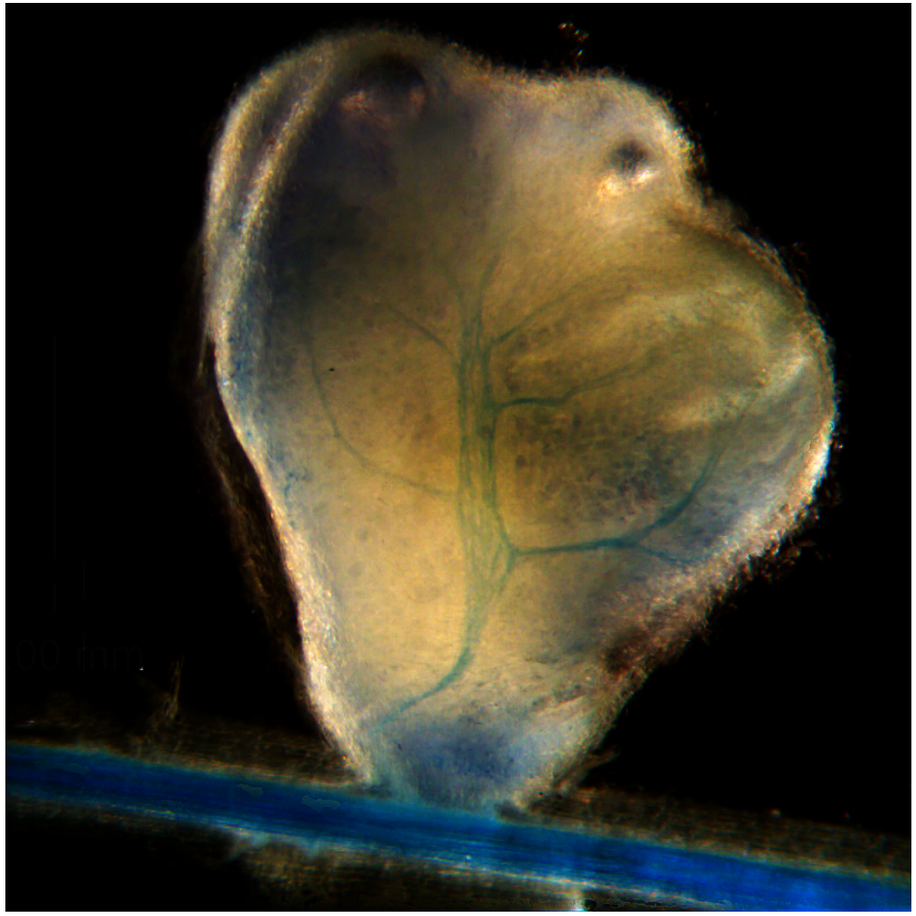

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SYMBIOSIS

The Canadian Society of Plant Biologists - Eastern Regional Meeting & Plant Development Workshop 2012
Délibérations du Congrès de la Société Canadienne de Physiologie Végétale - Congrès Régional de l'Est & Congrès de Développement Végétal 2012

The Canadian Society of Plant Biologists Eastern Regional Meeting and Plant Development Workshop
November 30th and December 1st, 2012
Wilfrid Laurier University, Waterloo, Ontario, Canada

Organizing Committee
Allison McDonald (Chair)
Frédérique Guinel
Matthew Smith
Jennifer Baltzer
Kevin Stevens

The organizing committee acknowledges financial assistance from the following:

Dr. Scott Ramsay, Chair of the Department of Biology, Wilfrid Laurier University
Dr. Paul Jessop, Dean of the Faculty of Science, Wilfrid Laurier University
Dr. Deborah MacLatchy, Vice-President: Academic and Provost, Wilfrid Laurier University
The Office of Research Services, Wilfrid Laurier University
The Centre for Women in Science, Wilfrid Laurier University
The Canadian Botanical Association
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Regent Instruments Inc.
Nelson Education Ltd.

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Elma Schweigert
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Communications, Public Affairs, and Marketing, Wilfrid Laurier University
Our wonderful student and post-doc volunteers

Cover image: A parasitic plant from the family *Cuscuta* (dodders), Mihai Costea
Back cover image: A nodule of *Pisum sativum* (pea), Frédérique Guinel

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Schedule Overview

Friday, November 30th, 2012

Science Building

5:30-7:00 p.m.	Registration
7:00-7:10 p.m.	Welcoming remarks
7:10-8:00 p.m.	Plenary Lecture - Dr. Mary Rumpho "Unraveling the mystery of photosynthetic sea slugs: Giving new meaning to "going green" N1001
8:00-10:00 p.m.	Welcome reception and 50th Anniversary Celebration of Laurier Biology

Saturday, December 1st, 2012

Science Building

7:30-8:30 a.m.	Registration and poster set-up
8:30-8:40 a.m.	Welcoming remarks
8:40-9:30 a.m.	Plenary Lecture - Dr. Debashish Bhattacharya "Genomic methods elucidate the origin of plastids and endosymbiotic gene transfer in photosynthetic eukaryotes" N1001
9:30-10:00 a.m.	Refreshment Break
10:00-12:00 p.m.	Concurrent oral presentation sessions 1A, 1B, 1C N1001, N1002, N1044
12:00-1:30 p.m.	Lunch and poster session, CSPB Executive Lunch
1:30-1:35 p.m.	Introductory Remarks
1:35-2:25 p.m.	Plenary Lecture - Dr. Simon Chuong "Going Solo: the art of single-cell C4 photosynthesis in plants"
2:25-2:30 p.m.	Movement between session rooms
2:30-3:30 p.m.	Concurrent oral presentation sessions 2A, 2B, 2C N1001, N1002, N1044
3:30-4:00 p.m.	Refreshment Break
4:00-5:30 p.m.	Concurrent oral presentation sessions 3A, 3B, 3C N1001, N1002, N1044
5:30 p.m.	Presentation of student awards and closing of the conference

Concurrent Oral Sessions

Session 1A: Environmental Stress
Chair: Harold Weger
Room N1001

Time	Speaker	Title
10:00-10:15 a.m.	Joseph Stinziano	**Elevated temperature eliminates the decline in photosynthetic capacity during a simulated autumn in Norway spruce (*Picea abies*)**
10:15-10:30 a.m.	Katharina Bräutigam	**Genetic and epigenetic impacts on the poplar drought response**
10:30-10:45 a.m.	Vera Marjorie Velasco	**The effect of low phosphate conditions on the growth of the extremophile crucifer, *Thellungiella salsuginea***
10:45-11:00 a.m.	Mitchell MacLeod	**Comparative physiology and transcriptome sequencing to identify genes implicated in the drought tolerance of *Thellungiella salsuginea***
11:00-11:15 a.m.	Laura Verena Junker	**Isoprenoid-related mechanisms of drought tolerance in field-grown Douglas-fir provenances**
11:15-11:30 a.m.	Purushothaman Natarajan	**Correlation network analysis identifies major gene regulatory pathways involved in the temperature and photoperiod response of *Pinus banksiana* needles during cold hardening**
11:30-11:45 a.m.	Harold G. Weger	**Pathways for iron acquisition by iron-limited cyanobacterial cells**
11:45-12:00 p.m.	Peter Ryser	**Effects of early-season soil temperatures on wetland graminoid root length growth**

Session 1B: Plant–microbe interactions
Chair: Sheila Macfie
Room N1002

Time	Speaker	Title
10:00-10:15 a.m.	Melanie P Columbus	**Plant Growth-Promoting Rhizobacteria Appear to Become Deleterious in Growth-Limiting Conditions**
10:15-10:30 a.m.	Christian Huynh	**Assessing rhizobial symbiosis efficiency in low-nodulating pleiotropic mutants of *Pisum sativum***
10:30-10:45 a.m.	Hwi Joong Yoon	***SUNERGOS1*, a *Lotus japonicus* gene required for proper accommodation of rhizobial infection**
10:45-11:00 a.m.	Mandana Miri	**Role of cytokinin receptors during symbiotic root nodule organogenesis in Lotus japonicus**
11:00-11:15 a.m.	Yue Wu	**The Arabidopsis NPR1 Protein Is a Receptor for the Plant Defense Hormone Salicylic Acid**
11:15-11:30 a.m.	Huoi Ung	**Characterizing the role of two TTM family members, *AtCYDP1* and *AtCYDP2*, in programmed cell death, linking immunity and senescence.**
11:30-11:45 a.m.	Kimberley Chin	**Suppressor mutant S171 identifies a link between pathogen defense and floral transition mediated by Cyclic Nucleotide-Gated Channel 2**
11:45-12:00 p.m.	May Yeo	**Investigating Disease Resistance and Priming in *Thellungiella salsuginea***

Session 1C Plant Development Workshop: Development and Regulation of Organs and Organelles
Chair: Ishari Waduwara
Room N1044

Time	Speaker	Title
10:00-10:15 a.m.	Lisa Amyot	**The translation elongation factor subunit eEF1Bβ1 affects cell wall formation and vascular development in Arabidopsis.**
10:15-10:30 a.m.	Julia Nowak	**Abaxial greening phenotype in hybrid aspen (*Populus tremula* x *tremuloides*)**
10:30-10:45 a.m.	Reynald Tremblay	**Finding needles in haystacks: Combining phylogenetic reconstruction, expression analysis and meta-analysis to isolate potential ancestral functions of the *IDD* gene family**
10:45-11:00 a.m.	Kiah Barton	**Differential colouring using a photo-convertible fluorescent protein suggests that stromules do not fuse to form a plastid network**
11:00-11:15 a.m.	Michael E Stokes	**Integration of nutrient and hormone signalling drives root meristem development in Arabidopsis.**
11:15-11:30 a.m.	Madhulika Sareen	**Understanding ARP 2/3 complex mediation in F-actin dependent Endoplasmic Reticulum organization in plant cells**
11:30-11:45 a.m.	Zaheer Ahmed	**Post-translational modification and protein-protein interactions among enzymes of starch biosynthesis in high-amylose barley genotypes from diverse genetic backgrounds**

Session 2A Herbivory and Pollination
Session Chair: Tariq Akhtar
Room N1001

Time	Speaker	Title
2:30-2:45 p.m.	Catherine Martel	**Tomato defense responses to herbivory by two closely related spider mite species**
2:45-3:00 p.m.	Tawhidur Rahman	**Utilization of the genome sequence data of *Tetranychus urticae* (two-spotted spider mite) to create pest-resistant transgenic plants targeting various pest genes**
3:00-3:15 p.m.	Tariq A. Akhtar	**Veratrole Biosynthesis in *Silene latifolia* (White campion)**
3:15-3:30 p.m.	Anja Geitmann	**A tight grip causes sperm release ..... in plants**

Session 2B Subcellular Organization
Session Chair: Lynn Richardson
Room N1002

Time	Speaker	Title
2:30-2:45 p.m.	Ashley Jaipargas	**Live Cell Imaging Reveals the Early Subcellular responses of *Arabidopsis thaliana* seedlings challenged by *Colletotrichum destructivum***
2:45-3:00 p.m.	Lynn G.L. Richardson	**Understanding the role of ESCRT in mitochondrial outer membrane re-modeling during *carnation Italian ringspot virus* infection**
3:00-3:15 p.m.	Firas Bou Daher	**The ARP2/3 mutants link actin organization and cell wall integrity to the maintenance of cell-cell connectivity.**
3:15-3:30 p.m.	Rongmin Zhao	**Cosuppression of the chloroplast localized molecular chaperone AtHSP90.5 impairs plant development and chloroplast biogenesis in *Arabidopsis***

Session 2C Plant Development Workshop Stress and Defense Responses
Session Chair: Terry Lung
Room N1044

Time	Speaker	Title
2:30-2:45 p.m.	Matthew Ierullo	**A New Suite of Pathogenesis-Related Gene Markers in *Arabidopsis thaliana***
2:45-3:00 p.m.	Alena Mammone	**Peroxules provide increased interactivity between peroxisomes and mitochondria during high subcellular ROS induced stress**
3:00-3:15 p.m.	Shailu Lakshminarayan	**Role of carotenoid cleavage dioxygenases in volatile emissions and insect resistance in Arabidopsis**
3:15-3:30 p.m.	Behnaz Najafi Majd Abadi Farahani	**Histone Marks Associated with Adaptation to Salt Stress in Arabidopsis**

Session 3A Enzymology
Session Chair: Bill Plaxton
Room N1001

Time	Speaker	Title
4:00-4:15 p.m.	Sarah Massey	**Investigation of protein phosphorylation and protein complex formation in a TILLING mutant of maize and its influence on starch synthesis**
4:15-4:30 p.m.	Thomas A. DeFalco	**Regulation of *Arabidopsis* cyclic nucleotide-gated ion channels by calmodulin**
4:30-4:45 p.m.	Micaela G. Chacon	**Amino Acids Important for Conferring Chain-Length Substrate Specificity of Two Arabidopsis Alcohol-Forming Fatty Acyl Reductases**
4:45-5:00 p.m.	Charlotte de Araujo	**Evidence for Multiple Carboxysomal Bicarbonate Dehydration Complexes based on the Form and Function of Carbonic Anhydrase**
5:00-5:15 p.m.	Qianru Zhao	**Starch biosynthesis in *Arabidopsis thaliana***
5:15-5:30 p.m.	Fushan Liu	**Phosphorylation of Starch Branching Enzymes and Protein-Protein interactions in Maize Amyloplasts**

Session 3B Molecular Biology and Genetics
Session Chair: Barry Micallef
Room N1002

Time	Speaker	Title
4:00-4:15 p.m.	Shaowei Dong	**Identification Of Carbohydrate-binding Ability Based On Proteome-wide Tertiary Structure Prediction: *in vitro* Validation**
4:15-4:30 p.m.	Ann Meyer	**Using polymorphic loci to investigate the evolution of gene expression regulation**
4:30-4:45 p.m.	David Maj	**Elucidation of promoter elements governing the jasmonate-responsiveness of the Calmodulin-Like Gene *CML39* in *Arabidopsis***
4:45-5:00 p.m.	Dhani Kalidasan	**Functional analysis of a conifer protein that interacts with the global transcriptional regulator, *Ab*scisic acid *I*nsensitive 3 (ABI3)**
5:00-5:15 p.m.	Barry J. Micallef	**Harnessing the Anabolic Properties Of Dark Respiration Using *Arabidopsis thaliana* With Partially-Suppressed Mitochondrial Pyruvate Dehydrogenase Kinase**
5:15-5:30 p.m.	Maye Chin Saechao	**Investigating a new link between polyamine metabolism and vascular development**

Session 3C Plant Development Workshop Flower Development and Self-Incompatibility
Session Chair: Emily Indriolo
Room N1044

Time	Speaker	Title
4:00-4:15 p.m.	Preetam Janakirama	**An insight into the function of the *HUA2* gene family**
4:15-4:30 p.m.	Madiha Khan	***BLADE-ON-PETIOLE1/2* promotes TALE homebox genes, *ARABIDOPSIS THALIANA HOMEOBOX GENE1* and *KNOTTED-like from ARABIDOPSIS THALIANA6* to co-ordinate flowering and inflorescence architecture in *Arabidopsis thaliana***
4:30-4:45 p.m.	Darya Safavian	**Investigations in the cytological responses of stigmatic papillae to compatible and self-incompatible pollinations in the Brassicaceae**
4:45-5:00 p.m.	Emily Indriolo	**The role of ARC1 in the self-incompatibility pathway in *Arabidopsis spp.***

