 13NO2- tracer influx in *Atnrt2.1-2.2* and *Atnrt3.1* mutants showed reductions of 20 and 50% compared to WT, respectively. Influx of 13NO3- in the mutants was, on the other hand, reduced by 40 and 90% compared to WT, respectively. One would expect the same level of reduction of uptake of both NO3- and NO2- if the same transporters were responsible for the influxes of both nitrate and nitrite.

In the *Atnrt3.1* mutant the major high affinity nitrate transporters (NRT2.1/NRT2.2) are not functional; influx has been reported to be merely 5% of WT rates. The mutant was used to eliminate 13NO2- influx via NRT2.1and NRT2.2 transporters and facilitate evaluating the presence of a separate (discrete) nitrite-specific transporter. Studies of competition between NO3- and NO2- for transport showed that addition of NO3- did not affect NO2- influx. Comparison of "induction" by NO3- or NO2- of nitrate-deprived plants for up to 24 h showed a complete absence of induction by either NO3- or NO2-. . Decreasing pH, however, significantly increased NO2- influx in the *Atnrt3.1* mutant. These results suggest that permeation of uncharged nitrous acid across plasma membrane is a significant contributor to influx. Although the present results fail to provide conclusive data about the existence of a nitrite-specific transporter, we showed that significant amount of NO2- is taken up by plants even at low concentrations. This may be important in soils at low pH and environmental conditions that result in significant concentrations of NO-.

P9

Relationship between asparagine metabolism and protein content in soybean seed

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Soybean (*Glycine max* [L.] Merr.) is an important crop grown primarily as a protein and oil source. Asparagine is the main form of organic nitrogen that is transported to developing seeds during seed filling in soybean and other legumes. Asparaginase (ASPG) is considered to be the major enzyme metabolizing transported asparagine in the seed coat and developing cotyledon for the synthesis of other amino acids. Beside ASPG, there is evidence for concurrent expression of asparagine synthetase (AS) enzyme in developing seeds. The determination of free asparagine at mid-maturation in three genetically related cultivars (Maple Arrow, AC-Hercule and AC-Proteus) varying in protein content confirmed its positive correlation with seed protein content (SPC). Further, free amino acid profiles of two parental and eight

recombinant inbred lines (RILs) differing at four quantitative trait loci (QTL) determining SPC revealed that the high free asparagine trait is associated with two major QTLs. The relationship between ASPG and AS expression and SPC was investigated in Maple Arrow and AC-Proteus, in developing seed coat and cotyledons from growth-chamber grown plants, and in whole seeds from field-grown plants. ASPG and AS protein levels were characterized by immunoblots with specific antibodies. The contribution of specific ASPG and AS isoforms was evaluated in RT-PCR experiments with gene-specific primers. The results of these experiments will be discussed.

P10

Differential alterations in photosynthetic electron flux in a DpetE mutant of *Synechococcus* sp. PCC 7942 exposed to iron stress

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Plastocyanin and cytochrome *c*6 are the two soluble proteins which can alternately serve as electron donors to photosystem I (PSI) in cyanobacteria. Since the exposure of *Synechococcus* sp. PCC 7942 to iron stress restricts intersystem electron flow between PSII and PSI (*Ivanov et al. FEBS Lett. 485 (2000) 173-177*) a Δ*petE* mutant of *Synechococcus* lacking plastocyanin was used to assess the role of intersystem electron carriers under iron stress growth conditions. Photoautotrophic growth rates of a Δ*petE* mutant under control (iron sufficient) conditions was only slightly lower than that of wild type (WT) *Synechococcus* cells. The growth rate of WT cells was 12% lower under iron-deficient conditions, while the growth rate of Δ*petE* was reduced by 40%. Typical iron-stress-induced short wavelength shift in the main red Chl *a* absorbance peak from 679 nm to 674 nm was observed in iron-stressed WT *Synechococcus*. Under control conditions, the main red Chl *a* absorbance peak was shifted to 677 nm in the Δ*petE* mutant, while exposure to iron stress induced an even further blue shift to 673 nm. To examine the intersystem electron fluxes *in vivo*, far-red light induced P700 redox state transients at 820 nm of WT and Δ*petE* mutant cells grown under iron sufficient and iron deficient conditions were measured. The extent of the absorbance change ($\Delta A_{820}/A_{820}$) used for quantitative estimation of photooxidizable $P700^+$ indicated 2-fold lower level of $P700^+$ in Δ*petE* compared to WT cells under control conditions. This was accompanied by a 2-fold slower re-reduction rate of $P700^+$ in the Δ*petE* indicating a lower capacity for cyclic electron flow around PSI in the cells lacking plastocyanin. Thermoluminescence (TL) measurements did not reveal significant differences in PSII photochemistry between control WT and Δ*petE* cells. However, exposure to iron stress induced a 4.5 times lower level of $P700^+$, 2-fold faster re-reduction rate of $P700^+$ and a temperature shift of the TL peak corresponding to S_2/S_3Q_B- charge recombination in WT cells. In contrast, the iron-stressed Δ*petE* mutant exhibited only a 40% decrease of $P700^+$ and no significant changes in S_2/S_3Q_B- charge recombination. The physiological role of mobile electron carriers in modulating the photosynthetic electron fluxes in cyanobacteria under iron stress conditions will be discussed.

P11

Cold Acclimation of Winter Cereals Enhances CO_2 Assimilation under Short-term Elevated CO_2

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Since cold acclimation of winter rye, wheat and *Arabidopsis thaliana* is associated with enhanced rates of light saturated CO2 assimilation, we hypothesized that exposure of cold acclimated (5ºC) winter cereals (cv Norstar; cv Musketeer) to elevated CO2 (700 ppm) should stimulate photosynthetic capacity relative to non-acclimated controls (20ºC). Furthermore, cold acclimated winter and spring cereals (cv Katepwa; cv SR4A) should exhibit a differential stimulation of photosynthetic capacity upon exposure to elevated CO2. Short-term exposure to elevated CO2 stimulated photosynthetic capacity to a greater extent in cold acclimated winter rye and wheat (80-90%) than in non-acclimated controls (60-70%). However, exposure to elevated CO2 only compensated for the cold acclimation-induced inhibition of CO2 assimilation of spring cereals. These results for the four cultivars examined are consistent with the previous results showing that the unusual capacity of winter cereals to maximize CO2 assimilation appears as a consequence of global reprogramming of growth, development and carbon metabolism in response to low growth temperature. Concomitantly, we showed, for the first time, that this enhanced capacity for CO2 assimilation in cold acclimated winter cereals was also associated with increased rates of photosynthetic electron transport. Both cold acclimation and elevated CO2 substantially suppressed the stomatal conductance suggesting that the increased photosynthetic capacity of both winter cultivars associated with cold acclimation and/or elevated CO2 is not due to stomatal regulation. Since the maximum stimulation occurred in less than 30 min upon the shift from ambient to elevated CO2, we conclude that exposure to short-term elevated CO2 overcomes Rubisco CO2 substrate limitations present under ambient CO2 conditions. Furthermore, cold acclimated winter cereals exhibited a differential stimulation of photosynthetic capacity upon exposure to elevated CO2 relative to the spring cereals tested. However, this CO2-dependent stimulation of photosynthetic capacity was dependent upon temperature and time. The CO2-dependent stimulation was stable for the first 6 hours at 40 OC but declined subsequently by 80-95% after 80h at 40 OC in all cultivars. Although the CO2- stimulated rates of photosynthesis were inhibited by temperatures of 30ºC or greater in a time-dependent manner, elevated CO2 appeared to provide transient protection against high temperature inhibition of CO2 assimilation during the initial 6h. This protective phenomenon occurred independently of the acclimation state of the all cultivars tested.

P12

The role of nitric oxide in the nitrate-dependent induction of the alternative oxidase in *Chlamydomonas reinhardtii*

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In the green alga *Chlamydomonas reinhardtii* a shift in nitrogen source from ammonium to nitrate results in strong induction of alternative pathway respiration. Nitrate has been shown to increase both alternative oxidase (AOX) protein abundance within the mitochondrion as well as an upregulation of the nuclear gene, *Aox1*. In *C. reinhardtii* the genes required for nitrate assimilation such as various transporters, nitrate reductase and nitrite reductase are termed nitrate assimilation related or NAR genes. These genes, which are found in two clusters in the nuclear genome (Quesada et al. 1998) display coordinated regulation, being strongly induced by nitrate while being actively repressed by ammonium (Quesada and Fernandez 1994). Interestingly, *Aox1* was found to be located in one of the NAR gene clusters, originally being designated as *NAR5* (Quesada et al. 1998). The physiological basis for AOX induction by nitrate remains unresolved. It has been hypothesized by Quesada et al. (2000) that the induction of AOX during nitrate assimilation is in response to increased reductant formation in the chloroplast. However recent findings from our laboratory (Pakkiriswami et al. 2009) suggest that this is probably not the case. An alternative hypothesis is that AOX is induced by nitric oxide. It has been shown that nitrate reductase can catalyze the NAD(P)H-dependent reduction of nitrite to NO (Kaiser et al. 2002) and that this reaction is a major source of nitric oxide in *C. reinhardtii* (Sakihama et al. 2002). It follows that a shift into nitrate media would result in rapid induction of NR activity and would thus lead to NO accumulation. It has also been shown that while NO can inhibit the activity of cytochrome oxidase it does not inhibit the function of AOX (Millar et al. 1996, Zottini et al 2002) and furthermore, NO is a strong inducer of *Aox1* (Huang et al. 2002). The involvement of NO in nitrate dependent induction of AOX was studied by treating cells with sodium nitroprusside (SNP), a known NO specific donor and by using the NO scavenger cPTIO [2-(4-carboxy-2-phenyl)-4, 4, 5, 5,-tetramethylinidazoline-1-oxyl-3-oxide]. Cells incubated with 0.5 -1mM of SNP exhibited increased *Aox1* reporter gene activity compared to the untreated cells. Kinetic analysis of *Aox1* transcript and AOX protein abundance with SNP and nitrate treated cells in the absence and presence of cPTIO suggest that the nitrate-dependent induction of alternative pathway respiration is dependent upon increased intracellular NO.

P13

Alternative oxidase: Taxonomic Distribution and Protein Characteristics in Non-Angiosperm Plants

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Alternative oxidase (AOX) is a terminal ubiquinol oxidase present in mitochondrial electron transport chains that introduces a branch-point in respiration and can bypass two of the three proton pumping complexes (Complexes III and IV). This leads to a decrease in the amount of ATP that can potentially be generated by the ATPase. Plant AOX research to date has focused on angiosperms, such as *Arabidopsis thaliana*, tobacco, soybean, rice, and voodoo lily, where a good deal is known about its multigene family, gene expression, and the post-translational regulation of AOX activity. In contrast, little is known about AOX in other plant groups (e.g. gymnosperms, non-vascular plants). Bioinformatics has identified orthologous AOX sequences in liverworts, a moss, a lycopod, ferns, and several gymnosperms. We employed RT-PCR using degenerate primers as a strategy to amplify novel AOX sequences from plant phyla for which molecular data are not yet available (e.g. Psilotophyta, Equisetophyta, Ginkophyta, and Cycadophyta). A comparison of AOX sequences from angiosperms and non-angiosperms reveal similarities and differences which have direct implications for the evolutionary history of AOX and the post-translational mechanisms that regulate AOX activity in plants.

P14

The effect of anitoxidants on the tissue culture of *Nicotiana alata*, *Linus usitatissimum*, *Begonia auriculata* and *Saintpaulia inconspicua* explant growth and morphogenesis

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Our research looked at the action of reduced glutathione, ascorbic acid and β-hydroxyquinoline,on the tissue culture of on *Linus usitatissimum* (flax) and *Nicotiana alata* (tobacco) in the absence of exogenously applied hormones. Flax hypocotyls (0.75 cm length) and 7 mm diameter tobacco explants from the youngest leaves were placed on basal Murashige-Skoog media (1% sucrose, pH 5.7) with no hormones added and various concentrations of ascorbic acid, glutathione and β-hydroxyquinoline were added. All concentrations of ascorbic acid decreased shoot formation but 100 µM ascorbic acid increased root formation significantly in flax. Ascorbate appeared to inhibit callus formation in tobacco. The 100 µM glutathione increased root formation in tobacco. The 100 µM β-hydroxyquinoline appeared to induce shoot formation in flax and root formation in tobacco. A second experiment looked at glutathione and ascorbic acid *Begonia auriculata* (begonia) and *Saintpaulia inconspicua* (African violet) in the presence and absence of exogenously applied hormones. Response to antioxidants varied with species and the action of the antioxidants appeared to be concentration dependent.