List of Posters

Technologies for Plant Research

P1 Multispectral LED array for plant research

Michael Stasiak[1], Dave Hawley[1], Per Åge Lysså[2], Alan Scott[3] and Mike Dixon[1]
[1]*School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada*
[2]*Intravision Group AS, Snarøya, Norway*
[3]*COM DEV International, Ottawa, Ontario, Canada*

P2 Optical Sensors for the Ion-Selective Management of Hydroponic Nutrient Solution Quality

Cody G. Thompson[1], Mike Dixon[1], Tom Graham[1], Alan Scott[2], Serge Caron[3]
[1]*School of Environmental Science, University of Guelph, Guelph, ON*
[2] *COMDEV, Cambridge, ON*
[3] *INO, Quebec City, QC*

P3 RGB analysis detects phenological changes in Pinus strobus

Alyssa Molinaro*[1], Lisa Wingate[2] and Ingo Ensminger[1]
[1]*Department of Biology, University of Toronto at Mississauga, Canada*
[2]*School of Biological Sciences, University of Cambridge, Cambridge, UK*

Enzymology

P4 Post translational regulation of maize (*Zea mays* L.) starch synthase IIa by protein phosphorylation

Usha P. Rayirath[*1], Ian J. Tetlow[1] and Michael J. Emes[1]
[1] *Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada.*

Systems Biology

P5 Identification Of Carbohydrate-binding Ability Based On Proteome-wide Tertiary Structure Prediction: *in vitro* Validation

Shaowei Dong*[1], Andrew Doxey[2], William Willats[3], and Nicholas Provart[1]
[1]*Department of Cell and System Biology, University of Toronto, Toronto, Ontario, Canada*
[2]*Department of Developmental Biology, Stanford University, Stanford, California, USA*
[3]*Department of Plant Biology and Biotechnology, University of Copenhagen, Denmark*

P6 Global network analysis in *Populus* reveals variation in system-level responses to drought

Joseph Skaf*[†1], Erin T. Hamanishi*[†2] and Malcolm M. Campbell[1, 3]
[1]*Department of Cell & Systems Biology, University of Toronto, Toronto, Ontario, Canada*
[2]*Faculty of Forestry, University of Toronto, Toronto, Ontario, Canada*
[3]*Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto, Ontario, Canada*
[†]*Contributed equally*

P7 Whole plant NMR reveals metabolic adjustments in response to genetic perturbation of cell wall biosynthesis

Heather L. Wheeler[*1], Adolfo Botana[2], David J. McNally[2], Andre J. Simpson[2], and Malcolm M. Campbell[1,3]

[1]*Department of Cell & Systems Biology, University of Toronto, Toronto, Ontario, Canada*

[2]*Department of Chemistry, University of Toronto, Scarborough, Ontario, Canada*

[3]*Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto, Ontario, Canada*

Genetics

P8 Characterization and Target Validation of miR156 in *Medicago sativa*

Banyar Aung[1,2*], Lisa Amyot[2], Nusha Keyghobadi[1]and Abdelali Hannoufa[1,2]

[1]*Department of Biology, the University of Western Ontario, London, ON, N6A 5B7*

[2]*Agriculture and Agri-Food Canada, 1391 Sandford St., London, ON, N5V 4T3*

P9 Uncovering the molecular link between miR156 and carotenoid accumulation in Arabidopsis thaliana

Davood Emami Meybodi*[1,2], Anthony Percival-Smith[2] and Abdelali Hannoufa[1,2]

[1]*Agriculture Agri -Food Canada, London, Ontario, Canada*

[2]*Department of Biology, Western University, London, Ontario, Canada*

P10 IMPA2, a potential interactor of ABA2, is involved in Arabidopsis seed germination

Yi Zhang*, Eiji Nambara

Department of Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada

P11 Functional analyses of the ABA signaling genes at the cellular level.

Eric Nam*[1], Akira Endo[2], and Peter McCourt[1]

1 Department of Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada

2 Lowland Farming Research Division, National Agricultural Research Organization, Sapporo, Hokkaido, Japan

P12 Evidence for polygenic and regulatory evolution in rice

Megan House[1] and Lewis Lukens[1]

[1]*Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada*

P13 Regulation of FUSCA3 accumulation during seed Germination at high temperature in *Arabidopsis thaliana*.

*Rex S. Chiu and Sonia Gazzarrini

Department of Biological Sciences, University of Toronto, Toronto, ON, Canada, M1C 1A4 and Department of Cell and Systems Biology, University of Toronto, Toronto, ON Canada, M5S 3B2

P14 A Chemical Screen in *Arabidopsis* and *S. cerevisiae* Identifies Novel Compounds with Potential Strigolactone Activity

Duncan Holbrook-Smith*[1], Shigeo Toh[1], Yuichiro Tsuchiya[1], Peter McCourt[1]

[1]*Department of Cell and Systems Biology, University of Toronto, Ontario, Canada*

Crop Productivity

P15 H_2 oxidization increases soil ACC deaminase activity as part of rotation benefit

Jin Wang*[1], Chen Chen*[1], Zhongmin Dong[2]
[1] *Shaanxi Normal University, 199 South Chang'an Road, Xi'an, Shaanxi 710062, China*
[2]*Saint Mary's University, 923 Robie Street, Halifax, NS B3H 3C3, Canada*

P16 Profiling the root and seed surface lipids of the oilseed crop *Camelina sativa*

Fakhria M. Razeq*[1], Dylan K. Kosma[2], Owen Rowland[1], and Isabel Molina[3]
[1]*Department of Biology and Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada*
[2]*Department of Plant Biology, Michigan State University, East Lansing, Michigan, USA*
[3]*Department of Biology, Algoma University, Sault Ste. Marie, Ontario, Canada*

P17 The Effect of Long and Short-term CO_2 Enrichment on Gas Exchange, Water Use Efficiency, ^{14}C-Export and C-Partitioning in Iridoid Glycosides in *A. majus*

Ildiko Szucs, Mayhery Escobar, Demos E. Leonardos, and Bernard Grodzinski
Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada N1G 2W1

P18 Introduction of nitrogen fixing bacterium *Gluconacetobacter diazotrophicus* into corn

Nikita Eskin[1,2], Gary Tian[2*], Hugh Henry[1], Sylvia Yang[1,2], and Lining Tian[2]
[1] *University of Western Ontario, 1151 Richmond St. London, ON, Canada, N6A 3K7*
[2]*Agriculture and Agri-Food Canada, 1391 Sandford St. London, ON, Canada, S7N 0X2*

P19 CO_2 enrichment of *Arabidopsis thaliana* with partially-suppressed mitochondrial pyruvate dehydrogenase kinase enhances photosynthetic capacity of the inflorescence contributing to increased productivity.

Evangelos D. Leonardos[1], Sarathi M. Weraduwage[1], Shezad A. Rauf[1], Malgre C. Micallef[1], Elizabeth-France Marillia[2], David C. Taylor[2], Barry J. Micallef[1] and Bernard Grodzinski[1] [1]*Department of Plant Agriculture, University of Guelph, Guelph, Ontario, N1G 2W1, Canada;* [2]*National Research Council of Canada, Plant Biotechnology Institute, 110, Gymnasium Place, Saskatoon, Saskatchewan, S7N 0W9, Canada*

Environmental Stress

P20 Feeding Hungry Plants: the Secreted Purple Acid Phosphatase Isozymes AtPAP12 and AtPAP26 Play a Pivotal Role in Extracellular Phosphate-scavenging by *Arabidopsis thaliana*

Whitney Robinson[1], Hue Tran[1], Thomas McKnight[2] and William Plaxton[1]
[1]*Department of Biology, Queen's University, Kingston, Ontario, Canada*
[2]*Department of Biology, Texas A & M University, College Station, Texas, USA*

Coping with crowding: a role for ethylene production and response in *Oryza sativa* under density stress

P21 Misyura Max, Hudson Darryl, David Guevara, Joseph Colasanti, Steven Rothstein
The Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada

P22 Role of CMLs 38 and 39 in salt and freeze stress tolerance

Ubaid Midhat*[1], Kyle Bender[2] and Dr. Wayne Snedden[1]
[1]*Department of Biology, Queen's University, Kingston, Ontario, Canada*

P23 Growth Irradiance and Purine Ring Catabolism in *Thellungiella*
Varda M. Malik[1], Solmaz Irani[2], Christopher D. Todd[2] and Gordon R. Gray[1,3]
[1]*Department of Plant Sciences University of Saskatchewan, Saskatoon, Saskatchewan, Canada*
[2]*Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*
[3]*Depatment of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*

P24 Drought response and growth performance strategies in hybrid poplars
Katharina Bräutigam[1], Stefan Schreiber[2], Uwe Hacke[2], Barb Thomas[2] and Malcolm M. Campbell[1,3]
[1]*Centre for the Analysis of Genome Evolution & Function, Department of Cell & Systems Biology, University of Toronto, Toronto, ON, Canada*
[2]*Department of Renewable Resources, University of Alberta, Edmonton, AB*
[3]*Department of Biological Sciences, University of Toronto Scarborough, Toronto, ON, Canada*

P25 Cadmium-induced stress in *Arabidopsis thaliana* triggers up-regulation of phosphoenolpyruvate carboxylase (PEPC) isoenzymes and expression of PEPC specific kinase (PPCK)
Ian R. Willick*, Sheila M. Macfie
Department of Biology, Western University, London, ON

P26 Light Pollution Disrupts the Coordination between Circadian Rythms of Nitrate Update by Roots and Nitrate Assimilation in Shoots for Tomato (*Solanum lycopersicum* L.)
Maria Emilia Orozco-Gaeta, Malgre C. Micallef, James Robertson, Ling Tian, & Barry J. Micallef
Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

P27 Is *in vitro* generated *Eriophorum vaginatum* resistant to heavy metals?
Dylan Peddle[1], Sarah Bogart[2], Ewa Cholewa[3]
[1]*Department of Biology, Laurentian University, Sudbury, On*
[2]*Department of Biology, University of Lethbridge, Alberta*
[3]*Department of Biology, Nipissing University, North Bay, Ontario*

Climate Change Impacts

P28 Elevated growth temperatures alter hydraulic characteristics in trembling aspen: implications for tree drought tolerance
Danielle A. Way[1,2,#], Jean-Christophe Domec[2,3], Robert B. Jackson[1,2]
[1] *Department of Biology, Box 90338, Duke University, Durham, NC 27708, USA;*
[2] *Nicholas School of the Environment, Box 90328, Duke University, Durham, NC 27708, USA;*
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[#] *current address: Department of Biology, Western University, London, Ontario N6A 5B7, Canada*

P29 Climate change and permafrost degradation in a boreal forest: impacts on ground vegetation
Mélissa Fafard*, Jennifer Baltzer
Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, Canada

P30 Effect of simulated future climate on autumn freezing tolerance of *Pinus strobus*
Christine Chang*, Emmanuelle Fréchette, Tarek Bin Yameen and Ingo Ensminger
Department of Biology, University of Toronto at Mississauga, Mississauga, Ontario

Organelle Biology

P31 The C-terminus of *Bienertia sinuspersici* Toc159 receptor contains essential elements for its targeting and anchorage to the chloroplast outer membrane
Terry S.C. Lung[1], Matthew D. Smith[2], William Gwynne[1], Nathan Secord[1] and Simon D.X. Chuong[1]
[1]*Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada*
[2]*Department of Biology, Wilfrid Laurier University, Waterloo, Ontario N2L 3C5, Canada*

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James Campbell, Matthew Smith
Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, Canada

P33 TOC complex assembly: an examination of the interactions between TOC GTPases
Steven Siman*, Yi Chen, and Matthew D. Smith
Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, Canada

P34 A novel C-terminal dibasic motif mediates the targeting of a subset of tail-anchored proteins to the mitochondrial outer membrane
Naomi J. Marty[1], Yeen Ting Hwang[1], Howard J. Teresinski*[1], Eric A. Clendening[1], Satinder G. Gidda[1], Elwira Sliwinska[2], Daiyuan Zhang[3], Mark L. Johnstone[4], Jan A. Miernyk[4], Glauber C. Brito[5], David W. Andrews[5], John M. Dyer[3], and Robert T. Mullen[1]
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[3] *USDA-ARS, US Arid-Land Agricultural Research Center, Maricopa, Arizona, USA*
[4] *Department of Biochemistry, University of Missouri, Columbia, Missouri, USA*
[5] *Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada*

P35 A unique N-terminal sequence in the CIRV replicase protein p36 is responsible for recruiting Vps23 during ESCRT-mediated re-modeling of the mitochondrial outer membrane
Eric A. Clendening*, Lynn G.L. Richardson, and Robert T. Mullen
Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada

P36 Phosphatydylglycerol – Putative key component of chloroplast biogenesis at low temperature
Marianna Krol, Jessica Roche, Alexander G. Ivanov, Norman P.A. Huner
Department of Biology and the Biotron, University of Western Ontario, London, Ontario Canada N6A 5B7

Plant Defenses

P37 Mineral oil inhibits the movement of *Potato virus Y* in potato plants and reduces defense gene expression in tubers
Fadi Al-Daoud, M. A. Giguère, and Y. Pelletier
Potato Research Centre, Agriculture and Agri-Food Canada, Fredericton, New Brunswick, Canada

P38 PEP peptides and associated receptors in *Arabidopsis* defence response to spider mite feeding

Kristie Bruinsma*[1] and Vojislava Grbic[1]
[1]*Department of Biology, University of Western Ontario, London, Ontario, Canada*

Plant Development Workshop

P39 Functional characterization of flowering time orthologues in sugarcane

Carla Coelho*[1], Reynald Tremblay[1], Antonio Chalfun Junior[2] and Joseph Colasanti[1]
[1]*Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada*
[2]*Departmento de Biologia, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil*

P40 Functional analysis of a unique *ß-glucosidase* gene linked to the maize floral transition

Paul Kerrigan*[1], Viktoriya Coneva[1], Ali Livernois[1] and Joseph Colasanti[1]
[1]*Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada*

P41 Chemical screen uncovers link between one-carbon metabolism and sucrose signalling during Arabidopsis seedling development.

Michael E Stokes[1]*, Abhishek Chattopadhyay[1], Olivia Wilkins[2] & Malcolm M Campbell[1,3]
[1]*Department of Cell & Systems Biology,* [3]*Centre for the Analysis of Genome Evolution and Function, University of Toronto, 25 Willcocks St., Toronto, ON M5S 3B2, Canada*
[2]*Present Address: Center for Genomics and Systems Biology, New York University, 12 Waverly Place, New York, NY 10003, USA*

Invited Speaker Abstracts

Friday, November 30th, 2012
7 p.m. N1001

Unraveling the mystery of photosynthetic sea slugs: Giving new meaning to "going green

Dr. Mary Rumpho
Dept. of Molecular & Cell Biology, University of Connecticut, Storrs, CT 06269-3125, USA

The "solar-powered" sea slug *Elysia chlorotica* has fascinated scientists for years because of its ability to retain algal chloroplasts and carry out photosynthesis. These emerald green molluscs feed early in their life-cycle by sucking out the cellular contents of their algal prey (*Vaucheria litorea*). As a result of retaining the green chloroplasts in cells lining their digestive gut, they survive for months on only sunlight and air by carrying out photosynthesis as if they were a plant. This is perplexing because chloroplast activity requires the nuclear genome to make most of the chloroplast proteins and there are no algal nuclei in the sea slug. In addition, it remains unknown how the animal obtains essential nutrients such as nitrogen and vitamins. We are taking a systems level approach using a combination of several 'omics and visualization approaches to understand how this unique symbiosis becomes established, as well as functionally maintained. Currently, we are focusing on the role of lipid accumulation, as well as the roles of other microbial symbionts in contributing metabolically to the success of the symbiotic association and/or the production of novel secondary compounds. Understanding this unusual organism gives a whole new meaning to "going green"!

Saturday, December 1st, 2012
8:40 a.m. N1011

Genomic methods elucidate the origin of plastids and endosymbiotic gene transfer in photosynthetic eukaryotes

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Much of our understanding of the natural world has come from experiments done on model species manipulated under controlled laboratory conditions. The advent of modern high-throughput genomics and bioinformatics allows researchers to explore genetic diversity in natural systems, opening the way for exploring organism biology *in situ*. Here I will discuss work from our lab on the genomes of key algal groups that help us understand plastid and eukaryote evolution. One focus wil be on the recently sequenced genome of the glaucophyte *Cyanophora paradoxa* that allowed us to unite the Plantae (also known as Archaeplastida) as a monophyletic group [1]. I will also discuss how we have developed single-cell genomic methods to interrogate DNA associated with individual cells captured in nature [2]. We target unicellular eukaryotes in their natural environment and generate draft genome assemblies to discover novel biodiversity, to reconstruct their biotic interactions, and to generate *de novo* assemblies of associated symbiont and pathogen genomes [3].

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[2] Yoon, H.S., D.C. Price, R. Stepanauskas, V.D. Rajah, M.E. Sieracki, W.H. Wilson, E.C. Yang, S. Duffy, and D. Bhattacharya. 2011. Single cell genomics reveals trophic interactions and evolutionary history of uncultured protists. *Science* 332: 714-717.

[3] Bhattacharya, D., D.C. Price, H.S. Yoon, E.C. Yang, N.J. Poulton, R.A. Andersen, and S.P. Das. 2012. Single cell genome analysis supports a link between phagotrophy and primary plastid endosymbiosis. *Nature Sci. Rep*. 2: 356.

Saturday, December 1st, 2012
1:35-2:25 p.m. N1001

Going Solo: the art of single-cell C4 photosynthesis in plants

Simon Chuong
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Bienertia cycloptera, B. sinuspersici, B. kavirense and *Suaeda aralocaspica*, are four species of family Chenopodiaceae known to perform single-cell C_4 photosynthesis. These discoveries challenge the central dogma proposing that Kranz anatomy is a required feature for C_4 photosynthesis in terrestrial plants. These species are able to achieve this novel solution of C_4 photosynthesis through partitioning of organelles including dimorphic chloroplasts, mitochondria and peroxisomes and key photosynthetic enzymes in distinct cytoplasmic compartments within single chlorenchyma cell. In this talk, I will present our recent research aims at elucidating the cellular and molecular mechanisms responsible for the establishment and maintenance of this unique intracellular compartment in these single-cell C_4 plants. We have recently demonstrated that the differentiation of dimorphic chloroplasts in the single-cell C_4 species appears to involve a distinct protein import pathway and this may account for their differential protein accumulation. In addition, the interaction of chloroplasts with actin filaments and microtubules has also shed light on the importance of these cytoskeletal components in the intracellular organization of organelles in plants.

Concurrent Oral Session Abstracts

Concurrent session 1A
Environmental Stress

Elevated temperature eliminates the decline in photosynthetic capacity during a simulated autumn in Norway spruce (*Picea abies*)

Joseph Stinziano*[1], Leonid Kurepin[1,2], Danielle Way[1], Vaughn Hurry[2], Gunnar Öquist[2], and Norman Hüner[1]
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[2]*Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, Umeå, Sweden*

Global surface temperature at high latitudes is projected to increase more severely in the next century than lower latitude regions on Earth, which could have negative consequences on the boreal forest system. The boreal zone is one of the largest forested areas on Earth; any large changes to this region would have a notable effect on global carbon flux. Warming is likely to lead to changes in growing season length via changes in the winter dormancy of boreal species. Winter dormancy induction during fall is important for the overwintering survival of boreal tree species. We investigated the effect of elevated temperatures on Norway spruce *Picea abies* photosynthetic capacity during a simulated autumn with photoperiod and ten year average air temperature conditions for London, Canada, from August to November. We hypothesized that elevated temperature would slow or delay the fall decline in photosynthetic rate compared to ambient temperatures. Using a Licor-6400 to measure photosynthetic capacity, we found that photosynthetic capacity (maximum rates of Rubisco carboxylation (V_{cmax}) and electron transport (J_{max})) declined in trees exposed to ambient temperatures during the simulated autumn, and unexpectedly, photosynthetic capacity did not change for the elevated temperature-exposed trees. Our results suggest that elevated temperatures may lead to an extended growing season for Norway spruce by reducing or eliminating winter dormancy induction. Further research is needed to determine the relative importance of temperature and light in regulating winter dormancy induction, and the impact of changes to winter dormancy on overwintering physiology of boreal species.

Genetic and epigenetic impacts on the poplar drought response

Katharina Bräutigam*[1], Sherosha Raj[1], Olivia Wilkins[2], Yunchen Gong[1], Pauline Wang[1], and Malcolm M. Campbell[1,3]
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[2]*Center for Genomics and Systems Biology, New York University, New York, NY, USA*
[3]*Department of Biological Sciences, University of Toronto Scarborough, Toronto, ON, Canada*

Previous exposure to environmental conditions shapes future responses of an organism. This is likely to be particularly important for trees, which must contend with fluctuating, frequently challenging, environmental conditions throughout their long lifetimes. Here, the persistent influence of past experiences on the capacity of trees to respond to a current stress, drought, was investigated. Vegetatively-propagated trees of the genus *Populus* from different geographic locations were studied, i.e. genetically identical individuals with different clone histories were used. Analyses of transcriptome responses to drought under common garden conditions uncovered differences in transcript abundance patterns based on differences in geographic origin of poplar clones with identical genotypes. While classic fingerprinting confirmed genetic identity of the trees obtained from different locations, whole genome sequencing is currently under way to detect possible somatic mutations that might have arisen independently in different geographic locations. Differences in total DNA methylation based on differences in clone history were found to parallel differences in drought transcriptomes. A more detailed analysis by fingerprinting detected some loci with variable methylation patterns based on geographic location within the same genotype, while genotype-specific patterns were also detected. These data provide insight into the interplay between genotype and the local environment, and hint that epigenetic mechanisms may add an additional layer of plasticity. Moreover, the data add

to our understanding of long-standing applied questions related to the nursery source of poplar clones and how that impacts on future clone performance in plantations.

The effect of low phosphate conditions on the growth of the extremophile crucifer, *Thellungiella salsuginea*

Vera Marjorie Velasco*, John Mansbridge, Peter Summers, Elizabeth Weretilnyk
Department of Biology, McMaster University, Hamilton, ON

We study an accession of *Thellungiella* that thrives on saline, alkaline and low Pi soils of the Yukon Territory. At the 6 to 8-leaf stage, *Thellungiella* seedlings on media containing 0.5 mM Pi showed a 1.7-fold greater shoot and root biomass relative to seedlings lacking added Pi with no change in the shoot:root ratio. Under identical conditions, Arabidopsis seedlings showed 2- and 3.5-fold reductions in shoot and root biomass, respectively, yielding a higher shoot:root ratio with Pi deficient media. Plants were grown four weeks on low nutrient potting media treated once with a solution lacking Pi (0 mM Pi) or containing Pi (up to 2.5 mM). With 0 Pi, Arabidopsis rosettes were smaller with senescent older leaves while *Thellungiella* plants showed no change in shoot biomass and no senescence relative to plants treated with 2.5 mM Pi. However, leaf free Pi content was 2-fold lower in *Thellungiella* plants receiving the 0 mM Pi treatment relative to 2.5 mM Pi and qPCR analyses of *Thellungiella* orthologues of Induced by Phosphate Starvation *At4*/*IPS2*, high affinity phosphate transporter *PHT1;4* and purple acid phosphatases *PAP17* showed up-regulated expression in plants treated with 0 mM Pi. While these measurements were consistent with a Pi deficiency, some genes (*PHR1*, *WRKY75* and *RNS1*) were not Pi-responsive in *Thellungiella* compared to *Arabidopsis* plants. Rather, these genes were constitutively expressed at a higher level under all Pi conditions. Thus Pi homeostasis is likely regulated differently in *Thellungiella* compared to *Arabidopsis* yielding a plant more tolerant to low Pi conditions.

Comparative physiology and transcriptome sequencing to identify genes implicated in the drought tolerance of *Thellungiella salsuginea*

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We use *Thellungiella*, an extremophile crucifer, to study plant responses to water deficit. Two accessions, one from the subarctic, semi-arid, salt flats of the Canadian Yukon and the second native to saline soils of a temperate region of Shandong Province in China, have differing responses to water stress. We subjected plants from each accession to simulated drought by withholding water. The rate of rosette water loss, relative water content, water/solute potential and time for wilting to occur were measured. When wilted Yukon plants re-establish turgor with re-watering, they do so at a lower solute potential than pre-drought levels and recovered plants take longer to wilt upon a second exposure to drought. In contrast, Shandong plants show no comparable change in solute potential or wilting behaviour with drought exposure. Due to these differences in physiology we selected the Yukon accession for gene expression profiling in response to drought. Transcriptome sequencing of leaf cDNA libraries from Yukon plants was used to identify differentially expressed genes (DEGs) using the program DESeq. We identified 1065 DEGs by comparing profiles of well-watered and drought-stressed Yukon plants grown in controlled environment cabinets. Comparisons between transcriptomes of plants collected from field sites in the Yukon during years of drought (2003) and abundant precipitation (2005) identified 1661 DEGs. Only 111 DEGs were drought-responsive under both cabinet and field conditions. This overlapping category of DEGs is of particular interest as it likely includes products essential for tolerance to water deficits independent of where stress is experienced.

Isoprenoid-related mechanisms of drought tolerance in field-grown Douglas-fir provenances

Laura Verena Junker*[1,2], Ingo Ensminger[1,2]
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Douglas-fir is common to an extensive latitudinal range of North America, and its two subspecies, Interior Douglas-fir (*Pseudotsuga menziesii var. glauca*) and Coastal Douglas-fir (*var. menziesii*) adapted to dry and humid habitats, respectively. Within subspecies, provenances can be defined which vary in their drought tolerance due to local adaptation. The aim of this project is to determine isoprenoid-related mechanisms of drought tolerance in Douglas-fir provenances originating from different habitats. Under drought conditions, plants are particularly susceptible to photo-oxidative damage, as excess energy impairs the photosystems and produces reactive oxygen species (ROS). Essential isoprenoids comprise accessory pigments and antioxidants, which mediate different photo-protective processes including dissipation of excess energy via heat and ROS scavenging. We hypothesize that Douglas-fir-provenances adapted to drought stress show enhanced biosynthesis and abundance of these photo-protective isoprenoids. We studied carbon fixation and photo-protective capacity of fifty-year-old trees in four Douglas-fir provenances adapted to contrasting (dry or humid) environments at two field sites in southern Germany. Measurements of photosynthetic capacity conducted in well-watered trees during the spring season and drought-stressed trees during summer reveal different physiological drought responses among Douglas-fir provenances. Under drought stress, the Interior provenance Salmon Arm can maintain higher assimilation rates, whereas Coastal provenances from humid habitats show lower photosynthetic rates and consequently enhanced NPQ. Results were confirmed by analyses of isoprenoid content in sampled needles which reveal an enhanced xanthophyll cycle pigment pool size under cold spring and dry summer conditions. Our results suggest that Coastal provenances seem to be more susceptible to prolonged drought stress.

Correlation network analysis identifies major gene regulatory pathways involved in the temperature and photoperiod response of *Pinus banksiana* needles during cold hardening

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As the climate warms over the coming century, an increased length of the growing season for boreal forest trees is predicted, potentially altering the carbon sink of conifer forests. To understand the role of increased temperature on the autumn cold hardening, we isolated the effects of temperature and photoperiod on Jack pine seedlings (*Pinus banksiana*) in factorial experiments. Using the ARBOREA 9k spruce microarray we studied the gene expression in pine needles. Improved annotation, pathway mapping, functional classification, gene ontology enrichment analysis and weighted gene correlation network analysis identified major gene regulatory pathways involved in the response of *Pinus banksiana* to autumn photoperiod and low temperature, which control the cold hardening process in this conifer species. Our analysis revealed 989 differentially expressed genes and most of the genes are exclusively up- or down regulated under naturally cool autumn conditions. Compared to summer conditions, seedlings acclimated to natural autumn conditions showed a major metabolic switch with significant changes in the expression of genes involved in the GO categories for metabolism of carbohydrates, lipids, amino acids, nitrogen and thiamine. Cold hardening also induced significant changes in the GO categories of photosynthesis, plant development, defence, transport and hormone signaling. In addition, we detected co-expression of several transcription factors and GO categories for modification of cellular membranes. The observed changes in the needle transcriptome data were

confirmed by functional changes in photosynthetic capacity. Together, control of the cold hardening process by photoperiod in pine appears to negate any potential for an increased carbon gain associated with higher temperatures during the autumn season.

Pathways for iron acquisition by iron-limited cyanobacterial cells

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Cyanobacterial cells are generally considered to acquire extracellular iron through a siderophore-dependent system, although evidence has started to accumulate that other, as yet poorly-characterized, iron acquisition systems may also play a role. Iron-limited cells of the cyanobacterium *Anabaena flos-aquae* are well-known to produce the relatively low Fe(III) affinity dihydroxamate siderophore schizokinen. In this set of experiments we show that iron-limited *A. flos-aquae* cells: 1) acquired iron at substantial rates in the absence of the schizokinen, and 2) acquired iron from a bacterial siderophore (the trihydroxamate molecule desferrioxamine B [DFB]), and also a synthetic chelator (DTPA), with substantially higher affinities for Fe(III) than schizokinen, indicating that a schizokinen-independent iron acquisition pathway was operating. We suggest that there exists a siderophore-independent iron acquisition system that is able to acquire Fe(III) from high stability Fe(III)-chelates that are not accessible to iron-limited cells via the schizokinen-based system. As well, we present two possible models for iron acquisition by iron-limited *A. flos-aquae* cells. Both of these models suggest that there are two major routes for Fe(III) entry into the periplasm of iron-limited cells: 1) the well-characterized siderophore (schizokinen)-dependent process and 2) a siderophore-independent process that is able to access Fe(III) sources not biologically available to the schizokinen system.

Effects of early-season soil temperatures on wetland graminoid root length growth.

Peter Ryser* and Nicole Gagnon
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Fine roots of many Northern Ontario wetland graminoids survive the winter, but roots of some graminoids completely senesce at the end of the growing season. As soil temperatures in these wetlands favour growth only for a relatively short time, we hypothesized that species with annual root systems are able to grow better under cool substrate temperatures than species with perennial roots. Three experiments were conducted: two in a growth chamber and one outdoors. Four species found co-occurring in a fen were studied: *Rhynchospora alba* and *Xyris montana* with annual roots, and *Carex lasiocarpa* and *Carex magellanica* with perennial roots. Additionally, *Sparganium androcladum*, a marsh species with annual roots, was included in the two growth chamber experiments. The experiments were conducted over 32 to 56 days, with substrate temperatures ranging from 8.3 to 17.5°C. Combined data of all three experiments indicate exponential increase in root length as a function of growing-degrees days (base = 7°C), except for the coldest treatment, in which most species suffered a disproportionate decline in root growth. No clear association between root turnover type and temperature response was found. Low substrate temperatures had a large effect on the two small species from open environments and with annual roots, *R. alba* and *X. montana*, under the relatively low light conditions of the growth chamber, but not in the full sunlight of the outdoor experiment. On the other hand, *S. androcladum*, with annual roots, showed a similar temperature response in the growth chamber as species with perennial roots.