P15

Environmental Regulation of the Photosynthesis and Transpiration Rates and Leaf Protein Profiles of Western Redcedar, Pine, Red Oak and Red Maple during the Fall and Winter Periods

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During the fall and winter periods, evergreen trees such as Western Redcedar (*Thuja plicata*), and Pine (*Pinus)* do not lose their leaves and stay green, rather than deciduous trees such as Red Oak (*Quercus rubra*) and Red Maple (*Acer rubrum*) which follow the pattern of seasonal senescence. The objective of this study was to investigate the effect of environmental changes on gas exchange and leaf protein profiles in various species of trees such as, Oak, Cedar, and Maple as representatives of evergreen and deciduous trees. Leaves of evergreen trees (Western Redcedar, and Pine) showed a higher photosynthesis, and transpiration rates than those of the deciduous trees (Red Oak, Red Maple) during the period of study. The Western Redcedar leaves had the highest LHCIIB and RUBISCO protein levels during the August - February period. The physiological and biochemical basis of environmental stress-induced changes in these tree species will be discussed.

P16

Growth temperature modulates sucrose cleaving enzyme activities through time allowing for more starch to accumulate in the sink organ of trout lily at the lower temperature regime

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Spring ephemerals produce larger bulbs at cool than at warmer growth temperatures. Increased bulb size at low temperatures appears to be related to changes in the carbon metabolic pathway at the sink level, rather to changes at the source level. We monitored the activity of the sugar cleaving enzymes – sucrose synthase (SuSy) and the neutral, acid and cell wall invertases (Inv) – along with ADP-glucose pyrophosphorylase (AGPase) activity in the bulb throughout the season at three growth temperatures: 18/14, 12/8 and 8/6°C. Early in the season, neutral Inv and cell wall Inv activities were high while cells were increasing in size. Neutral Inv activity reached higher maximum at higher temperatures. SuSy and acid Inv activities increased much more slowly during bulb development; SuSy activity remained high up to the end of the growth period whereas acid Inv activity decreased as leaf senesced. SuSy activity has been previously associated with the cell maturation stage and its maximum did coincide with the termination of cell elongation. Maximum SuSy activity was slightly reduced at lower temperatures whereas the acid Inv activity was strongly stimulated at lower temperatures. AGPase activity peaked very early in the season and remained high throughout the season in accordance with the very high starch concentration that accumulates in these bulbs (around 80% of dry mass), regardless of growth temperature. As shown in other species, sucrose cleaving enzymes appears to play a significant role in determining sink strength in spring ephemerals, and their activities are coupled with bulb developmental stages. Early induction of SuSy activity at warmer temperatures possibly shortened the cell elongation period, strongly reducing the capacity to accumulate starch and thus biomass. Furthermore, accumulation of hexoses in the vacuole through high acid Inv activity might avoid feedback inhibition at lower temperatures. Growth temperature appears to modulate the activity of some of the key enzymes of the C metabolism through time in such a way that it strongly influences the duration of starch accumulation and thus the final bulb size and biomass of the plant.

P17

High stability ferric chelates result in decreased iron uptake via several interacting mechanisms

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Cells of the green alga *Chlorella kessleri* use a reductive mechanism for iron acquisition. Iron-limited cells acquired iron more rapidly from a chelator with a lower stability constant (HEDTA) for Fe3+ than from a chelator with a higher stability constant (HBED). Furthermore, iron uptake rates decreased with increasing chelator concentrations at constant iron concentration. The negative effects of elevated HBED levels on iron uptake could be partly alleviated by the addition of Ga3+; this suggests that iron-free chelator has a negative effect on iron acquisition by competing with the ferrous transport system. Furthermore, ferric reductase activity progressively decreased with increasing concentrations of both chelators (in the iron-free form); this effect was not alleviated by Ga3+ addition and was apparently caused by the direct inhibition of the reductase. Overall, we conclude that chelators with high stability constants for Fe3+ decrease iron acquisition rates by Strategy I organisms via three separate mechanisms: 1) chelation of the Fe2+ produced by ferric reductase activity (partially alleviated by Ga3+), in competition with the Fe2+ transport system, 2) decreased ferric reductase activity compared to lower stability ferric chelates, and 3) progressive inhibition of ferric reductase activity at increasing concentrations of iron-free chelator (not alleviated by Ga3+).

P18

pH affects ammonium, nitrate and proton fluxes in the apical region of conifer and soybean roots

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The effect of pH on nitrate and ammonium uptake in the HATS and LATS ranges was compared in two conifers and one crop species. Many conifers grow on acidic soils, thus their preference for ammonium vs. nitrate uptake can differ from that of crop plants, and the effect of pH on nitrogen (N) uptake may differ. Proton, ammonium and nitrate net fluxes were measured at seedling root tips and 5, 10, 20 and 30 mm from the tips using a non-invasive microelectrode ion flux measurement system in solutions of 50 or 1500 µM NH4NO3 at pH 4 and 7. In *Glycine max* and *Pinus contorta*, efflux of protons was observed at pH 7 while pH 4 resulted in net proton uptake. *Pseudotsuga menziesii* roots consistently

showed proton efflux, and thus appear better adapted to maintain proton efflux in acid soils. In all three species, net nitrate uptake was greatest at neutral pH. In *P. menziesii* and *G. max*, net N uptake was greater in 1500 than 50 µM NH4NO3 solution. Net ammonium uptake in *G. max* and net nitrate uptake in *P. menziesii* were greatly reducedat pH 4, particularly at the higher N concentration. N concentration should be considered, therefore, when determining optimum pH for N uptake.

P19

Investigation of a potential role of Phospholipase D Zeta in *Arabidopsis thaliana* seed oil accumulation

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Seed oils are important commodities used primarily for human consumption, but also for a wide range of industrial applications (e.g. production of lubricants, paints and specialty chemicals). With the decline of crude oil supplies, seed oils are also generating a lot of interest as a "green energy source" for biofuel production. Given the overall demand for oil, understanding the factors that limit seed storage oil accumulation is an essential first step in an effort to increase oil content by genetic engineering. We have focused our research on investigating a potential role of phospholipase D Zeta (PLDZ) in oil deposition in developing *Arabidopsis* seed. Two PLDZ genes, *PLDZ1* and *PLDZ2*, have been annotated in *Arabidopsis* and they are homologous with the mammalian *PLD1* and *PLD2* which encode enzymes that specifically hydrolyze phosphatidylcholine (PC) to produce phosphatidic acid (PA). Besides being a second messenger in cell signaling, PA is a substrate for seed oil biosynthesis. Expression analysis of *PLDZ1* and *PLDZ2* in the developing embryo revealed that both genes are highly expressed during storage oil biosynthesis (7-10 days after fertilization). Oil content in single *pldz1* or *pldz2* mutants was decreased by about 4%, while *pldz1pldz2* double mutant exhibited a10% reduction oil content. These results indicate that both genes/enzymes are required for storage oil accumulation. GLABRA2 (GL2) homeodomain protein is known to negatively regulate both seed oil levels and PLDZ expression leaves. To assess a possible role of the GL2 transcription factor in *PLDZ1* and *PLDZ2* expression during oil production, we have analyzed *PLDZ1* and *PLDZ2* expression in the *gl2* mutant and constructed the *gl2pldz1pldz2* triple mutant. These studies indicate that GL2 and PLDZ1 and PLDZ2 independently regulate oil biosynthesis.

P20

Insight into the redox regulation of the phosphoglucan phosphatase SEX4 during starch degradation

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Starch is a branched polyglucan that is stored in chloroplasts during photosynthesis and then hydrolyzed into glucose as an energy source for growth at night. Rates of starch synthesis and degradation respond to changes in diurnal regimes indicating starch metabolism is linked to photoperiod. Over the last decade, several kinases/phosphatases have been identified and are involved in the reversible phosphorylation at the granular surface during starch breakdown. In *Arabidopsis thaliana*, one of these enzymes includes the dual-specificity protein phosphatases (DSPs) starch excess 4 (SEX4). Mature leaves of *sex4* mutants contain 4-fold more starch than wildtype. Typically DSPs dephosphorylate both protein phosphotyrosine and phosphoserine/phosphothreonine residues (through a cysteinyl-phosphate intermediate) but SEX4 is unique as it targets phosphoglucans. This phosphatase activity can be modulated between reduced (active) and oxidized (inactive) states *in vitro* suggesting SEX4 is redox regulated. Mass spectroscopy of SEX4 indicates that oxidation promotes disulfide bond formation between Cys130 and the catalytic Cys198 within the DSP phosphatase domain. This redox-mediated structural switch may regulate the phosphatase activity of SEX4 within the chloroplast and/or protects the catalytic cysteine from irreversible oxidation. This mechanism will be further resolved using Cys130Ser variants.

P21

Photoinhibitory Relationships Between Various Wheat Cultivars

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Photosynthesis is an elaborate process by which plants convert sunlight into usable chemical energy through photochemical and biochemical reactions. However, under certain conditions a decrease in photochemical and photosynthetic efficiencies can occur, also known as photoinhibition. Two spring wheat (*Triticum aestivum* L.) cultivars (MacKenzie and Snowbird) and one winter wheat (CDC Raptor) were grown under non-acclimating (20°C), cold-acclimating (5°C) and high CO_2 (750 ppm) conditions. Photosynthetic rate was measured as CO_2 assimilation was monitored over varying irradiance and leaf internal CO_2 concentration. Response curves were modeled to determine various photosynthetic parameters. Leaf samples were also subjected to photoinhibition for 8 hours while photochemical efficiency (F_V/F_M) was monitored. Depending on growth regime, plants from all three cultivars had similar responses to photoinhibition. Leaves from 20°C/350 ppm (50% decrease in F_V/F_M) were more susceptible to photoinhibition than leaves grown at 20°C/750 ppm (40% decrease in F_V/F_M). Plants from 5°C/350 ppm were more tolerant to photoinhibition than 20°C/350 ppm grown plants with a 25% decrease in F_V/F_M. Additionally, plants from 5°C/750 ppm were the most tolerant of all with an F_V/F_M decrease of 3-10%. Therefore, the most tolerant plants were those grown under cold acclimating and CO_2 enriched conditions. In addition, maximal CO_2 assimilation rates also correlated with photoinhibitory tolerance.

P22

Functional characterization and regulation of asparaginases in Arabidopsis

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In higher plants, asparagine (Asn) is an important form of nitrogen transport and storage, which can be delivered to sink tissues, or utilized during seed germination and seedling growth to support the synthesis of other nitrogenous compounds. Asn can be hydrolyzed by asparaginase (ASPG), releasing ammonia which can be re-assimilated by the glutamine synthetase-glutamate synthase cycle. There are two genes coding for ASPGs in Arabidopsis, belonging to two subfamilies differing in substrate preference and activation by monovalent cations. The K^+-independent enzyme (At5g08100) has a similar catalytic efficiency with Asn and beta-aspartyl dipeptides, while the K^+-dependent enzyme (At3g16150) exhibits a strong substrate preference for Asn. There is relative lack of information on ASPG function *in vivo*. To address this gap, T-DNA knock-out alleles were isolated, and a double mutant was obtained through crosses. The T-DNA insertions led to the absence of detectable transcript, as determined by RT-PCR experiments with gene-specific primers. The phenotype of seedlings grown in the presence of Asn or other nitrogen sources was characterized. The effect of the mutations on amino acid composition in seed was also evaluated. Transgenic lines expressing translational *PASPG*:*GUS* fusions were generated and characterized, which provided a detailed map of *ASPG* expression during development. The effect of different nitrogen sources on reporter gene expression in seedlings was also characterized. The results of these experiments will be discussed.