Concurrent Session 1B
Plant –microbe interactions

Plant Growth-Promoting Rhizobacteria Appear to Become Deleterious in Growth-Limiting Conditions
Melanie P Columbus* and Sheila M Macfie
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Plant growth-promoting rhizobacteria (PGPR) are commonly used to promote plant growth under stress conditions. The PGPR *Pseudomonas putida* has received attention for its ability to reduce plant stress by simultaneously 1) metabolizing the plant stress hormone ethylene through the enzyme 1-aminocyclopropane-1-carboxylate deaminase (AcdS) and 2) producing the plant growth hormone indole 3-acetic acid (IAA). This study used *P. putida* UW4/AcdS$^+$ and the mutant strain *P. putida* UW4/AcdS$^-$ to determine the extent to which each bacterial strain, and therefore growth-promoting pathway, affected *Arabidopsis thaliana* under cadmium stress. In the system used, neither bacterial strain affected plant health but both strains significantly reduced plant growth for every variable measured. For example, shoot biomass was reduced by up to 50% depending on treatment and total root length was reduced by 30% at 7.5 □M $CdCl_2$ or higher. Further investigation revealed that the bacteria could not grow directly on the plant media, indicating that the conditions were stressful to the bacteria. However, in the presence of plants both strains survived in the media. Confocal microscopy combined with BacLight Live/Dead staining confirmed that bacterial strains were alive on the plant root in all test conditions and light microscopy ruled out mechanical damage of the plant root as an explanation of reduced growth. We propose that the use of PGPR to improve agricultural plant growth will be successful only if the field conditions are conducive to bacterial growth. Further work is needed to determine the mechanism responsible for reduced plant growth in the system used.

Assessing rhizobial symbiosis efficiency in low-nodulating pleiotropic mutants of *Pisum sativum*
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Legumes form a symbiosis with rhizobia, which results in the formation of nodules. My lab has been studying several *Pisum sativum* mutants including E107 (*brz*), E132 (*sym21*), E151 (*sym15*), and R50 (*sym16*) that are defective in nodulation. Our research focus has been on nodule organogenesis and we are now beginning to examine the physiology behind the mutant defects. Determining if these mutants differ in their mutualistic relationship from their wild-type counterpart Sparkle will give insight into the basis of their symbiotic dysfunction. Peas from each line were inoculated with *Rhizobium leguminosarum* and harvested 14, 21, 35, 28 and 42 days after inoculation. The host and total nodule dry weights (DW) were measured. The efficiency of symbiosis was determined by calculating three known parameters, specific nodulation, specific nodule DW, and nodule construction cost. From these, we concluded that at all time points, Sparkle had a more efficient symbiosis than the mutants. In search of a different approach to measure nodule efficiency, a regression analysis was done to compare the relationship between host DW and nodule DW. We found that though mean DWs differed among plant lines, this relationship was similar for mutants and wild-type. This suggests first that nodule efficiency remains uncompromised in these mutants and second that the defect in symbiosis is related to nodule formation rather than to a deficiency in host-symbiont nutrient exchange. These two approaches give different perspectives on mutualism and therefore multiple strategies should be used to obtain a better understanding of the mutant defects.

***SUNERGOS1*, a *Lotus japonicus* gene required for proper accommodation of rhizobial infection.**

Hwi Joong Yoon*[1,2], Md Shakhawat Hossain[1], Loretta Ross[1], Mark Held[1,2], Marilyn Kehl1[1,2], Shusei Sato[3], Satoshi Tabata[3], and Krzysztof Szczyglowski [1,2].

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Nitrogen is an essential nutrient for plant growth. Unfortunately, the vast majority of nitrogen exists in the atmosphere in a very stable di-elemental state, which is unusable by most organisms. Legumes, however, are able to efficiently use atmospheric nitrogen to support their growth and development as a result of successful symbiosis with beneficial nitrogen-fixing bacteria, commonly known as rhizobia. This mutualistic interaction is known as nitrogen-fixing root nodule symbiosis (NFS). By conducting a screen for genetic suppressors of the *L. japonicus har1-1* hypernodulation phenotype, a novel symbiosis-relevant locus, called *SUNERGOS 1 (SUNER1)*, was identified. The *suner1* mutant was found to have a rather subtle symbiotic phenotype. It was able to support epidermal infection and initiate the cortical program for nodule primordia organogenesis as in wild-type. However, the infection process was temporarily stalled in the root cortex, such that infection threads failed to ramify within the root cortex and timely release of bacteria inside the nodule primordia cells did not occur. This apparent symbiotic defect was found to be ephemeral and with additional time, functional nodules were formed by the *suner1* mutant. Using a combined approach of map-based cloning and next-generation sequencing, a candidate gene for the *SUNER1* locus was identified. It is predicted to encode a topoisomerase VI subunit A. The data obtained suggest that the SUNER1 topoisomerase VI mediates endoreduplication of nodule primordia cells and that this is essential for normal differentiation of functional, nitrogen-fixing nodules.

Role of cytokinin receptors during symbiotic root nodule organogenesis in *Lotus japonicus*

Mandana Miri [*1,2], Hongwei Hou[1], Mark Meld[1,2], Loretta Ross[1], Jillian Perry[3], Trevor Wang[3] and Krzysztof Szczyglowski[1,2]

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Cytokinins are essential plant hormones that control many aspects of plant development. They participate in responses to endogenous cues and play an important role as messengers for external stimuli related to diverse biotic and abiotic conditions. During beneficial root symbiosis between legumes and nitrogen-fixing rhizobia, cytokinin signaling constitutes the key endogenous signal for the nodule structure organogenesis. In *Lotus japonicus*, activation of the LHK1 histidine kinase cytokinin receptor is required and also sufficient for this process to occur. However, the fact that some residual nodulation still occurs in *lhk1-1* loss-of-function mutants indicates the presence of an LHK1-independent signaling pathway. In this study, we have tested a hypothesis that additional *L. japonicus* cytokinin receptors, such as LHK1A, LHK2 and LHK3, might function in a partially redundant manner to mediate nodule formation. A combined approach involving reverse genetics and expression analyses was used for this purpose. Based on the obtained data, a working model is presented in which LHK1 has a primary function in the root epidermis but acts redundantly with LHK1A and LHK3 in the root cortex to mediate differentiation of nodules in *L. japonicus*. Together with results of double mutant analyses, our data suggest that superimposition of epidermal signalling over cortical responses, as mediated by cytokinin receptors, has constituted an important step in the evolution of nitrogen-fixing root nodule symbioses.

The Arabidopsis NPR1 Protein Is a Receptor for the Plant Defense Hormone Salicylic Acid

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Salicylic acid (SA) is an essential phytohormone required during signal transduction leading to gene activation and deployment of a broad-spectrum and long-lasting immunity termed Systemic Acquired Resistance. Despite SA's central role in plant disease resistance, its receptor has remained elusive for decades. The transcriptional coregulator NPR1 is central to the activation of SA-dependent defense genes, and we previously found that Cys521 and Cys529 of *Arabidopsis* NPR1's transactivation domain are critical for coactivator function. Here, we demonstrate that NPR1 directly binds SA, but not inactive structural analogs, with an affinity similar to that of other hormone-receptor interactions and consistent with *in vivo Arabidopsis* SA concentrations. Binding of SA occurs through Cys521/529 via the transition metal copper. Mechanistically, our results suggest that binding of SA causes a conformational change in NPR1 that is accompanied by the release of the C-terminal transactivation domain from the N-terminal autoinhibitory BTB/POZ domain. While NPR1 is already known as a link between the SA signaling molecule and defense-gene activation, we now show that NPR1 is the receptor for SA.

Characterizing the role of two TTM family members, *AtCYDP1* and *AtCYDP2*, in programmed cell death, linking immunity and senescence.

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The members of the triphosphate tunnel metalloenzyme (TTM) superfamily are a group of enzymes that can hydrolyze a variety of polyphosphate substrates. They perform divergent functions in different organisms, such as RNA capping, thiamine metabolism, and cAMP formation. TTM members exist in all domains of life; however, no information is currently available about the function of any members in plants. In *Arabidopsis*, 3 genes are annotated as TTM family members (*AtCYDP1*, *2*, and *3*). In order to understand the biological function of these genes, knockout (KO) mutants for *CYDP1* and *CYDP2* have been characterized. Pathogen infections using *AtCYDP2* knockouts (KOs) showed an enhanced resistance phenotype to *Hyaloperonospora arabidopsidis* and *Pseudomonas syringae* strains that are recognized by TIR class R proteins. Recognition by these proteins leads to restricted pathogen growth as well as hypersensitive cell death, a form of programmed cell death at the site of infection. Epistatic analysis using double mutants of the *AtCYDP2* KO and known defense mutants (*pad4*, *sid2*, and *npr1*) has been conducted. Current data show that *AtCYDP2* acts upstream of these defense genes and salicylic acid is required for the enhanced resistance in *AtCYDP2* KOs. Interestingly, *AtCYDP1*, which has 66% amino acid sequence identity with *AtCYDP2*, does not appear to be involved in pathogen defense, but rather, seems to play a role in senescence. Though they play distinct roles, both CYDP1 and CYDP2 possess phosphatase activity. This suggests an intriguing possibility that both may contribute to programmed cell death in different aspects (*i.e.* immunity and senescence).

Suppressor mutant S171 identifies a link between pathogen defense and floral transition mediated by Cyclic Nucleotide-Gated Channel 2

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While the involvement of cyclic nucleotide-gated channels (CNGCs) in signal transduction in animals is well documented, the role of CNGCs in mediating physiological responses in plants is still at its infancy. Through forward genetic approaches, multiple CNGCs have been identified to be involved in plant immunity. One of the earliest responses upon pathogen recognition is reported to be Ca^{2+} flux changes within the plant cell. The *Arabidopsis* genome sequencing project identified over 150 cation transport proteins of which CNGCs have

emerged to be strong candidates for generating a Ca^{2+} flux after pathogen recognition. Here, we aimed to identify novel downstream components of *AtCNGC*-mediated pathogen defense signaling using *AtCNGC2*, in which the loss of this gene induces multiple defense responses such as constitutive expression of *Pathogenesis Related* (*PR*) genes, accumulation of salicylic acid (SA), and broad spectrum pathogen resistance in the *Arabidopsis* conditional lesion mimic mutant, defense, no death1 (*dnd1*). A genetic screen for mutants that suppress *dnd1*-conferred phenotypes identified suppressor S171 based on intermediate wildtype-like morphology. Positional cloning strategies have mapped S171 to the upper arm of chromosome 5. In addition, S171 partially suppresses *PR* gene expression, SA accumulation, and *dnd1*-mediated enhanced resistance to both bacterial and oomycete pathogens. Interestingly, S171 also suppresses the late flowering phenotype observed in *dnd1* plants. Taken together, our data presented here identifies a novel downstream signaling component of *AtCNGC2* that acts to regulate both disease resistance and floral transition in *Arabidopsis thaliana*.

Investigating Disease Resistance and Priming in *Thellungiella salsuginea*

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Thellungiella salsuginea is an extremophile displaying tolerance to numerous abiotic stresses and has become a valuable model plant for abiotic stress studies. The Yukon ecotype is native to northern Canada and grows on alkaline salt flats and can survive in freezing conditions, while the Shandong ecotype is found in temperate eastern China where it thrives on highly salinized soil. *Thellungiella's* tolerance to abiotic stress led us to investigate its capacity to resist biotic stress such as pathogen infection. Plants have numerous defense pathways including Systemic Acquired Resistance (SAR) in which an initial infection primes distant tissues to respond to a subsequent infection in a resistant manner. *Arabidopsis* and *Thellungiella* are close relatives, therefore we used the *Arabidopsis* bacterial pathogen *Pseudomonas syringae* to investigate *Thellungiella* disease resistance. Compared to Arabidopsis (Col-0), *Pseudomonas* growth was suppressed and few disease symptoms were observed in Yukon and Shandong, suggesting that both ecotypes are resistant to *Pseudomonas*. RT-PCR and next generation sequencing studies suggested that Shandong plants are primed for resistance as healthy, uninfected plants expressed modest levels of *PR1*. After inoculation with *Pseudomonas*, Shandong plants rapidly and abundantly accumulated *PR1* transcripts and were highly resistant to *Pseudomonas.* These observations suggest that Shandong plants are primed for SAR allowing them to respond to subsequent infections in a resistant manner. Understanding how healthy Shandong plants maintain a primed state will reveal fundamental information that can be used to improve disease resistance in crops.

Concurrent Session 1C – PDW
Development and Regulation of Organs and Organelles

The translation elongation factor subunit eEF1Bβ1 affects cell wall formation and vascular development in Arabidopsis.

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Cell wall digestibility is a major obstacle in the production of cellulosic biofuel. Our goal is to identify candidate genes that could be targeted for improving saccharification in bioenergy crops. Results from our microarray study, which compared transcript levels in high vs. low lignin stem segments of *Brassica napus*, revealed elongation factor eEF1Bβ1 as a potential regulator of lignin biosynthesis. Through *eEF1Bβ1*-promoter-*GUS* and *35S*-promoter-*eEF1Bβ1-YFP* experiments we demonstrated that *eEF1Bβ1* is ubiquitously expressed in the Arabidopsis seedling, and that its protein localizes in the plasma membrane and cytoplasm. Analysis of inflorescence stem sections from the *GUS*-expressing plants revealed that *eEF1Bβ1* gene activity is mainly confined to the xylem vessels and interfasicular cambium. To learn more about the role of this gene in vascular development we examined an Arabidopsis *eEF1Bβ1* loss-of-function mutant, *efβ*. In addition to a respective 38% and 20% reduction in total lignin and crystalline cellulose, the *efβ* mutant has a lower S/G lignin monomer ratio relative to wild type plants. Furthermore, histochemical staining showed that the *efβ* mutant has a reduced vascular apparatus, including smaller xylem vessels in the inflorescence stem. We also demonstrated that over-expression of *eEF1Bβ1* in the *ectopic lignification 1* (*eli1)* mutant restored a wild type phenotype and abolished ectopic lignin deposition as well as cell expansion defects. Taken together, these data strongly suggest a role for eEF1Bβ1 in cell wall formation and vascular development in Arabidopsis.

Abaxial greening phenotype in hybrid aspen (*Populus tremula* x *tremuloides*)

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The genus *Populus* contains two leaf types: bifacial and isobilateral. The former is fairly representative of the angiosperm leaf, including *Arabidopsis*, and contains palisade mesophyll cells in association with the top or adaxial surface while the bottom or abaxial surface consists of spongy mesophyll cells. Isobilateral leaves, on the other hand, contain palisade mesophyll on the top and bottom surfaces of the leaf, making the two sides virtually indistinguishable at the macroscopic level. This type of leaf in poplars has been termed to exhibit the "abaxial greening" phenotype. The internal tissue distribution in isobilateral leaves very much resembles that of the previously characterized *ASYMMETRIC LEAVES2* (*AS2*) mutant in *Arabidopsis* with altered dorsiventral or adaxial-abaxial leaf polarity. This gene, as well as other genes involved in setting the dorsiventral axis of leaves were investigated in two poplar species (*Populus trichocarpa* and *Populus tremula* x *tremuloides*), representative of each leaf type (bifacial and isobilateral, respectively). Expression patterns in leaf blade tissue show that poplar orthologs of *AS2, ATS* (*ABERRANT TESTA SHAPE*), and *YAB3* (*YABBY3*) were significantly differentially expressed in *P. tremula* x *tremuloides* suggesting their possible roles in underlying the isobilateral leaf phenotype.