Environment, Poster Abstracts

P23

Salinity tolerance of *Cornus sericea* (L.) from three southeastern Manitoba sites

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Cornus sericea (red-osier dogwood) from Alberta has been shown to tolerate moderate levels of salt, although this woody species is often classified as salt sensitive. Salinity tolerance may vary within a species due to the differences in environmental conditions between the sites. *Cornus sericea* prefers moist habitats although it is also found growing in relatively dry habitats. Exposing plants to drought stress, which shares similar stress response pathways with salt stress, has been found to increase their tolerance to salinity. The objective of this study was to compare the salinity tolerance of *C. sericea* from three different provenances in Manitoba. The first site, selected as the control site, was dominated by large shrubs growing near a stream. The second site was a jack pine stand with *C. sericea* having shorter internodes and thinner stems compared to the control site. The third site was a wetland, with short shrubs exhibiting abundant lateral shoot growth. Although there were differences in growth form in the field, there were no apparent differences in the growth of the cuttings under the same conditions in a greenhouse. Six-month-old cuttings were treated hydroponically for four weeks with 0, 25, 50, or 100 mM NaCl. There were no significant differences in the photosynthetic rate, transpiration, and biomass among the *C. sericea* of the three sites in response to the NaCl treatments. The sodium, chloride, potassium, magnesium, and calcium content in the roots, stems, and leaves of *C. sericea* exposed to NaCl were also not significantly different between the sites. Overall, the photosynthetic rate of the 50 mM NaCl treated plants were reduced by 22% after four weeks of treatment. The root biomass was not reduced by 50 mM NaCl. The results suggested that the salinity tolerance of *C. sericea* from the three different sites in Manitoba were similar to the salinity tolerance of seedlings from Alberta. The similarity of the climatic conditions (temperature and rain) of the three sites may have been more influential than the differences in the edaphic conditions.

P24

Effect of Freeze-Thaw Cycles on the Rheology of Protein Extracts From Winter Rye

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Winter rye (*Secale cereale* L. cv Muskateer) is a freezing-tolerant, overwintering, annual plant that can survive temperatures below -30oC by forming ice in intercellular spaces. Most studies on winter rye are focused on the freezing tolerance of the plant. Little research has been done on the rheology of winter rye protein extracts. We hypothesized that the rheologic properties of winter rye leaf protein extracts will be changed after subjected to freeze-thaw cycles. Both cold-acclimated (CA) and non-acclimated (NA) winter rye leaf tissues were utilized in this study. The protein extracts were suspended in borate buffer (pH 12) and subjected to 3 to 4 freeze-thaw cycles. The extracts from both CA and NA leaf tissues started to form a gel after this treatment. Experiments optimizing the extraction conditions and the number of freeze-thaw cycles required for gel formation were also performed. The gel was composed of several proteins in the extracts as determined by SDS-PAGE. Studies are currently underway to identify specific components in the protein extracts required for gel formation.

P25

Analysis of aluminium resistance background in wheat cultivars from Madeira, Portugal

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The Portuguese Archipelago of Madeira located 630 km west of North Africa has soils of volcanic origin, with predominantly low pH (ranging from 3.81 to 7.87; average 5.21), which results in high aluminium bioavailability in soil solution, especially in cultivated soils located at elevations between 20 and 600 meters above sea level. These soil conditions make local wheat germplasm a potential source of genetic material for study and improvement of aluminium resistance of this crop. Over fifty local landraces of wheat were screened for resistance to aluminium toxicity with several identified genotypes exhibiting considerably elevated aluminium resistance. Populations of two wheat cultivars displaying drastically differing responses to aluminium have been selected for further studies on the mechanism of aluminium resistance. To produce homozygous plants, which would facilitate elucidation of Al background of the Madeiran wheats, we have developed a set of double haploid (DH) lines as well as advanced the wheat cultivars to the F3 generation. Aluminium resistance of all developed generations was screened in hydroponic culture using the erichrome cyanine root staining, root elongation, biomass and callose production tests as indicators. We observed increased resistance and increased susceptibility among the developed lines originating from genotypes initially deemed as resistant and sensitive, respectively. Field trials in acidic and neutral soils were conducted to evaluate grain yield and the aboveground vegetative biomass production. Presently, the collected data are being analysed to explore correlations between resistance and phenotypic traits. Plant tissues obtained from the field trials and hydroponic assays will be used to study physiological and molecular mechanisms involved in the response to aluminium toxicity. Currently, aluminium uptake by roots of the two lines grown in the field and in hydroponic cultures is being quantified. Simultaneously, efforts to identify changes in the expression patterns of root proteins have been made. The methodology for detection of proteins, specifically expressed in the presence of aluminium, includes separation of soluble proteins using 2D gel electrophoresis, the isolation of the root proteomes and the isolation of proteins that co-segregate with aluminium resistance in wheat roots. Their subsequent identification using the MALDI-ToF MS technique and preliminary data obtained from these analyses will be presented and discussed.

P26

Physiological characterization and stability of leaf carbon isotope discrimination as a measure of water use efficiency in barley

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Temporal and seasonal water deficit is one of the major factors limiting crop yield on the Canadian prairies. Selection for low carbon isotope discrimination ($\Delta^{13}C$) or high water use efficiency (WUE) can lead to improved yield in semi-arid environments. Twelve barley (*Hordeum vulgare* L.) genotypes with contrasting levels of leaf $\Delta^{13}C$ were investigated for performance stability across locations and years in Alberta. Two of those genotypes were also grown in a greenhouse under well-watered and water deficit conditions to characterize the relationship between gas exchange parameters and leaf $\Delta^{13}C$. Ten days of water deficit was imposed at the stem elongation and booting stages followed by stepwise re-watering of pots to pre-deficit level. Genotypic variation in $\Delta^{13}C$, WUE, specific leaf area (SLA), and leaf gas exchange (assimilation rate, transpiration rate, stomata conductance and internal CO_2 concentration) in response to water deficit and recovery were investigated. Genotypic ranking in leaf $\Delta^{13}C$ across locations and years was highly consistent in several of the genotypes tested. During the stem elongation stage, water deficit significantly increased WUE, and SLA was strongly correlated with WUE and leaf $\Delta^{13}C$ (r=-0.98 and 0.94, n=4). 'CDC Cowboy' - a low leaf $\Delta^{13}C$ genotype had significantly higher WUE ($WUE_{integrative}$, ratio of dry matter to transpired water, $WUE_{instantaneous}$, ratio of assimilation rate to transpiration and $WUE_{intrinsic}$, ratio of assimilation rate to stomatal conductance) and lower percentage decline in assimilation rate and stomata conductance than 'kasota' - a high leaf $\Delta^{13}C$ genotype. Results of this study suggest that low leaf $\Delta^{13}C$ of CDC Cowboy was achieved by maintaining a balance between moderate decline in assimilation rate and stomata closure, which are important for maintaining productivity and yield stability under water limiting conditions on the Canadian prairies.

P27

Hormonal regulation of chilling tolerance in lettuce (*Lactuca sativa*) and cucumber (*Cucumis sativus*) seedlings

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The objective of this study was to investigate the role of brassinolide (BL), abscisic acid (ABA) and salicylic acid (SA) in inducing chilling tolerance in lettuce (*Lactuca sativa*) and cucumber (*Cucumis sativus*) seedlings. Lettuce seedlings were more cold tolerant that cucumber seedlings. Cucumber seedlings were shown to die after less than 24 hours exposure to 3^0C, but a combination of ABA, BL and SA treatments considerably enhanced the chilling tolerance to increase survival in the cold to more than 7 days. It is suggested that a combination of ABA, BL, and SA could be beneficial for increasing the chilling tolerance of chilling-sensitive plants. The possible mechanisms of ABA, BL, and SA in chilling tolerance will be discussed.

P28

Physiological and Biochemical basis of Autumn Leaf Senescence in Climbing Hydrangea (*Hydrangea anomala*)

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Climbing Hydrangea (*Hydrangea anomala*) exhibits

natural autumn leaf senescence. The objective of this study was to investigate key physiological and biochemical changes viz. chlorophyll content, photosynthesis and transpiration rates, and protein profiles in hydrangea (*Hydrangea anomala*) leaves during development and autumn leaf senescence. Our results indicate a dramatic reduction in the levels of chlorophyll, key photosynthetic proteins and photosynthesis and transpiration rates with the progress of autumn senescence in hydrangea leaves. We also analyzed the levels of stress proteins, including heat shock and dehydrin in hydrangea leaves of various maturity levels. Stress proteins e.g. dehydrin were more abundant in senescent than green hydrangea leaves. The transpiration and photosynthesis rates for leaves during natural senescence are noted to support the link between natural senescence and the accompanying protein profile changes, especially the photosynthetic proteins and the biochemical processes in the leaves. The physiological and biochemical mechanisms regulating autumn senescence in climbing hydrangea will be discussed.

P29

Do higher temperatures, enhanced ultraviolet -B radiation and water stress promote methane emission from plants?

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We examined the effects of temperature, ultraviolet-B radiation and watering regimes on aerobic methane (CH4) emission from six crops – faba bean, sunflower, pea, canola, barley and wheat. Plants were grown in controlled-environment growth chambers under two temperature regimes (24/20 and 30/26oC), three biologically effective UVB radiation (0, 5 and 10 kJ m^{-2} d^{-1}) and two watering regimes (well watered and water stressed). A gas chromatograph with a flame ionization detector was used to measure methane emission rates (ng g-1 dw h-1) from detached fresh leaves of each species. We found that methane emissions differed among crop plants. Methane emission was highest for pea and lowest for barley. Our results also showed that both higher temperature and water stress significantly enhanced methane emissions, whereas UVB radiation had smaller effect on methane production. We suggest that methane emissions may increase under conditions of climate change and this extra source might contribute to the 'greenhouse effect'.

P30

Proteomics of stress tolerance in seed germination of the*7B-1* mutant of tomato (*Solanum Lycopersicum)*

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Seed germination is the first obstacle a new plant overcomes. This initiation of plant growth and emergence from the seed is in part dependent on protein reserves in both the embryo and endosperm, more so under stress conditions. With the continuing environmental changes, understanding the methods by which plants are able to thrive under adverse conditions is of great importance. In the male-sterile *7B-1* mutant of tomato, seed germination is resistant to a number of osmotica, (mannitol, polyethylene glycol), salts (NaCl and K_2SO_4), and low temperature as compared to the wild type (WT). The goal of this study was to identify proteins associated with stress tolerance in the *7B-1* mutant by using the proteomic approach. Proteins were extracted from the *7B-1* mutant and WT seeds (whole, embryo, and endosperm) post-imbibition from one to 48 hours and with or without exposure to mannitol. Proteins were separated by two- dimensional gel electrophoresis (2-DE) and differential in-gel electrophoresis (DIGE), analyzed by mass spectrometry (MALDI-TOF, MS/MS-Q/TOF), and identified using search engines (NCBI, Mascot). Comparison of protein profiles revealed differential expression of proteins in the *7B-1* mutant and WT. Storage proteins were up-regulated in the *7B-1* mutant in comparison to the WT, and four protein spots that were detected in the *7B-1* mutant were absent in WT, including a LEA-like and a cystatin-like protein. Two protein spots that were present in WT were lacking in the *7B-1* mutant. Investigation into the distribution of these six protein spots revealed differential presence in the embryo and endosperm/testa but none was completely absent from either tissue. Comparison of seed germination up to 48 hours under control and mannitol stress did not reveal differences in the protein profiles between the *7B-1* mutant and WT gels. This may be attributed to a number of factors including proteins of low abundance, proteins occluded by highly abundant storage proteins, and post-translational modifications. Further investigations into the effects of mannitol on seed germination and the differences between the *7B-1* mutant and WT are underway.

P31

Virus-induced genome instability in tobacco plants

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Previously our experiments showed that local infection of *Nicotiana tabacum* plants with tobacco mosaic virus (TMV) leads to an increase in homologous recombination frequency (HRF) in non-infected tissue. The progeny of such plants had global genome hypermethylation and showed an increase in the frequency of rearrangements in resistance gene loci. In our new study, we show evidence that tobacco plants infected with TMV exhibited an increase in HRF in two consecutive generations. Analysis of global genome methylation confirmed the hypermethylated genome in both generations of plants. Analysis of methylation via 5-MeC antibodies showed hypomethylation in euchromatic areas and signs of hypermethylation in heterochromatic areas. Analysis of the response of the progeny of infected plants to TMV revealed that the first and second generation of infected plants have a significant delay in symptom development and lower viral titre. Infection with TMV and *Pseudomonas syringae* showed higher induction of PR1 gene expression, especially noticeable at 48 hours after infection. The progeny of infected plants also had higher tolerance to methyl methane sulfonate (MMS). While analyzing possible mechanisms of stress tolerance, we found the progeny of

infected plants to have a higher level of callose deposition and a higher amount of various metabolites, including phenolic compounds and sugars, which allow plants to tolerate stress. To test the involvement of epigenetic machinery in establishment of transgenerational changes in HRF in infected plants, we analyzed *dcl2*, *dcl3* and *dcl4 Arabidopsis thaliana* mutants. Infection with oilseed rape mosaic virus (ORMV) showed that *dcl4* is impaired in the increase of transgenerational HRF. Collectively, our findings suggest the existence of stress-induced adaptive inheritance in plants, which probably depends on the function of DCL4 and is established through changes in the genome methylation patterns.

P32

An Examination of Freezing Tolerance in *Arabidopsis* and *Thellungiella* Using Re-growth, Electrolyte Leakage and Chlorophyll Fluorescence Analyses

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Freezing stress destabilizes the integrity of cell membranes and organelles, perturbing metabolic process including photosynthesis. The extent of freezing tolerance is conventionally assessed through the electrical conductivity test of the tissues or re-growth scoring of plants. Both of these processes are cumbersome and time consuming, limiting their utility for screening of large number of genotypes. As stresses rapidly perturb photosynthesis, the degree of disturbance in photochemical efficiency (Fv/Fm) can be a potential indicator of the severity of freezing stress. In this study, a potential use of chlorophyll fluorescence imaging as an alternative to the electrical conductivity or re-growth method is examined. Non-acclimated and cold-acclimated plants of *Arabidopsis* and *Thellungiella* (or their excised leaves) were exposed to low temperatures between 5oC and -25oC and the effect of freezing assessed. A very high degree of correlation ($r > 0.85$) was found between the photochemical efficiency of frozen plants after 8 hours thawing and their re-growth scores after two weeks of recovery. Electrolyte leakage and plant survival percentage were only moderately correlated. It is evident that the plants displaying Fv/Fm values greater than 0.60 upon freezing exposure tended to survive the stress while those displaying Fv/Fm values of less than 0.40 failed to survive. The freezing temperature at which the Fv/Fm values of about 0.50 occur, or the Fv/Fm is reduced by one-third from that of the unfrozen values, can be an approximation of lethal temperature for 50% plant survival (LT50) in *Arabidopsis* and *Thellungiella*. These results clearly demonstrate that chlorophyll fluorescence imaging can be used as an alternative tool for freezing tolerance assessment. With some species-specific fine-tuning of the freezing tolerance assessment procedure, chlorophyll fluorescence imaging promises to be an efficient technique for screening large numbers of breeding lines.

P33

Developing molecular markers for drought resistance in *POPULUS*

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Poplar (*Populus* spp) trees are an important component of Canada's forests and are highly valued for their fast growth. Poplar is commercially important as a source of raw material for the pulp and paper industry, plywood and lumber, as well as for the production of bio-fuels. Moreover, poplar is a prominent component of programs to optimize carbon sequestration and so aid Canada in meeting its international commitments to reduce greenhouse gas emissions. All of these applications rely on the rapid growth capacity of poplar. However, rapid growth is problematic, particularly for poplar grown on the Canadian prairies due to the occurrence of sporadic summer droughts. Poplar is the only tree with a completely sequenced genome and this has allowed for the development of an array of molecular tools and afforded us the opportunity to study stress responses in a deciduous tree of economic importance. We are screening *Populus* hybrid genotypes that represent those grown on the Canadian prairies for their response to drought stress so as to identify drought tolerant and susceptible genotypes to aid in the identification of expression markers for drought tolerance. Our efforts to identify expression markers are guided by analyses of the *Populus* drought transcriptome, which has provided candidate genes for analyses in drought susceptible and tolerant *Populus* genotypes. Among the candidate genes tested *NCED3* (nine-cis-epoxycarotenoid dioxygenase 3-like), *EIN3* (ethylene-insensitive 3), *PP2C* (protein phosphatase 2C) and *ABF2* (abscisic acid responsive elements-binding protein 2) were highly up-regulated in drought stressed leaves and the level of gene expression varied among *Populus* genotypes. This research will permit the identification of molecular markers that have importance in breeding and selection programs for the development of robust drought tolerant poplar genotypes that are adapted not only to the Canadian prairies but to other locations that, as a result of predicted climate change, are faced with drier growing seasons.

P34

Proteomic Analysis of Hybrid Poplar Xylem and Phloem Sap

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We have undertaken proteomic analyses of phloem and xylem sap in poplar. 2-D gel analysis showed the presence of a large number of distinct proteins in both of these biological fluids, and with mass spectrometry techniques (LC-MS/MS), 48 phloem proteins and 97 xylem proteins could be clearly identified. Xylem sap contained many glycolytic enzymes, which might be important for specific metabolic challenges of xylem in large plants such as trees, including localized anaerobic stresses. Poplar phloem contained a significant number of pest and pathogen defense-related proteins, unlike previously characterized phloem proteomes of herbaceous plants. Tree adaptation to environmental conditions often requires rapid local and plant-wide responses to stress. Phloem, and to some extent xylem, are

strongly implicated in such systemic stress signals and responses. Wounding poplar leaves to mimic herbivory is known to cause dramatic local and systemic changes in gene expression in leaves, but this stress provoked only a few changes to the phloem protein profile. However, two defense proteins, pop3-like and thaumatin-like (TLP) proteins were present at higher levels in the phloem exudate collected from leaf-wounded compared to control plants. Immunolocalization confirmed the presence of these proteins in sieve elements and other phloem cells. These proteins will be characterized further as to their potential roles.

P35

Regulation of energy partitioning during cold acclimation of Lodgepole pine is oxygen-dependent

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One year old seedlings of Lodgepole pine (*Pinus contorta* L.) were exposed to simulated summer, autumn and winter conditions. As a result of cold acclimation the proportion of linear electron transport utilized in carbon assimilation (ETR_{CO2}) was 40% lower in both "autumn" and "winter" pine when compared to the “summer” needles. Alternatively, the proportion of photosynthetic linear electron transport (ETR_{excess}) not used for carbon assimilation within total ETR_{Jf} was similarly increased by 30% in both "autumn" and "winter" pine. However, while in “autumn” pine the increased amount of “excess” electrons were directed equally to 21%O_2- and 2%O_2-dependent alternative electron transport pathways, in “winter” needles 60% of photosynthetically generated “excess” electrons were utilized through 2%O_2- dependent electron sink and only 15% by photorespiratory (21%O_2) electron pathway. The estimated energy partitioning of absorbed light under ambient p(CO_2) of 35 Pa and p(O_2) of 21 KPa conditions and saturating light irradiance indicated that the fractions of excitation light energy utilized by PSII photochemistry (PSII), thermally dissipated through Φ_{NPQ} and dissipated by additional quenching mechanism(s) ($\Phi_{f,D}$) were the same in “summer” and “autumn” pine. In contrast, “winter” adaptation led to the 3-fold lower Φ_{PSII}, 2-fold higher $\Phi_{f,D}$ and only marginal increase in Φ_{NPQ}. As expected, lowering the p(O_2) concentration to 2 KPa resulted in a sharp decrease of Φ_{PSII} accompanied by significant increase of Φ_{NPQ} in summer and autumn seedlings. In contrast, although winter seedlings also exhibited decreased Φ_{PSII} under low p(O_2) concentration, the fraction of Φ_{NPQ} remained unchanged. However, the fraction of constitutive quenching ($\Phi_{f,D}$) in winter needles was further increased by 50% under low p(O_2). This demonstrates that in winter pine oxygen plays an important role in the regulation of energy partitioning which occurs independently of antenna-based non-photochemical quenching. In addition, up-regulation of photorespiration can play more significant role in utilizing “excess” electrons in “autumn” than in “winter” pine, where the photorespiratory electron flow is strongly reduced and other oxygen-dependent “photochemical” sinks are involved. A model to account for the structural and functional changes in cold acclimated Lodgepole pine is discussed.

Cell and Development, Poster Abstracts

P36

The Role of a Microtubule Associated Protein, Ending Binding 1, in Plant Responses to Touch/Gravity Stimuli

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Microtubules play important roles in cell elongation and division. To understand how microtubules influence growth in plants we are investigating the function of a microtubule associated protein, End Binding 1 (EB1). EB1 is a +TIP that localizes to the more rapidly growing or plus ends of microtubules. In Arabidopsis, there are three members of the EB1 family and the Bisgrove lab has isolated mutants carrying T-DNA insertions in each of the three EB1 genes. On inclined agar plates, *ateb1* roots tend to form more loops than wild type plants and they exhibit delays in bend formation in response to gravity/ touch stimuli. Based on these phenotypes, we hypothesize that EB1 has a role in touch/gravity response. To address the relative contribution of touch stimuli to the *ateb1* phenotype, seedlings are grown on tilted agar plates. In this assay, mutants form additional loops at a higher angle suggesting a defect in responding to touch stimuli. The mutants are also being analyzed for their growth responses to changing agar hardness. To determine whether the *ateb1* phenotype is dependant on asymmetric touch, seedlings are grown inside the agar and assayed for their ability to respond to a change in the gravity vector. In this assay mutants no longer exhibit gravitropic delays suggesting that asymmetric touch is necessary for the phenotype. Crosses between *eb1* and mutants with defects in touch or gravity responses are underway.

P37

Is the function of the microtubule end binding protein EB1 linked to auxin?

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Plants are sessile and must adapt to environmental conditions by altering their growth. At the cellular level, changes in growth involve cell division and elongation. These processes require microtubules (MTs), which are regulated by MT associated proteins (MAPs). To understand how MTs influence growth and development, I am studying a MAP, End Binding 1 (EB1). There are three EB1 genes in the Arabidopsis thaliana genome: EB1a, EB1b, EB1c. I am analyzing plants carrying mutations in each of these genes, as well as a triple mutant. Mutant roots form loops and have delayed responses to touch and gravity. These growth responses are regulated by the plant hormone auxin. To test if the function of EB1 is linked to auxin, plants were grown on different concentrations of auxins or chemicals that inhibit

polar auxin transport and auxin signaling. I measured root lengths, and proportions of roots forming loops. I found that root lengths decreased by a similar amount in all genotypes. However, with respect to root looping, two of the ateb1 mutants (ateb1b and the triple) formed more loops than the wild type in the presence of auxin inhibitors. When different auxins were added to the media (IAA or NAA), the proportion of roots making loops increased by similar amounts in both wild type and ateb1b. In contrast, mutants impaired in auxin transport were more sensitive to NAA. Taken together, these results suggest that EB1b is linked to an auxin pathway.

P38

Regulation of secondary cell wall biosynthesis in Arabidopsis by a KNAT7 transcription factor complex

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The plant secondary cell wall is a composite network of complex polymers (cellulose, lignin, and hemicellulose) that provides protective and structural properties to the cell wall. The *Arabidopsis KNOX* gene *KNAT7* has been identified in transcriptional profiling and other experiments as a member of a transcriptional network regulating secondary wall formation during in xylem and fiber cell differentiation in *Arabidopsis* inflorescence stems. We have characterized the phenotypes of *knat7* mutants, which display an irregular xylem (irx) phenotype, as well as increased fiber wall thickness. KNAT7 interacts with members of the Ovate Family Protein (OFP) transcription co- regulators. We confirmed the KNAT7-OFP1 and KNAT7-OFP4 interactions by yeast two hybrid analysis and by biomolecular fluorescence complementation analyses *in planta*, and showed that the interaction enhances KNAT7 transcriptional repression activity. Furthermore, an *ofp4* mutant exhibits similar phenotypes as *knat7*, and the pleiotropic effects of *OFP1 and OFP4* overexpression depend upon *KNAT7* function. Co-expression and yeast two hybrid analyses suggest that the homeodomain transcription factor BHL5 could be part of a KNOX-BELL-OVATE transcription factor complex, together with KNAT7 and OFP1/4. We are further investigating the functional interaction of BLH5 with KNAT7 and OFP proteins through yeast two hybrid and *in planta* biomolecular fluorescence complementation analyses, and by analyzing *bhl5* and *knat7* double mutant phenotypes. In order to identify target genes whose expression is directly regulated by KNAT7, we have generated transgenic Arabidopsis *knat7* lines expressing a KNAT7-GR gene fusion and will use microarray expression profiling following dexamethasone (DEX)-induced nuclear localization of KNAT7 to assess changes in transcription.

P39

Genetic and biophysical analysis of a subfamily of CMLs in *Arabidopsis thaliana*

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Plants are faced with a multitude of biotic and abiotic environmental stresses and must also coordinate and equally diverse array of developmental cues. Eliciting appropriate responses to environmental and developmental cues is achieved through signal transduction pathways leading to shifts in metabolism or changes in gene expression. In plants many of these pathways are mediated by rapid, transient, and spatially and temporally distinct influxes of Ca2+ into the cytosol termed calcium signals. These signals are 'decoded' by calcium sensors, the most well characterized of which is calmodulin (CaM). The *Arabidopsis* genome encodes a family of 50 putative calcium sensors known as calmodulin-like proteins (CMLs) which share a minimun 16% identity with CaM at the amino acid level. Three of these CML37, CML38 and CML39 show distinct and overlapping developmental and stress responsive expression patterns but their physiological functions remain unknown. Our lab is using a broad range of genetic, biochemical and biophysical approaches in order to understand the physiological functions of these CMLs. Here we provide a detailed biophysical characterization of these CMLs indicating that they are bona fide Ca2+ sensors and analysis of single and double knockout lines which suggest that there is functional redundancy among members of the CML family.