Finding needles in haystacks: Combining phylogenetic reconstruction, expression analysis and meta-analysis to isolate potential ancestral functions of the *IDD* gene family.

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The progression through different phases of a plant's life cycle requires the coordination of a variety of complex, interdependent external and internal factors. Flowering, for example, requires integration of information from external factors, such as temperature and light, and internal factors, such as carbohydrate status and size, to induce the floral transition under optimal conditions. The *INDETERMINATE DOMAIN* (*IDD*) family of zinc finger proteins are transcription factors found in land plants that are involved in regulating a variety of development processes. We have found a link between sequence and expression patterns within the IDD gene family from taxonomically divergent species and propose an ancestral role in carbohydrate metabolism. A phylogenetic reconstruction of the *IDD* family in land plants, from the moss *Physcomitrella patens* through monocots such as *Brachypodium distachyon* and dicots such as *Glycine max*, allows for the assignment of 7 subfamilies based on newly identified conserved motifs. We have also found that several of the subfamilies share similar patterns of gene expression in both monocots and dicots, with 3 subfamilies having highest expression within seeds. A meta-analysis of data for published IDD members in monocots and dicots reveals a potential role for the entire family in the regulation of carbon balance in a variety of important developmental processes, such as flowering, root growth, gravitropism and seed development. These results provide a framework for the classification of newly identified *IDD* genes into subfamilies and should allow for a forward genetics approach to understand the roles of this important gene family.

Differential colouring using a photo-convertible fluorescent protein suggests that stromules do not fuse to form a plastid network

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Stromules are stroma-filled dynamic tubular extensions of plastids. It has long been speculated that stromules might also function to interconnect plastids and form a network through with proteins and genetic material may be exchanged. Photo-bleaching experiments that showed green fluorescent protein diffusing between interconnected plastids provided support for this idea. Interestingly, the actual fusion of two stromules has never been directly observed since the plastids utilized in the photo-bleaching experiments were already connected. Nevertheless stromule fusion is a pre-requisite for this proposed function and raises the question of possible mechanisms by which this can occur. We have developed a photo-convertible Eos fluorescent protein to differentially colour individual plastids. Using this tool we show that there is a distinction between elongated plastids, which may appear to possess more than one plastid body and be interconnected by a thin tube, and the stromules extended from independent plastids. Imaging over extended time periods shows that although stromules from two different plastids may appear to touch or overlap, they remained distinct, neither fusing nor exchanging fluorescent protein. The observations remained true under conditions that increase stromule frequency and in mutants that display numerous extremely elongated stromules. We thus propose that a distinction be made between stromules extended by independent plastids and the isthmus that can be seen as tubular regions of an elongated or dividing plastid. While negating the idea of stromules as plastid inter-connectors we present additional observations that support their role in increasing the interactive surface area between a plastid and its surrounding milieu.

Integration of nutrient and hormone signalling drives root meristem development in Arabidopsis.

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Nutrient sensing at the root tip helps drive the meristematic activity that ultimately shapes root growth. Given the sensitivity of the root meristem to changes in nutrient abundance, as well as the established role that hormone signalling plays in meristem development, the root meristem offers a unique platform through which the interaction between nutrient- and hormone-signalling pathways can be explored. Recently, a chemical screen uncovered the role of sulfamethoxazole, a folate biosynthesis inhibitor, to perturb seedling responses to sucrose. Here, sulfamethoxazole and sucrose are used to probe the effect of nutrient abundance on hormone signalling at the root meristem. When dark-grown seedlings are raised in the presence of the sulfamethoxazole, minimal effects on meristem organisation are observed. In contrast, treatment with the compound in the presence of sucrose induces pleiotropic effects at the root meristem, including a disruption of cell-cycle progression and changes in auxin signalling that ultimately lead to a loss of meristem integrity. This suggests that the effects of sucrose on meristem development are dependent on folates, and that meristematic activity is influenced by metabolite signalling. Transcriptome analysis uncovered multiple components of auxin signal transduction that respond differently to the compound when administered in the presence of sucrose. It is hypothesised that these signalling components may shape hormone responses at the meristem in response to metabolic cues.

Understanding ARP 2/3 complex mediation in F-actin dependent Endoplasmic Reticulum organization in plant cells

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The plant cytoskeleton is composed mainly of an arrangement of fibrous polymers, microtubules and microfilaments. The rapid organization and behavior of actin microfilaments in a cell is determined by a number of actin interacting proteins. One of the major regulators of the actin cytoskeleton is the seven subunit Actin Related Protein (ARP) 2/3 complex implicated in actin nucleation and polymerization. In plants observations on mutants in different subunits of the ARP2/3 complex and its upstream regulators have suggested their essential roles in determining the shape of cells. However, the secretory as well as the endocytic pathways, both of which involve the endoplasmic reticulum (ER), are also known as major regulators of cell shape. In this context, the link between the ARP2/3 complex and endoplasmic reticulum (ER) organization remains unclear. We have addressed this issue by using fluorescent protein probes and transgenic plants to label and track F-actin and ER organization simultaneously in living cells. The initial comparison of F-actin organization in hypocotyl cells of wild type *Arabidopsis thaliana* and its different mutants suggests a highly bundled F-actin organization in the arp2/3 complex mutant *dis2* but a much finer F-actin configuration in the *klunker* mutant in its upstream regulator. A comparison of the ER between the mutants and the wild type shows differences in ER polygon networks and cisternal features. Understanding the fundamental ARP2/3 complex based mechanism for ER organization and shape development helps in elucidating a very basic facet of eukaryotic cell biology.

Post-translational modification and protein-protein interactions among enzymes of starch biosynthesis in high-amylose barley genotypes from diverse genetic backgrounds

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Resistant starches (RS) have potential benefits for human health as a source of low-glycemic carbohydrate and as a prebiotic for the large colon. Such starches are often referred to as high-amylose though this may reflect changes in amylopectin as well the proportion of amylose in the starch granule. The present study investigated the role of protein phosphorylation, and protein complex formation between enzymes of starch synthesis, in a range of barley genotypes exhibiting "high amylose" phenotypes. The genotypes used in this study were either starch branching enzymes mutants (*sbeiia*$^-$ and *sbeiib*$^-$), starch synthase (*ssiii*$^-$) *amo1* mutants, *amo1* derived lines, and *sex1* (starch mutants). Mutation in either starch branching enzyme (SBE) IIa or IIb has pleiotropic effects on the activities of starch synthases (SSs), starch phosphorylase (SP), isoamylases (iso) and pullulanase (pul) but had no effects on measurable SBEIc and amylase activities. Mutation in either of (SBEIIa or SBEIIb) resulted in formation of novel protein complexes containing (SSI, SSIIa, SBEIIb and SP) and (SSI, SSIIa, SBEIIa and SP) respectively. It was also found that in (*sbeiib*$^-$) mutant there was a > 95% loss of measurable, soluble SP activity but this did not affect the incorporation of SP into a heteromeric protein complex with (SSI, SSIIa and SBEIIa). Seed structure and starch packing in the endosperm were also unaltered. It was also found that in *sbeiia*$^-$ and *sbeiib*$^-$ mutants, different protein complexes are involved in the synthesis of A- and B- granules. *Amo1* and *sex1* mutations resulted in pleiotropic effects on SSs and SBEs but no effects on SP, isoamylases, pullulanase and amylase activities. In *amo1* and *sex1* mutants only one type of complex was found containing (SSI, SSIIa, SBEIIa and SEBIIb) compared to wild type. Phosphorylation studies (with pro Q diamond staining) of different granule bound proteins revealed that (GBSS, SSIIa, BEIIa, SBEIIb and SP) are phosphorylated in their granule bound state. Overall this study provides a better understanding of the contribution of post-translational modification of proteins to production of starches with desired phenotypes.

Concurrent session 2A
Herbivory and pollination

Tomato defense responses to herbivory by two closely related spider mite species
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Spider mites are a major agricultural pest feeding on a variety of plants. *Tetranycus urticae* and *Tetranycus evansi* are two closely related spider mite species that show interesting differences in feeding behavior. *T. urticae* is a generalist, feeding on up to a thousand plant species, whereas *T. evansi* feeds uniquely on Solanaceae, including economically important crops such as tomato and potato. Although both species are able to feed on tomato (*Solanum lycopersicum*), the detailed mechanism by which each interact and evade/tolerate tomato plant defenses remains unclear. The complete genome sequences of both *T. urticae* and *S. lycopersicum* have recently been published, opening the door to large scale genomic studies of both species and their interactions. Using a tomato microarray platform, we characterized how tomato defense is affected in response to attack by *T. urticae* and *T. evansi*. Microarray analyses revealed a set of genes whose expression level changes in response to feeding by spider mite, regardless of the species. This common response is characterized by an up-regulation of transcription factors, hormone biosynthetic genes, and secondary metabolite genes. Our analysis also uncovered species-specific gene expression changes reflecting difference in response to each spider mite species. Interestingly, *T. evansi* attack seems to interfere with a number of tomato defense responses, minimizing both gene induction and repression when compared to *T. urticae* response. This suggests that *T. evansi* is able to manipulate tomato defense responses to reduce the production of anti-herbivory compounds. The differences and commonalities in tomato defense response to *T. urticae* and *T. evansi* shed light on the mechanisms involved in plant herbivore interactions and how each mite species is dealing with them.

Utilization of the genome sequence data of *Tetranychus urticae* (two-spotted spider mite) to create pest-resistant transgenic plants targeting various pest genes
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Tetranychus urticae, the two-spotted spider mite, is one of the major pests in agriculture that can feed on numerous host plants including many greenhouse, field and horticultural crops. Spider mites develop resistance to major group of agricultural pesticides within 2-4 years of their use, creating the challenge to control them. The sequenced genome of *T. urticae* has provided the opportunity to test if the expression of double-stranded RNAs (dsRNAs), complementary to selected spider mite genes, in transgenic plants may generate effective RNA interference (RNAi) and suppress the pest survival or development. To achieve these aims, a number of *T. urticae* genes bearing genes- and species-specific sequence tags (GSTs) were selected according to their putative essential roles in primary metabolism or development. Twenty-five GSTs, representing twenty five genes, were amplified from the spider mite genome and cloned in hairpin RNA expression vector. The 25 hpRNA expression vectors were introduced into an *Agrobacterium tumefaciens* strain, and were transformed into *Arabidopsis thaliana* (ecotype Kondara). The primary transformants of *A. thaliana* for each construct were isolated and grown for two more generation to isolate multiple-insertion and homozygous single-insertion transgenic lines. The expression analyses by real-time PCR showed high level of expressions of the GST constructs in both multiple- and single insertion *A. thaliana* lines. The processing of short-interfering RNAs (siRNAs) in transgenic *A. thaliana* was confirmed by Northern blot analysis. The analysis of the level of suppression of target genes in spider mites feeding on homozygous RNAi lines for each

GST is in process. The data available have shown a consistent decrease of the target gene expression in spider mites fed on a homozygous RNAi line expressing the GST for *ABC transporter*. The present study promises to generate a method for RNAi-targeting of selected spider mite genes in transgenic *A. thaliana*. The method could be used in the future to generate transgenic crop varieties resistant to spider mite attack.

Veratrole Biosynthesis in *Silene latifolia* (White campion)

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Silene latifolia (white campion) is a dioecious plant that emits an array of volatiles from both its male and female flowers. Among these volatiles is 1,2-dimethoxyphenol (veratrole) which is a particularly potent pollinator attractant to the nocturnal moth, *Hadena bicruris*. Little is known about veratrole biosynthesis although methylation of 2-methoxyphenol (guaiacol), another volatile produced by *S. latifolia*, is an *a priori* possibility. Here we describe a biosynthetic pathway to veratrole and the enzyme responsible for the pathway's final step. When *S. latifolia* flowers were treated with the phenylalanine ammonia lysase (PAL) inhibitor, 2-aminoindan-2-phosphonic acid (AIP), guaiacol and veratrole levels were reduced by 50 and 63%, respectively. Feeding with benzoic acid or salicylic acid resulted in an approximately two-fold increase in veratrole emission. Stable isotope labeling experiments indicated that the phenyl ring of both guaiacol and veratrole can be derived from either salicylic acid or benzoic acid. We further report the presence of guaiacol *O*-methyltransferase activity (GOMT) in the flowers of *S. latifolia* and the protein responsible was purified to apparent homogeneity. Peptide sequencing of the purified protein matched a recently identified guaiacol *O*-methyltransferase (*SlGOMT1*). Screening a small population of *S. latifolia* for floral volatile emission revealed that not all plants emit veratrole nor possess GOMT activity and that *SlGOMT1* expression is only associated with veratrole emitters. Collectively these data suggest that veratrole is derived by methylation of guaiacol which itself originates from benzoic acid via salicylic acid, and therefore implies a novel branch point of the general phenylpropanoid pathway.

A tight grip causes sperm release ..... in plants

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The pollen tube is a cellular transport system that is generated to connect the male gametophyte with its female counterpart. Through this cellular protuberance the sperm cells are delivered from the pollen grain to the ovule nestled deep within the pistillar tissues. To be competitive, the pollen tube elongates extremely rapidly and it has to do so against the impedance of the apoplast of the transmitting tissue. To reach its target, the pollen tube therefore needs to exert significant penetrative forces. In plant and fungal cells, invasive forces are generated by the hydrostatic turgor pressure. Using a microfluidic device we tested the pollen tube's ability to navigate mechanical obstacles and to exert penetrative forces by guiding them through microscopic gaps made of elastic polydimethylsiloxane (PDMS) material. Depending on the size ratio between tube and gap, the tubes either deformed the gap walls completely, became deformed themselves while passing, or stalled. Based on the deformation of the gaps the extrusive force exerted by the elongating tubes was determined using reverse engineering and finite element methods. Remarkably, tubes that successfully passed a narrow gap typically burst shortly after, releasing the sperm cells. This raises the questions whether the geometry of the micropyle is a factor in sperm cell release during the reproductive process in the flowering plants.

Concurrent Session 2B
Subcellular Organization

Live Cell Imaging Reveals the Early Subcellular responses of *Arabidopsis thaliana* seedlings challenged by *Colletotrichum destructivum*

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The composition of plant cell walls can dictate the success or failure of pathogenic invasions. Little is known, however, of what occurs in plants at the sub-cellular level as a pathogen is trying to invade the plant cell. We are using live-cell imaging to analyse the earliest phase of pathogen invasion whose outcome would determine a successful invasion of the plant cell by the pathogen or result in the attack being warded off by the plant. We have used *Colletotrichum destructivum*, an acknowledged fungal pathogen with a worldwide host range of economically important plants. The virulent strain has been engineered to expresses a Green Fluorescent Protein[1], making it appropriate for live cell imaging. To assess the early cellular responses to the pathogen, we used different stable transgenic lines of the model plant Arabidopsis thaliana expressing fluorescent proteins targeted to different sub-cellular compartments. Further, we have used mutants defective in cellulose deposition (any1), cuticle and epidermal cell structure (arp2/3 complex mutants). Our initial assessment reveals that organelles behaviour changes prior to penetration of the cell by the fungus. Mitochondria aggregate around the putative entry site and display altered morphology and dynamics. High callose deposition occurs in cells surrounding a penetrated cell suggesting an attempt to limit the spread of infection. The study will help in understanding early plant responses against similar pathogens and add to our knowledge of host -pathogen interactions.
Reference: 1) O'Connell *et al.*, MPMI 17(3) pp. 272, 2004.

Understanding the role of ESCRT in mitochondrial outer membrane re-modeling during *carnation Italian ringspot virus* infection

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Carnation Italian ringspot virus (CIRV) is a positive-strand RNA virus that assembles its membrane-bound replication complexes at mitochondria in plant cells. This process is accompanied by extensive invagination of the mitochondrial outer membrane (MOM), leading to the formation of spherules wherein viral RNA synthesis occurs. While it is known that CIRV replicase proteins are integral MOM proteins, how they participate in spherule formation at the MOM has not been investigated. Here, we provide evidence for involvement of the host-cell ESCRT (Endosomal Sorting Complex Required for Transport) machinery – a multi-protein complex involved in late endosome maturation, and in mammalian cells also participates in retroviral budding from the plasma membrane. We demonstrate that in cucumber protoplasts, expression of dominant-negative versions of several ESCRT components reduces the replication efficiency of CIRV. We also show that the ESCRT-I component, Vps23, is re-localized from late endosomes to mitochondria in plant cells expressing the CIRV replicase protein, p36. Furthermore, this re-localization is mediated by an interaction between a non-conserved region in the N terminus of p36, and the C terminus of Vps23. Notably, the C-terminus of Vps23 also interacts with another ESCRT-I component, Vps28, as part of normal ESCRT assembly; however, p36 does not appear to possess any known Vps23-binding motifs suggesting that it utilizes a novel mechanism for ESCRT recruitment. Together, these results not only provide important insight to how CIRV exploits ESCRT for spherule formation at the MOM, but also shed light on normal aspects of ESCRT function in plants.

The ARP2/3 mutants link actin organization and cell wall integrity to the maintenance of cell-cell connectivity.

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Plant cells can survive independently since each cell possesses a protective cell wall and is capable of photosynthesizing. During evolution, the transition from unicellular to multi-cellular plants required an intimate cell-cell connection that would allow individual cells to grow and develop in complete harmony with other cells. This harmonious development led to the formation of specialized tissues and organs. The fundamental basis for the development of cell to cell connectivity probably lies in its subcellular organization and cell-wall composition. We have investigated this basic aspect of plant organization through the use of several mutations in the actin related protein (ARP) 2/3 complex. This seven-subunit complex is implicated in actin nucleation and polymerization. Mutations in individual subunits of *Arabidopsis thaliana* ARP2/3 lead to morphological aberrations in epidermal cells. In addition the mutants exhibit a loss of connectivity between cells and this phenotype is most frequently observed in elongating cells of the hypocotyl. Electron microscopy showed that the mutants possess uneven cell wall deposition. Immuno-chemical labeling of the cell wall suggested that pectins are more abundant in the mutants. Callose, intimately implicated in response to wounding, was significantly higher in the mutants with patchy deposition around putative half-plasmodesmata. The aberrant depositions suggest correlations with abnormal hypocotyl and root development. Taken together our findings are beginning to provide insights that link actin-cytoskeleton integrity with cell wall compositions for maintaining cell-cell connectivity and growth harmony in higher plants.

Cosuppression of the chloroplast localized molecular chaperone AtHSP90.5 impairs plant development and chloroplast biogenesis in *Arabidopsis*

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Chloroplasts are the organelles that perform photosynthesis in higher plants and green algae. Similar to mitochondrion, the chloroplast contains its own genome and protein synthesis machinery. However, due to the small genome size in chloroplast, most of the chloroplast proteins are nuclear encoded and imported from the cytoplasm. The chloroplast therefore contains a cohort of proteins that make up the protein quality control system, including different families of molecular chaperones. AtHSP90.5 is one of the seven HSP90 family molecular chaperones in *Arabidopsis* and locates in the chloroplast stroma. To understand the role of AtHSP90.5 in chloroplast function and biogenesis, we generated transgenic *Arabidopsis* plants that express AtHSP90.5 driven by constitutive cauliflower mosaic virus (CaMV) 35S promoter. Interestingly, in addition to lines that over-express AtHSP90.5 proteins, three lines of transgenic plants were identified to have transgene-induced gene silencing for both transgenic and endogenous AtHSP90.5 genes. All silenced lines were noticed to show certain degree of albino phenotype in photosynthetic tissues. By analyzing the plant growth, chloroplast development and the altered AtHSP90.5 expression, we confirmed that chloroplast localized HSP90 is essential for plant development and chloroplast biogenesis, particularly in young developing leaves.

Concurrent Session 2C – PDW

Stress and defense responses

A New Suite of Pathogenesis-Related Gene Markers in *Arabidopsis thaliana*

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Understanding plant-pathogen interactions is fundamental to protecting the agriculture industry from pathogens. The current plant defence model indicates PAMP/MAMP-Triggered Immunity (PTI) as the first line of defense for plants; recognizing slowly evolving portions of pathogens in order to induce a defense response. Pathogens have responded with effectors to suppress or hide from the PTI. These effectors can be recognized by R proteins as the plant's second line of defence to induce Effector Triggered Immunity (ETI) followed by a stronger defence response. Currently, gene markers for specific infection conditions are poorly defined. The aim of this project is to identify genes that are highly expressed under specific pathogenesis-related conditions (eg. during PTI or ETI responses) in order to provide a novel set of markers for the study of host-pathogen interactions. By analyzing publically available microarray data, 19 gene candidates have been identified in *Arabidopsis thaliana* that show strong expression with specific strains of *Pseudomonas syringae* at specific time points. The validity of these genes as markers will be determined using reverse-transcription PCR to determine to confirm their expression profiles. The role of these genes in plant immunity will be determined based on the resistance of *A. thaliana* plants unable to express these genes.

Peroxules provide increased interactivity between peroxisomes and mitochondria during high subcellular ROS induced stress

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Peroxisomes and mitochondria have a complex relationship that helps maintain homeostasis in plant cells. Different reactive oxygen species (ROS) including H_2O_2 accumulate in both organelles. Whereas the detoxification of H_2O_2 by catalase in peroxisomes is well known it has been observed recently that under moderate stress such as a short exposure to high intensity light or low levels of H_2O_2, peroxisomes extend thin projections called peroxules. We undertook a live-imaging based approach to investigate whether interactions between mitochondria and peroxisomes are increased during the stressed state of a cell and whether the two organelles display physical interactions. A number of fluorescent protein probes including GFP, YFP, and mCherry targeted to peroxisomes and mitochondria were introduced into *Arabidopsis thaliana* to create single and double stable transgenic lines. Under normal conditions both organelles displayed rapid subcellular motility with largely coincidental interactions. However, when stressed by H_2O_2 or high light, mitochondria exhibited rapid fission to form 0.5-0.8 μm spheres. Almost simultaneously, motile peroxisomes were observed extending peroxules with mitochondria aggregating around them. Alternatively several peroxisomes localized to chloroplasts and extended peroxules that connected with mitochondria streaming past them. These observations suggest that ROS transfer from mitochondria to peroxisomes may involve physical contacts via peroxules. For testing this conjecture further we have created a HyPer-GFP based peroxisomal probe that increases in fluorescence following exposure to H_2O_2. In addition our experiments are being extended to *Arabidopsis* mutants impaired in their ability to manage ROS. Our observations provide new insights into physical interactions between mitochondria and peroxisomes during stress.

Role of carotenoid cleavage dioxygenases in volatile emissions and insect resistance in Arabidopsis

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Plants have evolved specialized biochemical signalling pathways to manage insect interactions. Plant volatiles have been shown to act as repellants or attractants to herbivores. Among the various plant volatiles, terpenoids have been documented to act as toxins or feeding deterrents to a range of insects. Carotenoid degradation by enzymatic oxidative cleavage produces an array of terpenoid products that are collectively known as apocarotenoids, which include volatile and non-volatile compounds. In *Arabidopsis thaliana*, a family of nine genes known as carotenoid cleavage dioxygenases are involved in apocarotenoid production in plants. Of these, four encode carotenoid cleavage dioxygenases (*CCD*s – *CCD1*, *CCD4*, *CCD7*, and *CCD8*), and five encode 9-*cis*-epoxycarotenoid dioxygenases (*NCED*s – *NCED2*, *NCED3*, *NCED5*, *NCED6*, and *NCED9*). Research involving *CCD1* overexpression in Arabidopsis revealed that transgenic Arabidopsis plants had significantly enhanced β-ionone emissions. These transgenic plants were included in an insect feeding assay to observe the changes of feeding damage in these plants. It was clearly evident that the *CCD1* lines showed significantly lower feeding damage by the crucifer flea beetle as compared to the wild type control. On the basis of this study, we have initiated a study to investigate the effects of overexpressing the other eight members of the *CCD/NCED* gene family on volatile apocarotenoid emissions, and further study the effects of their overexpression on insect-plant interactions. We will present our finding on the effects of *CCD*1 overexpression, and will provide an update of our research progress.

Histone Marks Associated with Adaptation to Salt Stress in Arabidopsis

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Epigenetic factors contribute to changes in gene expression under different physiological, developmental and environmental stimuli. Changes in the epigenetic status of plants under abiotic stress are well documented in the literature, but their stability and transmission to the next generation as epigenetic stress memory, and their incorporation into plant stress adaptation are still a matter for scientific debate. Using chromatin immuno-precipitation (ChIP) and Next Generation Sequencing (ChIP-Seq), we compared genome wide enrichment levels of two marks of histone acetylation and methylation in the progeny of salt stressed and control Arabidopsis plants. ChIP-Seq data showed that the chromatin of the progeny of salt stressed plants contained less enrichment levels of the H3K9ac mark compared to the chromatin of the progeny of control plants, but no changes were detected in the enrichment levels of the H3K4me2 mark. Regions with significantly reduced enrichment levels of the H3K9ac marks were selected and confirmed by ChIP followed by q-PCR. Furthermore, the expression levels of the genes corresponding to these regions were tested in the progeny of salt stressed and control plants. The results identified 10 genes that were significantly down regulated in the salt stressed progeny. Our findings demonstrate that histone modifications play a role in establishment of trans-generational stress memory. Moreover, we identified genes that are candidates to be involved in salt stress adaptation. Most of these are novel genes whose characterization will reveal their specific roles in the epigenetics of stress adaptation in plants.

Concurrent session 3A
Enzymology

Investigation of protein phosphorylation and protein complex formation in a TILLING mutant of maize and its influence on starch synthesis

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High amylose (resistant) starch is digested slowly in the small intestine, lowering the glycemic response and maintaining insulin sensitivity. Starch biosynthetic enzymes, such as starch synthases I and IIa and starch branching enzyme IIb (SBEIIb), associate in phosphorylation-dependent multi-subunit complexes in the maize endosperm and starch granules. A knockout mutation of the SBEIIb gene (*ae*) increases the apparent amylose content of the seed, but also causes a reduction in yield. This investigation aims to characterize new mutations in the *ae* gene using a TILLING technique, which may permit a more subtle phenotype in which starch structure is modified without a yield penalty. A single nucleotide substitution (G>A) in the *ae* gene was generated using EMS. The nucleotide substitution translates to a cysteine to tyrosine amino acid change (Cys_{663}>Tyr) and resides in a putative protein-protein interaction domain. Western blotting suggested that the Cys_{663}>Tyr mutation did not affect expression of SBEIIb, and native gel electrophoresis coupled with zymogram analysis showed similar soluble SBEIIb activity when compared to wild type. Starch granules from the Cys_{663}>Tyr mutant appear to possess SBEIIb, but also a number of other isoforms not normally found in the starch granule. It is argued this is indicative of the recruitment of other enzymes into heteromeric protein complexes. *In vitro* mutagenesis studies in this interaction domain demonstrated the loss of SBEIIb interaction with SSIIa consistent with this hypothesis. Elucidation of the regulatory mechanisms responsible for synthesis could lead to development of novel starches with application in the food industry.

Regulation of *Arabidopsis* cyclic nucleotide-gated ion channels by calmodulin

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Cyclic nucleotide-gated ion channels (CNGCs) have been recently implicated in numerous signalling pathways in higher plants, including *Arabidopsis*, where multiple CNGC isoforms have been found to play a role in pathogen response. Both animal and plant CNGCs have been shown to interact with the ubiquitous eukaryotic Ca^{2+} sensor calmodulin (CaM); however, the regulation of plant CNGCs is poorly understood in comparison to animal isoforms, which have been extensively characterized. The current model maintains that plant CNGCs interact with CaM at a CaM-binding domain (CaMBD) in the cytosolic C-terminus of the channel, within the putative cyclic nucleotide-binding domain (CNBD). In contrast, animal channels are generally regulated by CaM-binding to an N-terminal CaMBD, and this CaM-binding leads to channel closure. We have pursued a variety of studies to demonstrate that certain *Arabidopsis* CNGC isoforms interact with CaM via multiple, distinct CaMBDs, located at both the cytosolic N- and C-termini, and have found evidence for both Ca^{2+}-dependent and –independent CaM-binding. Furthermore, we will discuss the binding affinities for these sites, critical residues as identified by site-directed mutagenesis, and *in vivo* studies of truncated channels. We will also discuss growing evidence that plant CNGCs are highly divergent in both the sequence and location of CaMBD motifs, as well as possible physiological models of channel regulation by CaM. Ongoing work will further elucidate the contribution of CaM-binding to CNGC structure-function and physiology in plants.

Amino Acids Important for Conferring Chain-Length Substrate Specificity of Two Arabidopsis Alcohol-Forming Fatty Acyl Reductases

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Fatty alcohols play a variety of biological roles in bacteria, insects, plants, and mammals. In plants, long-chain (C16 and C18) and very-long-chain (>C18) primary fatty alcohols are found either as free alcohols or in a combined state (e.g. wax esters, phenolic lipids). Fatty alcohols are common chemical constituents of the plant surface lipid barriers cuticle, suberin, and sporopollenin. Alcohol-forming fatty acyl reductases (FARs) are responsible for the NADPH-dependent reduction of fatty acyl-coenzymeA or fatty acyl-acyl carrier protein to primary fatty alcohols via an unreleased fatty aldehyde intermediate. FAR enzymes have distinct substrate specificities with regard to chain length and saturation. The genome of *Arabidopsis* contains eight genes encoding FAR enzymes (FAR1-FAR8) that produce primary fatty alcohols ranging in chain length from C16:0 to C30:0, with each FAR possessing a distinct chain length specificity. FAR5 and FAR8 encode proteins that are 85% identical at the amino acid level but with strong specificity for C18:0 and C16:0 acyl chain length, respectively, when heterologously expressed in yeast. We have investigated the amino acid residues responsible for conferring these chain length substrate specificities. We have engineered chimeric proteins with FAR5 and FAR8, as well as single point mutations in FAR5 and FAR8, and have evaluated the resulting chain length specificities in yeast. A two amino acid substitution fully converted FAR5 specificity to C16:0 and a three amino acid substitution fully converted FAR8 specificity to C18:0. This study lays the way for the engineering of highly active FAR proteins with desired specificities and the production of fatty alcohols with significant industrial value.