P40

Understanding Regulation of Cuticular Wax Biosynthesis in Arabidopsis

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To successfully transition to life on land, plants developed a suite of necessary structural adaptations. One of them is a water-impermeable waxy layer called the cuticle which covers all aerial plant surfaces. The cuticle protects plants from non-stomatal water loss, UV radiation, pathogens, and other environmental stresses. The cuticle has also been shown to mediate plant-insect interactions and prevent organ fusions. Recently, we have discovered a novel mechanism involved in regulating cuticular wax formation. We hypothesize that at the onset of wax production the CER7 ribonuclease degrades an mRNA specifying a repressor of *CER3,* a key wax biosynthetic gene. In the absence of this repressor, *CER3* is expressed, leading to wax production. In order to identify this putative repressor target of the CER7 ribonuclease and learn more about the CER7 mediated regulation of wax production, we performed a screen for suppressors of *cer7.* Suppressor lines have waxy stems, suggesting restored cuticular wax levels and were therefore named *war* mutants *(wax restorer)*. Initial characterization of these suppressor lines will be presented.

P41

Function analysis of ArTrs120 and AtTrs130

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Rab proteins are key regulators of the various steps of intracellular membrane trafficking. They serve as molecular

switch for processes in vesicular transport because they can cycle between GTP-bound active state and GDP-bound inactive state. Guanine nucleotide-exchange factors (GEFs) are one upstream regulator of Rab proteins, which can activate Rab by exchanging GDP with GTP. Studies in yeast indicate that TRAPPII is a GEF for Ypt31/32, whose homologues in Arabidopsis, namely RabA, have a plant-specific radiation. Homologues of two TRAPPII specific subunits, Trs120 and Trs130 have been identified in Arabidopsis and both knock-out mutants have similar phenotype with severe defect in development. During embryogenesis, cotyledons of both mutants grow into the opposite space of the seeds and produce curling embryos. Shortly after germination, the mutants stop developing, ending up with a stunt structure. PI staining is performed to highlight the mutants' cell profile in root tip and the result turned out that comparing with wild type, the mutants have poor cell arrangement and the quiescent center cells are lost as well. Using Sec-GFP as a secretory marker, we found that GFP signal is retained in the mutant cells, indicating the secretory pathway in *attrs120* and *attrs130* mutants is defective. More putative cargoes will be tested to help understanding the molecular mechanism of the phenotype. Currently we are analyzing the expression pattern and sub-cellular localization of the AtTrs120 and AtTrs130.

P42

The Arabidopsis callose synthase gene, GSL8, is required for cytokinesis and stomatal patterning

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Cytokinesis is the division of the cytoplasm and its separation into two daughter cells. Cell plate growth and cytokinesis appear to require callose, but direct functional evidence is still lacking. Callose is a major component of the cell plate and, like cellulose, is synthesized by transmembrane proteins located in the plasmalemma. Twelve predicted *GLUCAN SYNYHASE-LIKE* (*GSL*) genes (aka *CALLOSE SYNTHASEs*) have been identified in Arabidopsis. Here we show that that *GSL8* is required for cell patterning as well as cytokinesis in Arabidopsis shoots. While *gsl8* mutations have been reported previously to be gametophytic lethal, we found that seedlings were produced in *gsl8*, although they were often deformed and stunted. They exhibited pleiotropic defects during embryogenesis and early vegetative growth. *gsl8* mutants, which are seedling-lethal, contain irregular tissue arrangements and cytokinesis defects such as incomplete cell walls and "stubs". Cells with cytokinetic defects have two nuclei suggesting that karyokinesis is normal. A 35S-driven GFP-GSL8 truncated fusion protein localizes to young cell plates supporting the idea that GSL8 is required for cell plate formation. Interestingly, *gsl8* mutants also display aberrant cell patterning phenotypes in the developing leaf epidermis including clusters of stomata in direct contact as well as islands of excessive cell proliferation (numerous islands of asymmetric divisions in the stomatal cell lineage). Many other cytokinesis-defective mutants (e.g. *knolle*, *keule*, and *scd1*) show division defects like *gsl8*, but to our knowledge, none display defects in stomatal patterning. Since these disruptions were observed in several different *gsl8* alleles, the patterning phenotype is not due to a second site mutation. It remains to be seen whether the patterning defects result from the severity of the cytokinesis defects or whether callose deposition is also essential for correctly distributing cell types in the shoot epidermis.

P43

Characterization of a Gene (*BnMicEmUP*) Upregulated in Embrogenic *Brassica napus* Microspore Cultures

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A mild heat stress of *Brassica napus* microspore cultures causes some of the cells to switch their developmental pathway from pollen maturation to embryo formation. Embryo formation in microspore cultures closely resembles zygotic embryo development in morphology, protein synthesis and physiology. In a microarray screen of ESTs from sorted embryogenic versus nonembryogenic cells 120 transcripts that were upregulated more than 2-fold in the embryogenic cells were identified, including a sequence that was homologous to the Arabidopsis gene AT1G73740. The function of this gene is unknown. The objectives of the current work were to characterize the complete gene and investigate the function of the gene by gene silencing and overexpression studies in Arabidopsis and *B. napus.* The complete gene was isolated and sequenced from *B. napus* by PCR and RT-PCR using primers designed from the AT1G73740 sequence and named as *BnMicEmUP* (*B. napus* microspore embryogenesis up regulated gene). Three distinctive *BnMicEmUP* genes with nucleic acid similarities ranging from 82 to 83% to *Arabidopsis* AT1G73740 were isolated from *B. napus* cv Topas. Real-time PCR showed that expression of one of these genes is expressed in induced (embryogenic) microspore cultures but not in noninduced cultures. In contrast, another form of *BnMicEmUP* is expressed constantly in induced and noninduced cultures and is also expressed in leaf, root, stem and pollen tissue. To further investigate the role of BnMicEmUP in embryogenesis, two silencing constructs were made with the Pfgc5941vector and vectors with a 35spromoter (pB1121) and an inducible promoter (pER8) were constructed for over- expression of the BnMicEmU gene. These constructs were delivered to Arabidopsis by Agrobacterium using the floral dip method. Their effects on gene expression and embryo formation are currently being investigated. The information from studies of microspore embryogenesis is immediately applicable to improving this system for producing homozygous plants for plant breeding. In addition, the basic information on embryogenic processes leads to a greater understanding of totipotency in plants.

P44

Structure-funtion analysis of a tomato PLD-alpha C2 Domain

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C2 domain is a calcium and lipid binding motif, first reported as one of the conserved regions in the classical protein kinase C (PKC) isoforms. It is responsible for recruiting proteins at the site of action in response to various stimuli, which is a key regulatory event of signal transduction. Phospholipase D (PLD) and phospholipase C (PLC) from green plants contain C2 domains at the N and C terminals, respectively. Approximately 130 amino acid long C2 domain folds in 4 pairs of anti-parallel beta strands, and is highly conserved in secondary and tertiary structures of animal and plant proteins. Multiple sequence alignment showed that calcium binding loop 1 (CBL-1) in plant PLD is diverse compared to that in PLC, and substitution of key residues has been reported to affect its calcium binding capacity. In this study, the sequence corresponding to the first 150 amino acids of the N terminal region of a full length cDNA of PLD-alpha from tomato was cloned in pET28(b) vector and expressed in *E.coli* as His-tagged protein. Expressed protein in inclusion bodies was efficiently refolded and purified using nickel based metal-ion affinity chromatography. In ligand binding study, recombinant C2 domain showed micromolar affinity towards Ca++ with a maximum of 2 high affinity binding sites. The conformational changes in C2 domain that occur upon binding with Ca++ was measured by the changes in intrinsic fluorescence of tryptophan. Interaction of C2 domain with synthetic unilamellar vesicles was studied by protein to lipid fluorescence resonance energy transfer (FRET). The C2 domain showed maximum affinity towards phosphatidic acid, and the binding varied based on the head groups of phospholipids. By contrast to animal systems, recombinant C2 domain showed no binding with phosphatidyl choline (PC). Phosphoinositide's binding affinity of C2 domain reduced with the degree of phosphorylation, indicating a direct role in signal transduction. Reconstituted unilamelar lipid vesicles isolated from tomato fruits at different developmental stages exhibited binding pattern corresponding to its total phosphatidic acid content. Acid and chaotropic salt titrations indicated an electrostatic rather than a hydrophobic mode of interaction between C2 domain and the membrane.

P45

DDB1A-DDB2 INTERACTION IN _ARABIDOPSIS_ DNA DAMAGE REPAIR

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In this study we examine the roles of *Arabidopsis Damaged DNA Binding protein 1A & 2* (*DDB1A* & *DDB2)* in DNA damage repair. No visible phenotype was found in *ddb1a*, *ddb2* or the double mutant *ddb1a ddb2* after irradiation with various doses of UV-C. However, 6-4 photoproduct repair analysis indicates that DDB1A-DDB2 is important in 6-4 photoproduct damage recognition 4 hours after UV exposure. In contrast, cyclobutane dimer repair 24 hours after UV exposure indicates an important role for the DDB1B-DDB2 complex. In addition, RT-PCR analysis indicates that *DDB2* is important for *DDB1A* and *DDB1B* induction following UV exposure. In conclusion, a subtle interaction was observed between *DDB1A*, *DDB1B* and *DDB2* in DNA repair and UV response.

P46

A Modifier Screen for the Identification of Mucilage (Pectin) Biosynthesis Mutants

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Plant cell walls are composed of a mixture of cellulose, hemicelluloses and pectins. Pectins form a complex matrix between cells that is involved in cell-to-cell adhesion, determining cell growth, cell shape, cell wall porosity and signaling events. They consist of complex polysaccharides, composed primarily of a backbone of galacturonic acid and rhamnose molecules. In cell walls, the pectin backbone can be very variable, branched with side chains composed of arabinose and galactose, and are highly modified with methyl and acetyl esters. In order to study pectin biosynthesis, the Arabidopsis seed coat is being used as a model system. The seed coat epidermal cells undergo a complex differentiation pattern, which culminates in the production of large amounts of mucilage composed primarily of relatively simple and un-branched pectins. When mature seeds are placed in an aqueous solution, the mucilage is released from the seed coat epidermal cells which form a gel-like capsule around the seed. Mucilage is easily observed using ruthenium red and is dispensable under laboratory conditions making the isolation of mucilage mutants feasible. Several genes required for normal mucilage extrusion,have been identified by mutation including five *MUCILAGE- MODIFIED* genes (*MUM1-5*). One of these, *MUM4* encodes a rhamnose synthase. Plants homozygous for the *mum4* mutation produce ~10% of the mucilage when compared to wild type seeds. This mutation has been used as a genetic background in a modifier screen to identify additional mutants specifically involved in mucilage biosynthesis or secretion. Our modifier screen has identified 8 mutants including 2 enhancers (*MEN*) and 6 suppressors (*SMM*). Preliminary characterizations of the mutant seed coat development and mucilage extrusion phenotypes are presented here.

P47

Dynamics of protein expression during pollen germination in canola (_Brassica napus_)

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This study was conducted to analyze the proteome of mature (MP) and germinating pollen (GP) of *Brassica napus* with the objective of identifying changes in protein expression and their potential roles in the pollen germination process. Proteins were extracted from the mature and *in vitro* germinated pollen, separated by 2-dimensional gel

electrophoresis (2-DE), and differential in-gel electrophoresis (DIGE) using fluorescent dyes. Over 2200 protein spots were detected in DIGE gel images of which 334 were differentially expressed (2-fold or greater change), and 165 spots were analyzed by mass spectrometry (MALDI-TOF/TOF analyzer) and identified using the NCBInr (virdiplantae) and Brassica EST databases. During pollen germination, the major proteins up-regulated were those involved in carbohydrate/energy metabolism, protein metabolism, and cell wall modeling. Others with higher expression in GP, relative to MP, were proteins with roles in cytoskeleton formation, nucleic acid and amino acid metabolism, cell signaling, and stress response. Proteins down-regulated in GP included catalases, and LEA proteins. Some proteins were also secreted in the germination medium, e.g., oleosin, cruciferin and enolase. The identified proteins have potential roles in pollen germination, early tube growth, and in pollen-stigma interaction. This study provides insights into fundamental mechanisms of cell growth, cell-cell communication, and aspects of the plant sexual reproductive process.