Evidence for Multiple Carboxysomal Bicarbonate Dehydration Complexes based on the Form and Function of Carbonic Anhydrase

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Despite possessing C_3 biochemistry, the physiological characteristics of cyanobacteria are similar to those for C_4 plants. It has been suggested that the suppression of the C_3 photorespiratory phenotype in cyanobacteria is due to the operation of the CO_2 concentrating mechanism (CCM). Carboxysomes are microcompartments essential for photosynthesis and the CCM in cyanobacteria. These organelles house Rubisco and carbonic anhydrase (CA) within a protein shell. In the cyanobacteria, *Synechocystis* PCC6803, CcaA is the functional CA and is complexed with two proteins, CcmM and CcmN – forming a HCO_3^- dehydration complex. CcmM acquires cytosolic HCO_3^- and delivers it to CcaA which catalyzes HCO_3^- dehydration within the carboxysome core [1]. However, many □-cyanobacterial strains such as *Thermosynechococcus elongatus* BP-1 lack a CcaA homolog. While the N-terminal region of CcmM is homologous to γ–CAs, this domain is inactive in *Synechocystis*. We demonstrated that CcmM from *T. elongatus* and *Nostoc* PCC7120 catalyzes ^{18}O exchange in CO_2, a hallmark of all CAs [2]. Using truncated forms of CcmM, biochemical analysis and homology modeling, we defined the minimal γ–CA catalytic domain of CcmM to encompass the N-terminal 201 amino acids. CcmM201 is active, suggesting an important stabilizing role for a C194-C200 disulfide bond, despite these residues playing no direct role in the catalytic mechanism. Consistently, CcmM209 C194S, C200S mutant was 55% less active than the wildtype. In addition, reducing agents inhibited the γ–CA activity of *Nostoc* PCC7120 suggesting that the C194-C200 disulfide bond is critical for the oxidative activation (and potentially regulation) of the enzyme.

[1] Cot, S.S.W., et al.. *J. Bacteriol*. 2008. 190(3): p. 936-945.
[2] Peña, K.L., et al.. *PNAS* 2010. 107(6): p. 2455-2460.

Starch biosynthesis in *Arabidopsis thaliana*

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In plants, starch is the most important carbohydrate storage polymer and is synthesized in plastids, such as chloroplasts and amyloplasts. Transient starch in *Arabidopsis thaliana* leaf chloroplasts turns over more rapidly than storage starch in amyloplasts. Previous studies in amyloplasts suggest that phosphorylation dependent assembly of multi-enzyme complexes is involved in storage starch biosynthesis. However, little is known in relation to transient starch synthesis. The present study investigated the post-translational regulation of starch synthases (SS), and starch branching enzymes (SBE) in leaf chloroplasts of *Arabidopsis thaliana* including protein phosphorylation and protein-protein interactions. The sub-organelle distributions of SSs and SBEs were detected among the wild-type and several mutant lines. SS2 and SBE2.2 were found to be trapped in the starch granule at the end of the day in 16/8 h light/dark photoperiod in the wild type. Analysis of mutants suggests that SS2 traffics other biosynthetic enzymes (SBE2.2) into the starch granule. For example, starch granules of *ss2$^-$ mutants* also lacked SBE2.2. The DNA sequences of SS and SBE isoforms were cloned into commercially available vectors and expressed with an affinity S-tag in Escherichia coli. γ- [^{32}P] –ATP labelling of the recombinant proteins showed that recombinant SBE 2.1 and SBE2.2 can be phosphorylated by chloroplast lysates extracted during the light period. This provides evidence that enzymes of starch biosynthesis in leaves are regulated by post-translational modification. This study in Arabidopsis will provide valuable insight for understanding starch synthesis and turnover in crops.

Phosphorylation of Starch Branching Enzymes and Protein-Protein interactions in Maize Amyloplasts

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Protein-protein interactions between starch synthases (SS) and branching enzymes (SBE) have a major role in controlling starch architecture. These interactions are strongly enhanced by protein phosphorylation. Protein complexes of SSI/SSIIa/SBEIIb and SBEI/SBEIIb have been observed in wild-type amyloplasts of maize. Previous studies (ref) support a model whereby granule-bound proteins involved in amylopectin synthesis are partitioned into the starch granule as a result of their association within protein complexes. The present work investigated phosphorylation of SBE isoforms in multi-enzyme complexes. Phosphorylated forms of SBEI and SBEIIb were detected within starch granules by Phos-tag SDS-PAGE in mutant and wild-type maize lines. We found that at least two serine (Ser^{748} and Ser^{793}) of SBEI can be phosphorylated. Truncations of recombinant SBEI proteins lacking these two phosphor-serine residues become dissociated from SBEIIb. SBEIIb is phosphorylated at Ser^{649} resulting in decreased affinity towards starch and amylopectin and disassociation from SSIIa. However, phosphorylation of SBEIIb at serine Ser^{649} is necessary in forming a heteromeric complex with SBEI. In conclusion, the formation of an SBEI/SBEIIb complex requires phosphorylation of SBEI Serine^{748} and SBEIIb Serine^{649}. This protein complex resides in the soluble stroma of amyloplasts, whereas the protein complex SSI/SSIIa/SBEIIb is dependent on phosphorylation of SBEIIb at two other sites, distinct from Ser ^{649}.

Concurrent session 3B
Molecular Biology and Genetics

Identification Of Carbohydrate-binding Ability Based On Proteome-wide Tertiary Structure Prediction: *in vitro* Validation

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Carbohydrates are often referred to as the third molecular chain of life and their interactions with proteins are thus worthy of attention. Carbohydrates are crucial storage and structural materials, and can also be used as information carriers. Carbohydrate-binding proteins are very diverse in structure and aromatic residues play a significant role in ligand binding. In my study, I am testing the carbohydrate-binding ability of proteins whose predicted tertiary structures contain co-planar aromatic residues at an appropriate spacing to bind carbohydrates. Forty eight expression constructs of the candidate proteins from *Arabidopsis thaliana* have been transferred into *Nicotiana benthamiana* and, after purification, the expressed, tagged proteins were applied to glycan arrays and different binding specificities were observed. The role of these novel carbohydrate binding proteins will be further elucidated through a combination of reverse genetics guided by online resources, hybridization of labeled proteins to sections of plant material, carbohydrate profiling, and other methods.

Using polymorphic loci to investigate the evolution of gene expression regulation

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Many of the trait differences among individual plant genotypes are due to differential gene expression. However, the genetic basis of this gene expression variation and the response of gene expression to selection are poorly understood. Here, we describe a multi-step data processing strategy to measure allelic transcript abundances from global RNASeq data. We will also describe how allelic transcript abundance estimates can address: 1) whether gene expression differences between genotypes are caused by mutations close to genes or by mutations in other regions of the genome, and 2) whether selection acting on different populations causes gene expression differences between the populations.

Elucidation of promoter elements governing the jasmonate-responsiveness of the Calmodulin-Like Gene *CML39* in *Arabidopsis*

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Observations of stimulus-specific $[Ca^{2+}]_{cyt}$ oscillations during signal transduction suggest that Ca^{2+} signals directly encode information. These stimulus-specific oscillations, known as Ca^{2+} signatures, can be interpreted by an array of Ca^{2+}-binding sensors and effectors which subsequently regulate appropriate cellular responses. While progress has been made regarding the classic Ca^{2+} sensor calmodulin, less research has been directed towards the calmodulin-like family of Ca^{2+} sensors (CMLs). This family, unique to plants, is suspected to regulate a multitude of stress and developmental pathways; however, to date very few members of this family have had their functions elucidated by the identification of downstream targets and upstream regulators. We have focused our research upon *CML39*, which has been shown to be significantly upregulated in response to jasmonates (a stress hormone) in *Arabidopsis thaliana*. A series of reporter-fused promoter deletion constructs in *A. thaliana* has been analyzed to identify regions of methyl jasmonate (MeJA) responsiveness in *CML39*. Intriguingly, while bioinformatics predicts the *CML39* promoter to have multiple MeJA motifs empirical evidence suggests that some respond in a tissue-specific manner.

Ongoing work aims to identify the specific cis-elements within the *CML39* promoter, as well as, ascertain why only a few of many identical motifs is functional within the *CML39* promoter.

Functional analysis of a conifer protein that interacts with the global transcriptional regulator, *Ab*scisic acid *I*nsensitive 3 (ABI3)

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The ABI3 (*Ab*scisic acid *I*nsensitive 3) transcription factor is an essential component of the ABA signal transduction pathway and is involved in seed development, stress signaling, and the induction and maintenance of seed dormancy. Our previous work led to the isolation of a conifer orthologue of the *ABI3* gene in yellow-cedar (*Callitropsis nootkatensis*), *CnABI3*. Like other ABI3/VP1 proteins, CnABI3 mediates its regulatory functions by physical interactions with other protein partners. Using a yeast two-hybrid approach, we have identified a protein (CnAIP1) in yellow-cedar that interacts with the B1 and B2 domains of CnABI3. Comparative analyses of the deduced amino acid sequence of CnAIP1 suggest that the *CnAIP1* gene encodes a putative heat shock protein associated factor. We are carrying out research to characterize CnAIP1 with a focus on its roles during seed development, germination and stress responses. *CnAIP1*::*GUS* reporter constructs are being expressed in Arabidopsis to discern the qualitative aspects of expression driven by the *CnAIP1* gene promoter, such as its tissue- and cell-specificity, and responsiveness to hormones and stress signals. *CnAIP1*-overexpressing Arabidopsis lines are further being used to elucidate the physiological roles of the CnAIP1 protein during seed development, seedling growth and stress responses. These lines show a hypersensitivity to ABA during the germination process.

Harnessing the Anabolic Properties Of Dark Respiration Using *Arabidopsis thaliana* With Partially-Suppressed Mitochondrial Pyruvate Dehydrogenase Kinase

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Transgenic Arabidopsis with partially-suppressed mitochondrial pyruvate dehydrogenase kinase (mtPDHK) show enhanced sink activity as evidenced by increased seed and oil weight at ambient CO_2. The mtPDHK is a negative regulator of mitochondrial pyruvate dehydrogenase (mtPDH); mtPDH provides acetyl-CoA for many anabolic processes including fatty acid synthesis. We hypothesized that suppressed mtPDHK will have its maximal effect on plant and oil productivity at high CO_2 due to the combination of increased source and sink strength. *Arabidopsis* having either constitutive or seed-specific expression of antisense mtPDHK were grown at either ambient or high CO_2. Assays were developed to allow efficient quantification of mtPDH activity in both leaf and seed extracts without mitochondrial isolation. At ambient CO_2, a hyperbolic relationship was found between leaf mtPDHK transcript levels and mtPDH. This contrasts with the negative linear relationship found in heterotrophic organisms. Seeds per silique, inflorescence size, seed number and weight, oil content, and harvest indices were significantly increased up to ~3 times at high CO_2; effects were greatest for constitutive lines 3^1 & 10^4. At high CO_2, lines 3^1 & 10^4 shared the following: (1) an intermediate elevation in seed mtPDH activity; (2) the highest retention of leaf mtPDH activity when incubated with ATP. These data indicate a dosage response between mtPDH activity and plant productivity. A significant correlation was found between enhanced net C exchange in rosette leaves and reduced mtPDHK transcript levels for constitutive lines. A model illustrating the effect of mtPDH on source and sink activities in plants will be presented.

Investigating a new link between polyamine metabolism and vascular development

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5'methylthioadenosine nucleosidase (MTN) is an essential enzyme in the salvage of methionine; disruption of the two MTN genes in *Arabidopsis thaliana* results in a pleiotrophic phenotype. *mtn1-1mtn2-1* double mutants retain only 14% of the MTN enzyme activity found in wild-type plants. They display altered vasculature, male and female sterility accompanied by increased 5'-methylthioadenosine (MTA) levels, reduced nicotianamine content and altered polyamine profiles. The vascular defects in *mtn1-1mtn2-1* mutants are similar to those in the thermospermine (Tspm) synthase mutant, *acl5*, leading us to hypothesize that accumulated MTA is causing feedback inhibition of Tspm synthase activity. The *acl5* vascular phenotypes are partially rescued by the second site suppressor mutant *sac51-d* that overproduces *SAC51*, a transcription factor that regulates genes involved in vasculature development. The *mtn1-1mtn2-1* vascular phenotype was partially rescued by overexpression of *SAC51*. Evidence from other groups suggests that changes in *SAC51* expression in stems are partly responsible for the abnormal *acl5* vasculature changes. Consequently, we determined the expression profiles of *SAC51*, and *SAC51*-responsive genes involved in xylem maturation and programmed cell death in various organs of MTN-deficient mutants – revealing an intriguing tissue-specific pattern that furthers our understanding of the relationship between Tspm, methionine salvage and vascular development.

Concurrent Session 3C –PDW
Flower Development and Self-Incompatibility

An insight into the function of the *HUA2* gene family
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HUA2 has been identified as a flowering gene regulating two MADS box genes, *FLOWERING LOCUS C (FLC)* and *AGAMOUS (AG)*, thus being implicated in the coordination of induction and maintenance of floral state. We have previously demonstrated that the natural variant of *HUA2-Sy-0* uncouples the HUA2 effects on *FLC* and *AG*. To gain more insight into the function of HUA2 protein, we used yeast two-hybrid and *in vitro* pull-down assays and identified HUA2 interacting with proteins involved in pre-mRNA splicing. The interaction of HUA2 with the splicing factors is associated with the effect of *HUA2* on the 3'-end processing of *FCA, AG* and *U1-70K.HUA2* is a member of a small gene family that includes *HUA2*-like genes *HULK1*, *HULK2* and *HULK3*. The members of the *HUA2* gene family share a high degree of similarity in sequence and protein structure. We show that they have overlapping expression patterns, localize in the nucleus and interact with the same set of splicing factors, indicating that they may act redundantly to specify the functions of this small gene family. Consistently, single mutations in the gene family (except for *HUA2*) do not produce a visible phenotype; however, double and triple mutants within the gene family members result in various pleiotropic phenotypes. Finally, the quadruple mutant is lethal, suggesting that the *HUA2* gene family is essential for *Arabidopsis* development.

BLADE-ON-PETIOLE1/2* promotes TALE homebox genes, *ARABIDOPSIS THALIANA HOMEOBOX GENE1* and *KNOTTED-like from ARABIDOPSIS THALIANA6* to co-ordinate flowering and inflorescence architecture in *Arabidopsis thaliana
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The flowering time and inflorescence architecture in flowering plants are optimized for reproductive success. Flowering is initiated when floral inductive signals allow the vegetative shoot apical meristem (SAM) to adopt an inflorescence meristem (IM) fate. In *Arabidopsis thaliana*, this switch promotes the production of flowers and the elongation of internodes to generate an inflorescence. Coordination of flowering and internode elongation is poorly understood but requires the activities of BEL1-like homeodomain proteins. Flowering can be blocked by mutations of PENNYWISE (PNY) and POUNDFOOLISH (PNF), whereas, internode elongation is suppressed by mutation of PNY. Here we show that both flowering and internode elongation defects are caused by the ectopic activity of four sets of genes acting in a linear pathway. This pathway is defined by the lateral organ boundary genes encoded by *ARABIDOPSIS THALIANA HOMEOBOX GENE1 (ATH1)* and *KNOTTED-like from ARABIDOPSIS THALIANA6 (KNAT*6) and *BLADE-ON-PETIOLE1* and *2 (BOP1/ 2)* whose expressions are tightly regulated in the SAM. BOP1/2 overexpression results in delayed flowering and shortened internodes similar to *pny* mutants. Here we show that overexpression of BOP1/2 results in a mis-expression of ATH1 and KNAT6. Preliminary results using glucocorticoid-based transcriptional induction of BOP1 suggest ATH1 may be a direct target of BOP1. We present a model and preliminary evidence to explain how mis-expression of this pathway with BOP1/2, ATH1 and KNAT6 negatively regulates the floral transition and internode elongation.