P48

RACK1 Negatively Regulates ABA Responses during Seed Germination and Early Seedling Development in Arabidopsis

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Abscisic acid (ABA) is one of the most important plant hormones regulating plant stress responses as well as many aspects of plant development. A previous study showed that loss-of-function of *RACK1A* (*Receptor for Activated C Kinase 1)*, one of the three *RACK1* homologues in Arabidopsis, renders ABA hypersensitivity. Here, we provide genetic and molecular evidence that all three *RACK1* genes are negative regulators of ABA signalling in seed germination and early seedling development. We found that loss-of-function mutations in *RACK1B* and *RACK1C* enhance the ABA hypersensitivity of *rack1a* mutant in the ABA inhibition of seed germination, cotyledon greening and root growth. Moreover, plants overexpressing *RACK1A* displayed ABA insensitivity. Consistent with their proposed roles in seed germination and early seedling development, all three *RACK1* genes were expressed in imbibed, germinating and germinated seeds. Expression analysis indicated that *ABI1* and *ABI2*, two key negative regulators of ABA signalling that act to reset ABA responses, displayed much lower response to ABA in *rack1a rack1b* seedlings, implying that RACK1 may be required for the proper desensitization of the triggered ABA signalling cascade. Furthermore, ABA induction of a group of potential physical interacting partners of RACK1 (identified by Arabidopsis Interaction Reviewer) which are known to be involved in ABA/stress signalling were dramatically altered in *rack1a rack1b* mutants. Taken together, our findings provide compelling evidence that *RACK1* genes are critical negative regulators of ABA responses.

P49

RACK1 Negatively Regulates Ethylene Biosynthesis in Arabidopsis

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Receptor for Activated C Kinase 1 (RACK1) is a versatile scaffold protein, serving as a nexus for multiple signal transduction pathways in mammals. The protein sequence of RACK1 is highly conserved in plants. However, the function of plant RACK1 remains poorly understood. Accumulating evidence suggested that RACK1 may be involved in hormone responses, but the exact role of RACK1 in any hormone signalling pathways remains elusive. Arabidopsis genome contains three *RACK1* homologous genes, designated as *RACK1A, RACK1B* and *RACK1C*, respectively. We found that *rack1a* single mutants and *rack1a rack1b* double mutants display short hypocotyls in the dark and epinastic cotyledons and leaves in light, resembling wild-type seedlings exposed to ethylene. The expression of *ACS2* and *ACS6*, which encode two key enzymes in ethylene biosynthesis, was up-regulated in *rack1a* single mutant and *rack1a rack1b* double mutant, suggesting that RACK1 may negatively regulate ethylene biosynthesis. Consistent with this view, ethylene biosynthesis inhibitors, AVG and AOA, rescued *rack1* mutants' phenotypes. On the other hand, *rack1a-1* mutation did not alter the responses of ethylene signalling mutants, *etr1-3* and *eni2-1*, to ACC (a precursor of ethylene) or Ag+ (an ethylene action inhibitor). Taken together, these findings suggested that RACK1 is a negative regulator of ethylene biosynthesis.

P50

Cortical microtubule organization influences the crystalline properties of cellulose microfibrils, and the movement of cellulose-synthase-complexes during cell expansion in *Arabidopsis thaliana*

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Growth anisotropy, in which cells grow predominantly in one direction, is common in plant cells, and an essential event for plant form and function. The direction and degree of growth anisotropy are governed by the mechanical properties of the primary cell wall. When aligned in a parallel manner, cellulose microfibrils accommodate great resistance in the direction of their alignment to expansion driven by isotropic turgor pressure. Microtubules have been considered to be the most likely candidates for controlling the orientation of cellulose microfibrils. Recent studies have indeed demonstrated a close association between cortical microtubules and the plasma membrane-localized cellulose-

synthase- complexes (CSCs) that produce cellulose. Despite this close association, microtubule disruption did not cause cellulose microfibrils to lose parallel alignment in the radial and inner periclinal walls of cells in the inflorescence stem, suggesting that microtubules influence mechanical properties of cellulose microfibrils other than orientation. X-ray diffraction analysis demonstrated that cellulose crystallinity in wild-type plants declines at the growth-promoting temperature of 29°C, whereas crystallinity fails to adapt and remains high in *mor1-1*, a temperature-sensitive mutant whose microtubule arrays become disorganized at its restrictive temperature (29°C). This finding suggests that organized microtubules are involved in reducing cellulose crystallinity that normally accompanies increased cell expansion. Live-cell imaging of CSCs by tracking a yellow fluorescent protein (YFP)-tagged CesA6 subunit in hypocotyl cells demonstrated that dynamic and well-organized microtubules affect the velocity, the direction of movement, and the density of CSCs, suggesting that there is a close relationship between microtubules and CSCs.

P51

Arabidopsis Mitogen-Activated Protein Kinase 4 Is Required for Male-Specific Cytokinesis

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Plant reproduction is crucial not only for the existence of the species itself but also for the world's food supply. Successful production of progeny relies on the formation of viable male and female gametes. Here we report that Arabidopsis mitogen-activated protein kinase 4 (AtMPK4) that was previously described as a regulator of disease resistance, also specifically regulates post-meiotic, male-specific cytokinesis. Although At*mpk4* mutants form smaller but complete flowers, the anthers contain fewer but highly enlarged pollen grains with reduced viability. Moreover, the mature At*mpk4* pollen grains have increased sets of the tricellular structure composed of two germ cells and one vegetative cell. We found that At*mpk4* cannot undergo proper cytokinesis after the completion of male meiosis, and that the callose walls that normally surround the tetrads in wild type plants are absent in At*mpk4*. Promoter-reporter analysis showed that *AtMPK4* is expressed in anthers, consistent with its role in male gametophyte formation. Because in yeast two-hybrid assays, AtMPK4 interacts with AtMKK6, a MAP kinase kinase that was previously shown to be involved in cytokinesis, we propose that AtMKK6 and AtMPK4 form a MAP kinase signalling module that specifically regulates post-meiotic, male-specific cytokinesis in Arabidopsis.

P52

Characterization of a Chloroplast Targeted PPP-Family Protein Phosphatase

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Reversible phosphorylation of proteins is a reaction controlled by the opposing activities of protein kinases and phosphatases. It is estimated that one third of all eukaryotic proteins are regulated by phosphorylation and current evidence indicates that protein phosphorylation within the chloroplast is a critical regulator of many aspects of plant biology, such as starch synthesis and degradation. However, despite the importance of reversible phosphorylation in regulating many biological processes in plants, research efforts to date have been particularly bias, focusing on protein phosphorylation by protein kinases, while largely neglecting protein de-phosphorylation by protein phosphatases. Recent bioinformatic research in the Moorhead group completed an inventory of plant phosphatases using publicly available genomic data. Through this bioinformatic work a previously uncharacterized *Arabidopsis thaliana* PPP-family serine/threonine phosphatase was identified and suggested to be Chloroplast-targeted; *Arabidopsis thaliana* Chloroplast Targeted Phosphatase 1 (AtCTP1). Successful heterologous protein expression in bacteria has facilitated the creation of mono-specific antibodies while *Agrobacterium* mediated transfection of *Arabidopsis thaliana* cell culture has successfully facilitated the expression of a fusion RFP-AtCTP1 construct. Cell imaging of RFP-AtCTP1 by confocal microscopy provides verification of the *in silico* predicted chloroplast localization of AtCTP1. A similar approach has also been implemented in identifying novel *in vivo* protein interactors of AtCTP1. Noteworthy biochemical characteristics of this protein phosphatase to date are discussed.

P53

Ribosomal protein family RPL23a isoforms have distinctive nucleolar localization abilities, impact on development and their translational status regulate auxin homeostasis

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Ribosomes are the largest enzyme complex in the cell. Together, the two subunits are comprised of four ribosomal RNAs (rRNAs) and in Arabidopsis 81 ribosomal proteins (r proteins). Ribosome biogenesis has been studied in detail in *E. coli* and yeast, where each r-protein is encoded by 1 or 2 genes. In *Arabidopsis,* each r-protein is encoded by a multiple gene family (2-7 members), from which only one isoform is incorporated into any one ribosome. The presence of these multi-gene families in plants adds a degree of complexity to ribosome biogenesis not seen in other organisms. We are working with the two-member gene family, *RPL23a*; RPL23aA is targeted to the nucleolus more efficiently than RPL23aB. Inducible RNAi knockdown plants showed that RPL23aA, and not RPL23aB, is required for normal development. The plants with reduced levels of RPL23aA develop a *pointed first leaf* (*pfl*) phenotype exhibiting reduced cell division, retarded growth, late flowering, irregular root and leaf morphology, fused leaf with first leaves having a pointed shape and loss of apical dominance. The *pfl* phenotype is typically associated with a

disruption in auxin mediated processes, auxin responsiveness or polar auxin transport mutants. The *pfl* phenotype also has intriguing similarities with microRNA biogenesis mutants, leading to the hypothesis that translational status regulates auxin homeostasis via miRNAs. Our model states that an inhibition of translation disrupts auxin homeostasis by disrupting the miRNA mediated regulation of auxin conjugating enzymes and auxin regulated transcription factors. Our current studies on auxin biosynthesis, conjugation, degradation or transport in RPL23a knockdown/knockout mutants, coupled with microRNA analyses will provide insights into the proposed model.

P54

Developmental and feedforward control of the expression of folate biosynthesis genes in tomato fruit

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Little is known about how plants regulate their folate content, including whether the expression of folate biosynthesis genes is orchestrated during development or modulated by folate levels. Nor is much known about how folate levels impact the expression of other genes. These points were addressed using wild type tomato fruit and fruit engineered for high folate content. In wild type fruit the expression of genes specifying early steps in folate biosynthesis declined during development but expression of other genes did not. In engineered fruit overexpressing foreign GTP cyclo-hydrolase I (GCHI) and aminodeoxychorismate synthase (ADCS) genes, the expression of the respective endogenous genes did not change but that of three downstream pathway genes (aminodeoxychorismate lyase, dihydroneopterin ald- olase, and mitochondrial folylpolyglutamate synthase) respectively increased by up to 7.8-, 2.8- and 1.7-fold, apparently in response to buildup of specific folate pathway metabolites. Together, these results indicate that folate pathway genes are not regulated coordinately during development, and that certain of them are subject to feedforward control by pathway intermediates. Microarray analysis showed that only 17 other transcripts (of 12,000 surveyed) changed in abundance by two-fold or more in high-folate fruit, demonstrating that the induction of folate pathway genes is relatively specific.

P55

The role of the intrinsically unstructured A-domain of Toc159 in mediating protein import into chloroplasts

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Plastids are characteristic organelles of plant cells, and are the site of photosynthesis and other essential biochemical pathways. Approximately 3000 distinct proteins (~95% of the total chloroplast protein complement) that support these essential processes are encoded in the nucleus, translated in the cytoplasm as preproteins, and imported into the organelle by the coordinate action of the Toc and Tic complexes of the envelope membranes that surround the organelles. Toc159 is an essential component of the Toc complex, and is believed to be the primary preprotein receptor. These receptors directly bind to transit peptides (TPs), which are N-terminal extensions that designate preproteins for import into chloroplasts; the mechanism of preprotein recognition is unknown. In *Arabidopsis*, Toc159 is part of a gene family. All members of the family have a tripartite domain structure consisting of a C-terminal membrane domain, a GTPase (G-) domain, and an N-terminal acidic (A-) domain. The amino acid sequences of the family members are most variable within their A-domains, suggesting it may contribute to the functional specificity of the receptors. The function of the A-domain is unknown, but it possesses physicochemical properties that are characteristic of intrinsically unstructured proteins (IUPs). Using circular dichroism (CD) and fluorescence spectroscopy we show that the A-domain of two members of the *Arabidopsis* Toc159 family (atToc132 & atToc159) are unstructured at physiological pH and temperature and show conformational changes at temperature and pH extremes that are characteristic of other known IUPs. Identification of the A-domain as an IUP domain suggests a role in protein-protein interactions, and may explain how these receptors can interact with a wide variety of preprotein substrates. We hypothesize that the A-domain confers preprotein specificity to the receptors, or plays a role in modulating the GTPase activity of the G-domain. This will be tested by examining binding interactions between recombinant A-domains and model preprotein TPs or GTPase domains using CD. Our most recent data will be presented.

P56

Probing the role of microtubules in plant cell wall formation

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Cellulose is the major component of plant cell walls that provides the rigidity of plant bodies. Traditionally, cellulose synthesis is hypothesized to be guided by microtubules and through the enzymatic activities of Cellulose Synthesis Complex. However, this could be an incomplete model not covering all complexity as both supporting and inconsistent experimental evidences exist. Previous analysis of the role of microtubules in cellulose synthesis relies on modifying microtubule behaviours by either treating with microtubule-disrupting chemicals like oryzalin or genetic disturbance such as the *mor1-1* mutant followed by observing cellulose depositions. These experiments have yield invaluable knowledge but may not be comprehensive enough as only one chemical, oryzalin, and one mutant (*mor1-1*) has been used. Here we present a study with larger scale that microtubule orientation was altered by mutations in tubulin

genes *(lefty1*, *lefty2*, *tua4V62I*, *tua5D251N*). Orientation of microtubules and cellulose microfibrils were measured in these mutant backgrounds together with Wild Type control using immunofluorescence and field emission scanning electron microscopy (FESEM) respectively. We found that cellulose microfibril oritentations in these mutant backgrounds are largely similar to WT, despite all of the mutants displaying severe changes in microtubule orientations. This result suggests that deficiency in microtubule organization does not necessarily cause failure to control orientation of cellulose deposition. Compared to previous studies which reported oryzalin treatment lead to immediate changes in cellulose synthase complex movement through imaging GFP-tagged CESA6, our FESEM assay provides a direct measurement of cellulose orientation and argues against the model that orientation of cellulose is strictly controlled by microtubules."

P57

A Conifer ABI3-interacting Protein, CnAIP2, Negatively Regulates ABI3 during Key Transitions in Plant Lifecycle

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ABI3 *(AB*scisic acid *I*nsensitive *3)*, a transcription factor of the ABA signal transduction pathway, plays a major role during seed development, dormancy inception and maintenance. ABI3 was initially considered seed-specific but later findings revealed that this protein appears to function as well in meristematic and vegetative plant tissues, and under certain stress conditions. We have isolated the *ABI3* gene ortholog (*CnABI3*) from yellow-cedar and found it was functionally similar to other *ABI3* genes of angiosperms. In addition, using a yeast two-hybrid approach, we have identified CnAIP2 protein of yellow-cedar that interacts with CnABI3. Functional analyses revealed that CnAIP2 interacts with (Cn)ABI3 in a negative manner during several key transitions in plant life cycle: (1) CnAIP2 negatively regulates ABI3 during seed development and germination; (2) In roots, CnAIP2 negatively regulates ABI3 by promoting root development, particularly in lateral root initiation and CnAIP2 expression is greatly enhanced by auxin. (3) During the transition from vegetative growth to reproductive initiation, CnAIP2 negatively regulates ABI3 by promoting flowering.

P58

Identification of Genes Expressed in Vascular Tissues Using NPA-Induced Vascular Overgrowth in *Arabidopsis*

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The genetic basis of vascular differentiation and function is relatively poorly understood, partly due to the difficulty of screening for mutants defective in internal vascular tissues. Here we present an approach based on a predicted increase in vascular-related gene expression in response to an auxin transport inhibitor-induced vascular overgrowth. We used microarray analyses to identify 336 genes that were up-regulated two-fold or more in shoot tissues of *Arabidopsis thaliana* showing vascular overgrowth. Promoter-marker gene fusions revealed that 38 out of 40 genes with four-fold or more up-regulation in vascular overgrowth tissues had vascular-related expression in transgenic *Arabidopsis* plants. Obtained expression patterns included cambial tissues and differentiating xylem, phloem and fibers. A total of 15 genes were found to have vascular-specific expression patterns in the leaves and/or inflorescence stems. This study provides empirical evidence of the efficiency of the approach and describes for the first time the *in situ* expression patterns of the majority of the assessed genes.

P59

Map positions of phenylpropanoid pathway genes in bean (*Phaseolus vulgaris* L.) and their association with seed coat color

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Common bean (*P. vulgaris* L.) market classes are defined by their seed coat colors and patterns. Previous genetic analyses have identified 15 genes that control seed coat color and pattern in beans. Many of these genes exhibit epistatic interactions with other genes and together define the large variability in seed coat patterns and colors observed in this species. Some of these genes have been positioned on the common bean linkage map. It has also been shown that seed coat color in common beans is determined by the presence and amounts of flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins). Therefore, it has been hypothesized that genes involved in the phenylpropanoid pathway correspond to some of the classical seed coat color genes in bean. In a previous study we cloned and sequenced fragments of thirty-five phenylpropanoid pathway genes from common bean. The purpose of the current work is to map the positions of these genes on the common bean linkage map and determine whether their position correspond to any of the loci for classical seed coat color genes. The core mapping population was used, consisting of recombinant inbred (RI) lines derived from a cross between 'BAT 93' and 'Jalo EEP558'. Polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLP) were identified for the phenylpropanoid gene sequences between parental lines. The segregation patterns of 24 phenylpropanoid pathway genes have been analysed in the RI population and their locations in the bean linkage map were determined by a JoinMap analysis. Linkages between 4CL1 (4-coumarate CoA ligase) and Z (8.7 cM), Myb15 (Myb AtMYB15) and P (9.8 cM), VT (Vacuolar transporter) and AS1 (Glutathione S-transferase) and C (4.2 cM) and CHR (Chalcone reductase) and G (14.9cM) on linkage groups 3, 7, 8 and 4, respectively were observed. The map positions of additional genes in this pathway will be identified in a similar way and cosegregation between phenylpropanoid and classical seed coat color genes will be tested.

Technology, Poster Abstracts

P60

Enhanced in planta expression of human therapeutic proteins, Glutamic acid decarboxylase-65 and Interluekin-4

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We developed an in planta expression protocol targeted to achieve enhanced expression levels of the human islet autoantigen, Glutamic acid decarboxylase (GAD-65) and the human immunoregulatory cytokine interleukin-4 genes in tobacco. Transgenic plants developed through this study could serve a potential source for oral administration of these proteins for the treatment of Type 1 diabetes. Plant expression constructs were made in pCAMBIA1300 using the *Phaseolus vulgaris*-arcelin promoter for driving the transgenes. For GAD-65 expression, both the full length coding sequence and a truncated version carrying an N' terminal deletion were used; five different constructs were developed with and without arcelin signal peptide and an endoplasmic reticulum retention signal. For IL-4 expression, constructs were made with and without arcelin signal peptide. These constructs were expressed in combination with a gene construct carrying the yellow cedar CnABI3-transcription factor. Coexpression of constructs resulted in transactivation of the arcelin promoter by the CnABI3 protein, leading to high levels of recombinant GAD-65 and hIL-4 mRNA and protein accumulation in leaves upon induction with absicic acid (ABA). Treatment of transgenic leaf tissues with ABA at 150-200 µM (+)-ABA or 700-800 mM glucose/mannose/sucrose or 400 mM sodium chloride, for 3-4 days of incubation at 22°C, significantly enhanced the yield of recombinant proteins. Various other stress-inducing treatments could also, to a lesser degree induced the expression of GAD-65 and hIL-4 proteins in leaf tissues. Further, the recombinant proteins were very stable in mature dry seeds.

P61

Creation of a Dual Luciferase Transactivation Assay to determine the ICE binding site in *Vitaceae CBF* genes

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The ability to cold acclimate is a quantitative genetic trait resulting from the input of many genes. The freezing tolerance pathway involved in cold acclimation is initiated by a cold stimulus resulting in the activation of a protein called inducer of CBF expression (ICE). The ICE protein is then able to bind and activate the promoter region of a *C-repeat binding factor* (*CBF*) gene. Eight potential *CBF* genes, and 3 potential *ICE* genes have been identified in grape plants (*Vitis* sp.), and at least *Vitis CBF1-4* were shown to be cold inducible *in planta* (Xiao et al., 2006, 2008). Although much is known about the players involved in the freezing tolerance cascade, how ICE interacts, and where it binds to a *CBF* promoter is still unknown. A 5' promoter deletion series for the *VrCBF4* gene, plus an artificial promoter containing putative regulatory elements in the *VrCBF4* promoter together with a minimal 35S promoter, were created to determine what promoter elements are necessary to induce its expression in a dual luciferase transactivation assay which is being developed. The dual luciferase assay will consist of one construct expressing an ICE protein, and another construct containing RiLUC and FiLUC reporter genes on a pCAMBIA-derived vector. The promoter being studied drives the expression of RiLUC, while 35S::FiLUC acts as a normalizer to minimize error caused by the infiltration. Preliminary results indicate the presence of enhancer sequences as RiLUC is constitutively active regardless of the promoter placed in front. To minimize this interference the 2x35S promoter driving hygromycin resistance will be replaced by a *nos* promoter, and a genetic "insulator" sequence will be placed between the RiLUC and FiLUC cassettes. Results illustrating the proper function of the Dual Luciferase construct as well as preliminary results with the *VrCBF4* deletion series and artificial promoter will be presented.

P62

TILLING for phenylpropanoid (lignin) biosynthesis pathway gene caffeic acid/5-hydroxyferulic acid O-methyltransferase (COMT)

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Caffeic acid/5-hydroxyferulic acid O-methyltransferase (COMT) is one of the key genes in the phenylpropanoid (lignin) biosynthesis pathway. Inhibition of COMT may alter lignin content in Hemp (Cannabis Sativa L.). Hemp could be developed as a sustainable alternative source of fiber and other biomaterials for pulp and paper products, textiles, and green bio-composites. To achieve this objective COMT was selected as one of the target genes to obtain mutants with altered lignin contents. Targeted Induced Local Lesions In Genomes (TILLING) was used for detection of mutations in 8000 M2 EMS mutagenized individual hemp plants. TILLING is an efficient, high-throughput screening method for altered genes of interest, in this case COMT. This is also the method of choice for isolation of non-GMO mutant plants altered in various characteristics. Currently several mutants of interest have been obtained after the screening. A number of these candidates will be grown and tested for desired fiber traits.

P63

Investigating regulation of isoprenoid production in lavender by transcript profiling

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The molecular, cellular, and biochemical mechanisms that control production of the isoprenoids (or terpenoids) in plants are poorly understood. We are developing *Lavandula angustifolia* (English Lavender, or lavender) as a model system for investigating regulation of isoprenoid metabolism in plants. Lavenders produce copious amounts of mono- and sesquiterpenes (C10 and C15, respectively) which are sequestered in the form of an aromatic essential oil in the storage cavity of leaf and floral glandular trichomes, also

known as oil glands. As an initial step towards building the necessary "genomics toolbox" for English Lavender, we have cloned and partially sequenced approximately 15,000 ESTs for this species. To gain insight into the expression of the cloned sequences, we then evaluated the expression pattern for the entire EST collection by transcript profiling in developing lavender leaves and flowers. The results highlighted a number of genes including terpene synthases whose expression correlates with essential oil production in lavender flowers. We are in the process of characterizing and evaluating the biological relevance of these genes to essential oil production in lavenders.

P64

Microarray analysis of Ripening-regulated gene expression and its modulation by 1-MCP and Hexanal

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Several techniques have been evaluated to extend the shelf life of fruits and vegetables including genetic modification, controlled atmosphere storage, and inhibition of ethylene action using the ethylene receptor blocker 1-methylcyclopropene (1-MCP). Preservation of cellular structure and compartmentalization by inhibition of the membrane phospholipid-degrading enzyme phospholipase D (PLD) provides an alternative approach for extension of shelf life. Hexanal, an inhibitor of PLD, has been successfully applied for the pre- and / post-harvest treatment of fruits, vegetables and flowers. To elucidate the gene expression changes induced by hexanal and 1-MCP in tomatoes, Microarray analysis was carried out using TOM2 tomato oligo-array containing approximately 12000 unigenes. Tomato fruits (commercial cultivar Growdena) at the mature green stage were treated with 1-MCP and hexanal and the RNA was extracted after 10 days. A large variation in the expression profile was observed in the 1-MCP treated fruits. The key components in the ethylene biosynthetic pathway such as ACC- synthase / oxidase; ethylene receptor and ethylene response factors were heavily down-regulated in the 1-MCP-treated fruits. In addition, these fruits also showed down-regulation of the main precursors of ripening and pigment development pathways viz., geranyl pyrophosphate synthase and phytoene synthase. Hexanal treatment significantly down-regulated ACC-synthase and to a lesser extent other components of the ethylene signal transduction. However, the genes involved in Spermine biosynthetic pathway were up-regulated and may potentially play a role in the inhibition of PLD action. Hexanal treated fruits gradually ripened and showed higher levels of lycopene and β-carotene. In addition, GC-MS analysis also showed higher levels of total volatiles in these fruits. The similarities in the modulation of gene expression by hexanal and 1-MCP suggest that hexanal, in addition to being a PLD inhibitor, may also act as a weak ethylene receptor blocker.

P65

"Fate of O-labelled metabolic water in plants"

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Respiration is typically measured by monitoring either carbon dioxide release or oxygen consumption. An alternative approach, used here, is to monitor the product of oxygen consumption, namely water. Although the metabolic water produced will, like CO_2, ultimately diffuse away from mitochondria or be carried off by other processes, it will do so at a much slower rate and may therefore be captured at or near the site of respiration. In this way, the amount and within limits the location of the label provided can be known. Plant species and tissues under investigation were vacuum infiltrated with $^{18}O2$ (99.97%) and kept devoid of light for a 30 minute period to prevent photosynthesis and allow sufficient time for the label to be incorporated. Labelled metabolic water was easily detected and recovered against a large background of normal tissue water, in *Medicago sativa* L. sprouts. We investigated four other species, representing different functional groups: a C3 plant (*Helianthus giganteus* L.), a CAM plant (*Crassula ovata* (Miller) Druce), a desert plant (*Disocactus flagelliformis* (L.) Barthlott.), and a tree (*Populus trichocarpa* Torr & Gray). The ^{18}O values of tissue water ranged from +7.93‰ to +216‰. Higher values were associated with tissues with high respiration rates per unit water content. In some experiments, labelling was followed by immersing the tissues in unlabelled water to monitor exchange (i.e. leakage) over periods of 5 to 60 minutes. Exchange of labelled water was more rapid during the first 5 minutes ($\bar{X}$ = 63.4% complete over all species, tissues and treatments) than over the 5-10 min interval, suggesting the existence of two pools of water available for exchange; namely apoplastic and symplastic water. Attempts to modify aquaporin activity failed to influence exchange. In conclusion, the movement of metabolic water across cellular membranes was very rapid, limiting the utility of ^{18}O labelling for pin-pointing sites of respiratory activity at a fine scale.

P66

Development of an EMS-mutagenized population of hybrid poplar trees to be used for mutant identification by TILLING

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The breeding of agricultural and horticultural species has been immensely successful. A key approach to plant breeding has been to induce genetic variation by various means followed by self fertilization and screening for desired phenotypes in the F2 generation. Due to the long generation time and the great difficulties associated with controlled large-scale self fertilization in forest tree species, this approach has never been undertaken in forest tree species. Targeting Induced Local Lesions IN Genomes (TILLING) can be used to screen thousands of individuals from a mutagenized population for single-base pair substitutions in genes of interest based on molecular lesion rather than phenotype. In

theory, a reverse genetics approach such as TILLING may provide an avenue for the application of mutational breeding in forest tree species. The sensitive molecular screen can be applied to a mutant population at an early stage to identify individuals harboring mutations in targeted genes that can then be brought into a greenhouse for treatments to induce early flowering, and allelic variants can be crossed to generate homozygosity in a targeted locus, while other mutant loci remain heterozygous. To provide proof of concept, we have established a mutagenized population for TILLING in hybrid poplar. To avoid chimeric mutant plants and maintain the genotype of the source hybrid line, we took the atypical approach of inducing mutations in cells of hybrid poplar calli followed by regeneration of a large number of trees from the mutagenized cells. We have generated approximately 5000 putative mutagenized plants and we are in the process of purifying DNA from these individuals. We are also comparing detection of mutation-induced polymorphism by single stranded digests resolved on PAGE gels versus double stranded digests resolved on agarose gels. We also aim at identifying mutants in potentially haploinsufficient loci, which could allow us to observe phenotypes without the need to advance to the next generation.

P67

Comparative SNP diversity across North American and European *Populus* species

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The genus *Populus* has become the model for perennial/woody plant genomic research. *Populus* occupies an enormous geographic range in the northern hemisphere, where it is adapted, both within and among species, to widely varying environmental conditions. Here, for the first time we report on comparative single nucleotide polymorphism (SNP) diversity among two North American "sister" cottonwood species (*Populus* balsamifera L. & *Populus* trichocarpa Torr. & Gray) along with the European aspen (*Populus* tremula L.) that are adapted to a similar range of climatic conditions while having different population history. SNP patterns along latitudinal gradients were examined across nine candidate genes involved in photoperiodism (*PhyB & GI*), wound inducible defense (*TI-3, TI-4, & WIN3*), stress response *(Dehy, PAL & PtCBF)*, and house-keeping *(Gapdh).* The SNP number and haplotypes were highest for European aspen compared to the North American cottonwoods. The photoperiod gene (*PhyB*) had low number of SNPs while the wound inducible kunitz trypsin inhibitor genes had highest number across all the three species. Overall haplotype diversity (*Hd*) was 0.770 for aspen followed by *(P. balsamifera)* (0.602) and *P. trichocarpa* (0.386). The total nucleotide diversity (πT) was similar among the cottonwoods (0.003) while aspen had twice the amount. Evidence for non-neutral evolution for the studied genes were substantiated using Tajima's *D*s. Linkage disequilibrium decayed rapidly in all three species, but the rate of decay was slowest in *P. trichocarpa.*

P68

Metabolic profiles of the freshwater alga *Chlamydomonas reinhardtii* determined by H NMR and cross model validation and permutation testing

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The effect of nutrition on metabolic profiles of *Chlamydomonas reinhardtii* was studied. The freshwater algae (wild type strain CC-125 m+) were grown in continuous culture systems, either autotrophically under continuous light (500 µmole/m2/sec PAR), or heterotrophically in the dark by adding sodium acetate (10 mM) to the nutrient medium. Polar metabolites were extracted according to published protocols (Ward, J.; *et al. Phytochem.,* **2003**, *62*, 949-957), with the addition of a 3-kDa cut-off filtration step (Wishart, D.; *Trends Anal. Chem.,* **2008**, *27*, 228-237) and analyzed using 1H NMR spectroscopy (400 MHz). Partial least squares discriminant analysis, PLSDA, (Barker, M.; *et al. J. Chemom*., **2003**,*17*, 166-173, Westerhuis, J. S.; *et al. Metabolomics*, **2008**, *4*, 81-89) with rigorous validation was used to identify statistically different spectral features. Twenty metabolites have so far been uniquely identified from the 1H NMR spectra (Weljie, A. M.; *et al. Anal. Chem.*, **2006**, *78*, 4430-4442). Based on our experiments 3-hydroxybutarate, cysteine and tyrosine have been identified as important biomarkers for the metabolic changes occurring when the algae are grown under the two different nutritional conditions.

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Plant Fatty acid desaturase enzymes synthesizing unusual monounsaturated and polyunsaturated fatty acids

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The most common monounsaturated fatty acid found in plants is oleic acid (cis-9 octadecenoic acid, $18:1^{\Delta}9$). This fatty acid is the substrate for the synthesis of polyunsaturated fatty acids by the sequential introduction of additional double bonds, generally towards the methyl end of the molecule. We have investigated the ability of a family of plant fatty acid desaturases to catalyse the introduction of double bonds at novel positions in a saturated acyl chain. Expression of various members of the *Arabidopsis* ADS desaturase family in yeast results in the accumulation of very long chain fatty acids (VLCFAs; chain length greater than 18 carbons) with double bonds in unusual positions. The double bonds in most polyunsaturated fatty acids produced in plants are separated by a single methyl group, and the double bond configuration is referred to as a "methylene interrupted" system. We have shown that the plant FAD3 desaturases can be used to produce novel non-methylene interrupted di-

unsaturated fatty acids in plants. These fatty acids could have novel applications as fatty acid feedstocks for industrial uses.

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Identification and analysis of seed coat epidermal specific promoters in *Arabidopsis thaliana*

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The mature seed coat epidermal cells of Arabidopsis contain large amounts of pectinaceous mucilage in a donut-shaped ring positioned between the primary cell wall and a volcano shaped secondary cell wall. During differentiation the epidermal cells undergo a successive series of cellular changes, involving cell growth, mucilage secretion, secondary cell wall synthesis and programmed cell death. Many genes with roles in *Arabidopsis* seed coat development have been identified, including *MUCILAGE-MODIFIED* genes (*MUM1-5*), *APETALA2*, *TRANSPARENT TESTA GLABRA1 AND GLABRA2.* These *Arabidopsis* seed coat epidermal cells represent an excellent platform to explore methods for modification of seed coats that could later be applied to canola and linseed flax. However, no seed coat epidermal cell- specific promoters have been identified in *Arabidopsis* or any other species. Our goal is to identify a seed coat epidermal specific promoter for use in manipulating *Arabidopsis* seed coat differentiation. Microarray data and RT-PCR experiments were used to identify potential candidate promoters. These promoters were fused to GUS/GFP reporter genes and transformed into *Arabidopsis* and we are currently using the transgenic plants to evaluate the specificity of expression. Further, the *MUM4* gene encodes an enzyme required to produce rhamnose, a substrate for mucilage. *MUM4* is up-regulated in the seed coat during mucilage biosynthesis. We are also attempting to identify, using deletion analysis of the *MUM4* promoter region, cis-acting sequences which are required for the up-regulation. Once a seed coat epidermal cell-specific promoter and *MUM4 cis*-elements have been identified, they will be used to express foreign proteins or modify seed coat features in *Arabidopsis,* and its economically important relative *Brassica napus*.

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Growth of Cell Suspension Cultures of *Arabidopsis thaliana* is Heterotrophic in the Light Despite the Presence of Chloroplasts and a Functional Photosynthetic Apparatus

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Sucrose is known to repress the expression of photosynthetic genes, and hence, inhibit the development of a functional photosynthetic apparatus. Thus, plant cell suspension cultures grown in the absence of sucrose typically exhibit a yellow-white phenotype devoid of mature chloroplasts and chlorophyll. However, suspension cultures of *Arabidopsis thaliana* grown in the presence of sucrose exhibited a normal green phenotype, the presence of mature chloroplasts and normal cellular ultrastructure based on transmission electron microscopy. In a preliminary characterization of these cell cultures, we report that these cell cultures grown in the light exhibited similar growth rates to those grown in the dark. However, dark grown cells did not accumulate chlorophyll. Furthermore, the addition of an external carbon source such as sucrose was essential not only for cell growth but also for the accumulation of chlorophyll in the light. Similar results were obtained for either glucose or fructose as the added carbon source. Light response curves for oxygen evolution indicated that cell cultures grown in the light were photosynthetically competent and exhibited the presence of Rubisco, the photosystem II reaction centre polypeptide, D1, as well as the major polypeptides (Lhcb1) associated with a normal photosynthetic apparatus even in the presence of sucrose as a carbon source. However, since the capacity for photosynthesis in these green cells did not appear to contribute to cell growth, we conclude that growth of the green cell suspension cultures of *Arabidopsis thaliana* remains heterotrophic despite the presence of a functional photosynthetic apparatus.

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42 is a calmodulin-related Ca2+ sensor that controls trichome branching

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Calcium is a key second messenger in eukaryotes where it regulates a diverse array of cellular processes in response to external stimuli. An important calcium sensor in both animals and plants is calmodulin (CaM). In addition to evolutionarily conserved CaM, plants possess a unique family of CaM-like (CML) proteins. The majority of these CMLs have not yet been studied, and investigation into their physical properties and cellular functions will provide insight into calcium signal transduction in plants. Here we describe the characterization of CML42, a 191-amino acid calcium-binding protein from *Arabidopsis*. Calcium-binding to recombinant CML42 was assessed by fluorescence spectroscopy, NMR spectroscopy, microcalorimetry, and CD spectroscopy. CML42 displays significant -helical secondary structure, binds three molecules of calcium with affinities ranging from 30-430 nM, and undergoes a calcium-induced conformational change that results in the exposure of a hydrophobic region(s). Gene expression analysis revealed *CML42* transcripts at various stages of development and in many cell types, including the support cells which surround trichomes (leaf hairs) on the leaf surface. Using yeast two-hybrid screening we identified a putative CML42 interactor; kinesin -interacting calcium-binding protein (KIC). As KIC is a protein known to function in trichome development, we examined transgenic CML42-knockout plants lacking CML42 expression and found that they possess aberrant trichomes with increased branching. Collectively, our data support a role for CML42 as a calcium sensor which functions during cell branching in trichomes.

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