Investigations in the cytological responses of stigmatic papillae to compatible and self-incompatible pollinations in the Brassicaceae

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In the Brassicaceae, pollination leads to a series of responses by the stigmatic papillae for either pollen acceptance or rejection. For compatible pollinations, exocytosis to the stigmatic papillar plasma membrane, under the pollen contact site, is proposed to be essential for the early stages of pollen acceptance. For self-incompatible pollinations, this polarized secretion is predicted to be inhibited as part of the pollen rejection response. In yeast and animal systems, polarized secretion has been shown to be promoted through the tethering of secretory vesicles to the plasma membrane by the exocyst complex. Exo70A1, a subunit of the exocyst complex, has been previously identified to be involved in the compatible pollen response in *Brassica napus* and *Arabidopsis thaliana.* Accordingly, we hypothesize that Exo70A1, functions in the stigma as part of the exocyst complex to mediate vesicle secretion at the pollen-papillae interface following compatible pollinations. Using transmission electron microscopy, we examined the presence or absence of secretory vesicles, following compatible and self-incompatible pollinations in *A. thaliana*, *A. lyrata* and *B. napus*. For compatible pollinations in *A. thaliana* and *A. lyrata*, vesicle secretion was observed at the stigmatic papillar plasma membrane under the pollen grain, while *B. napus* stigmatic papillae appeared to use multi-vesicular bodies (MVBs) for secretion. Also, secretory activity was examined in the *A. thaliana exo70A1-1* mutant and *B. napus Exo70A1-RNAi* plants where a loss of vesicle/MVBs tethering to the pollen contact site was observed. Similar results were seen for self-incompatible pollinations in *A. lyrata* and *B. napus*. Thus, by examining the cytological responses of stigmatic papillae at the ultrastructural level, we demonstrated that the regulation of exocytosis acts as a switch between compatible pollen acceptance or self-incompatible pollen rejection.

The role of ARC1 in the self-incompatibility pathway in *Arabidopsis spp.*

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Self-incompatibility is a complex process in flowering plants that facilitates genetic diversity by preventing self-fertilization. In the Brassicaceae, this process is regulated by a signalling pathway initiated by the stigma-specific, *S* Receptor Kinase (SRK), following binding of a pollen-specific ligand, SCR/SP11. In *Brassica* species, downstream signalling components of the SRK pathway have been identified as the *M* Locus Protein Kinase, the ARC1 E3 ubiquitin ligase, and the Exo70A1 subunit of the exocyst complex. While the functions of SCR/SP11 and SRK are conserved in the related *Arabidopsis* species, nothing is known about the downstream signalling pathway. As a result, we set out to investigate if the role of ARC1 is conserved in regulating pollen rejection in the naturally occurring *Arabidopsis lyrata* self-incompatibility system. We performed a genome wide survey of numerous species in the Brassicaceae and determined that ARC1 is frequently deleted in self-compatible species, indicating that ARC1 may have a conserved role in self-incompatibility signalling in the Brassicaceae. We identified an *A. lyrata ARC1* homologue to *Brassica ARC1*, and have generated transgenic *A. lyrata* with an *ARC1* RNAi construct. These plants were tested to determine what effect knocking down *ARC1* expression has on the self-incompatibility pathway. Thus, we have developed a transgenic system to investigate the self-incompatibility signalling pathway in *A. lyrata*. The RNAi in *A. lyrata* will allowed for the determination that *ARC1* is indeed necessary for the self-incompatibility signalling pathway in *A. lyrata*.

Poster Abstracts

Technologies for Plant Research

P1 Multispectral LED array for plant research

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In recent years the advances in light emitting diode (LED) technology have made the prospect of combining a variety of monochromatic lights to create a light source specifically tailored to plant photosynthetic requirements a reality. Rather than the sometimes crude facsimiles offered by more conventional technologies, LED irradiation can be adjusted to specific plant requirements based on criteria such as species and/or growth stage. In collaboration with Intravision AS (Norway) and COM DEV International, the Controlled Environment Systems Research Facility at the University of Guelph has developed a multispectral LED array designed specifically for plant studies utilizing our BlueBox precision growth chamber technology. The 'snowflake' array, consisting of 512 Philips visible LEDs, is capable of providing a full photosynthetically active radiation (PAR) spectrum at 2000 μmol m^{-2} s^{-1} over a 0.5 m^2 area from a distance of 60cm. Individual wavelengths are fully addressable and dimmable and can provide up to 1000 μmol m^{-2} s^{-1} over the same 0.5 m^2 growing area. This light source will allow the systematic study of the effect of specific changes in spectral quality and quantity and their impact on productivity parameters including photosynthesis, water use efficiency, yield, time to flowering, and plant structure.

P2 Optical Sensors for the Ion-Selective Management of Hydroponic Nutrient Solution Quality

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Traditional management practices for maintaining hydroponic solution quality waste considerable volumes of water and fertilizers. In general, hydroponic solutions are created to provide an ideal soup of nutrients to plants, the quality of which depletes as the plants grow. Because plant nutrient uptake is extremely variable and dependent on many factors, growers cannot know the composition of their nutrient solution without outsourcing expensive lab analysis. Typically, measures of electrical conductivity and pH help growers determine hydroponic solution quality (although crudely) and weak solutions are eventually discharged and replaced. However, if information describing the nutrient composition of a hydroponic solution was available to a grower, management practices could be developed whereby nutrient solutions are periodically adjusted to replenish ions that are depleted. Ion-Selective (IS) Sensor technologies have been developed the last few decades and are now realizing market availability. We are characterizing novel IS Optrodes alongside commercially available IS Electrodes in the hydroponic environment to assess the feasibility of each variety for use in an automated nutrient management system. The most suitable technology will be integrated into the design of an online IS Monitoring System. The efficacy of the IS Monitoring System will be tested in custom plant growth chambers at the University of Guelph as we monitor plant nutrient uptake in response to environmental variables. Ultimately this system could be used for feedback control of an IS Nutrient Dosing Apparatus and nutrient solutions could be maintained at an ideal composition continuously.

P3 RGB analysis detects phenological changes in Pinus strobus

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Climate is a main driver for the timing of plant phenological events. Observing and recording phenological events can be a tedious and time-consuming task. However, remote phenocam imaging provides a more convenient method for tracking phenological changes by capturing a time series of digital images for spectral analysis. Changes in colour signals (RGB) correspond to phenological events and likely indicate changes in pigment composition throughout the growing season. In order to exploit this technology it is important to understand the mechanisms that drive the changes observed in the spectral bands. We developed an experiment using a phenocam system in order to establish a relationship between the spectral bands and pigment composition. The use of remote phenocam imaging enabled the quantification of red, green and blue (RGB) colour signals on daily images of white pine seedlings taken from our experimental plots at Koffler Scientific Reserve. Subsequent pigment analysis was performed on needle samples taken from the same seedlings. A relationship between spectral and pigment data is most evident when comparing the strength of the green signal with the Chl a/Chl b and Chl a+b values. These findings suggest that remote phenocam imaging may provide a reliable means for tracking phenological changes and monitoring the effects of global climate change on plants over time.

Enzymology

P4 Post translational regulation of maize (*Zea mays* L.) starch synthase IIa by protein phosphorylation

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In maize (*Zea mays* L.) endosperm, starch biosynthesis occurs in the amyloplasts, through the co-ordinated actions of several enzymes including ADP-glucose pyrophosphorylase (AGPase), starch synthases (SSs), branching enzymes (SBEs) and debranching enzymes (DBEs). Previous studies indicate that in cereal endosperm protein phosphorylation plays a major role in regulating the formation of functional multi-enzyme complexes between SSs and SBEs during starch biosynthesis and that SSIIa forms the core of a functional protein complex with SSI and SBEIIb. The present study investigated whether maize SSIIa is regulated by protein phosphorylation. *In vitro* phosphorylation of maize SSIIa was detected by phosphate affinity gel electrophoresis and autoradiography following γ-^{32}P-ATP labelling. The activity of native SSIIa was assessed using zymograms, following treatment with ATP and alkaline phosphatase (to dephosphorylate SSIIa). Treatmentof amyloplast lysates with physiological concentrations of ATP (5-100µM) caused a marked shift in the mobility of SSIIa on native gels; while pre-treatment with potent protein kinase inhibitors prevented this ATP- dependent mobility shift suggesting that it is caused by protein phosphorylation. Substrate affinity electrophoresis using amylopectin and glycogen as substrates demonstrated that native SSIIa varied in its affinity towards the two glucans, and pre-treatment with ATP significantly reduced its affinity towards amylopectin. Size exclusion chromatography of amyloplast lysates pretreated with ATP and alkaline phosphatase was conducted to identify specific phosphorylation-dependent interactions of SSIIa with other starch biosynthetic enzymes. Future investigations aim to identify the phosphorylation sites within SSIIa and identify and characterize the protein phosphatases and kinases that modulate this post translational modification.

Systems Biology

P5 Identification Of Carbohydrate-binding Ability Based On Proteome-wide Tertiary Structure Prediction: *in vitro* Validation

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Carbohydrates are often referred to as the third molecular chain of life and their interactions with proteins are thus worthy of attention. Carbohydrates are crucial storage and structural materials, and can also be used as information carriers. Carbohydrate-binding proteins are very diverse in structure and aromatic residues play a significant role in ligand binding. In my study, I am testing the carbohydrate-binding ability of proteins whose predicted tertiary structures contain co-planar aromatic residues at an appropriate spacing to bind carbohydrates. Forty eight expression constructs of the candidate proteins from *Arabidopsis thaliana* have been transferred into *Nicotiana benthamiana* and, after purification, the expressed, tagged proteins were applied to glycan arrays and different binding specificities were observed. The role of these novel carbohydrate binding proteins will be further elucidated through a combination of reverse genetics guided by online resources, hybridization of labeled proteins to sections of plant material, carbohydrate profiling, and other methods.

P6 Global network analysis in *Populus* reveals variation in system-level responses to drought

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Plant responses to environmental perturbations, like drought, are underpinned by the concerted action of genes, giving rise to changes in the transcriptome. To date, most analyses of drought transcriptomes have not considered the regulatory interconnections between genes. Network analyses of drought transcriptomes offer the opportunity to explore the coordinated and hierarchical action of gene regulatory networks shaping environmental responses. Here we analyse transcriptome-level data collected from nine *Populus* genotypes at mid-day and pre-dawn to construct a representation of the drought-responsive, global co-expression network at two time-points during a diel cycle. Gene functional enrichment analysis revealed modules at mid-day and pre-dawn that were involved in different biological processes. This was reinforced by gene regulatory analysis that identified exclusive hub genes and *cis*-motifs within the two networks. We identified substantial shifts in regulatory networks underpinning the drought response. The findings provide insights into the multi-faceted nature of the *Populus* drought response, and suggest testable hypotheses that will further our understanding of the interplay between gene regulation and plant responses to environmental conditions.

P7 Whole plant NMR reveals metabolic adjustments in response to genetic perturbation of cell wall biosynthesis

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The secondary cell wall that surrounds xylem and fibre cells of vascular plants is primarily composed of cellulose, hemicellulose, and lignin. It is of high importance biologically and economically, and has been intensely studied. Nevertheless, some aspects of secondary cell wall formation remain poorly understood. Global metabolomic analysis of a secondary cell wall mutant provides an opportunity to address some of these gaps in knowledge. Here, Nuclear Magnetic Resonance (NMR) has been used to assess metabolic compensatory mechanisms in the *Arabidopsis thaliana* secondary cell wall mutant, *ectopic lignification1* (*eli1*). This mutant has a mutation in the gene encoding the CesA3 subunit of cellulose synthase. *Eli1* mutants have less cellulose in their secondary cell walls, but increased starch and lignin content compared to wild type. The *eli1* mutant has been well-characterized with respect to its morphology and lignification, but it has not previously been characterized in terms of overall metabolism. Intact wild-type and *eli1* plant samples were chemically profiled using NMR to holistically study the overall impact of cellulose synthase inhibition on carbon allocation. Key findings include differences between wild type and *eli1* in the processing of seed-derived lipids, differences in the abundances of many amino acids, and the potential discovery of novel signalling peptides invoking modified secondary cell wall deposition. Wild-type *Arabidopsis thaliana* seedlings grown in the presence of these peptides resemble *eli1* in their patterns of lignification. Current studies aim to confirm this function for the peptides.

Genetics

P8 Characterization and Target Validation of miR156 in *Medicago sativa*

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MicroRNAs (miRNAs), approximately 21-25 nucleotides in length, are sequence specific regulators of post-transcriptional gene expression. It has been shown that miR156 regulates many members of *Squamosa Promoter-Binding Protein-like* (*SPL*) genes and play important roles during vegetative to reproductive phase transition in plants. Although miR156 has been well characterized and studied in many plant species, information about its role in *Medicago sativa* (alfalfa) is still scarce. Thus, we conducted a study to investigate the function of miR156 in this forage and potential bioenergy crop. To determine the number of loci encoding miR156 paralogs in alfalfa, an *in silico* search was conducted using publicly available sequences. Alfalfa plants overexpressing miR156 were generated, and the miR156 cleavage targets were validated using a modified 5′-RACE technique. *In silico* analysis showed that some alfalfa sequence reads (~ 60 bp) were similar to the miR156 precursors but the hairpin secondary structure could not be produced from these short sequences. Of the five predicted target *SPLs* genes, three (*SPL6*, *SPL12* and *SPL13*) contained miR156 cleavage sites and their expression was downregulated in miR156 overexpression alfalfa plants. We also found that *WD40* gene was a potential target for miR156 cleavage. This study provides a foundation for future research into the effects of miR156 on biomass production and quality in alfalfa.

P9 Uncovering the molecular link between miR156 and carotenoid accumulation in Arabidopsis thaliana

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Carotenoid Cleavage Dioxygenases (*CCD*s) are an enzyme family that cleave specific double bonds in carotenoid molecules to produce secondary metabolites known as apocarotenoids. *MicroR156* in Arabidopsis regulates a network of genes by repressing 11 *SPL* genes, among which, *SPL15* was found to regulate shoot branching and carotenoid accumulation. In this study, we investigated whether expression of *CCD* and *NCED* genes is affected by changes in expression of *miR156* and *SPL15*. The expression of *CCD1, CCD4, CCD7, CCD8, NCED2, NCED3, NCED5, NCED6, NCED9* and *SPL15* were evaluated in siliques at 10 days post anthesis and in 10-day-old roots in WT, *sk*156 (miR156 overexpression mutant), *RS075* (miR156 overexpression line), *spl15* (SPL15 knockout mutant) and two 35S:SPL15 lines. Results from root analysis showed that the expression levels of *CCD4, CCD7, NCED2, NCED3, NCED6* and *NCED9* were lower in *sk156* and RS075 compared to WT, and *spl15* showed the lowest expression level of all *CCD* genes. Results from siliques showed that the expression level of *CCD7*, *NCED2, NCED3, NCED5*, and *NCED9* were lower in *sk*156 and *RS*075 relative to WT. Although 35S:SPL15 lines showed the highest expression of *SPL15*, some *CCD* genes showed lower expression in 35S:SPL15 compared WT, and even in comparison to *sk*156 and *RS*075. We conclude that increased *miR156* expression suppresses expression of *SPL15* resulting in a lower level of *CCD/NCED* expression and enhanced carotenoid accumulation.

P10 IMPA2, a potential interactor of ABA2, is involved in Arabidopsis seed germination

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Abscisic acid (ABA) is a plant hormone involved in many physiological processes in the plant, such as germination and various stress responses. One enzyme in the ABA biosynthesis pathway, ABA2, is a short-chain dehydrogenase/reductase that functions to convert xanthoxin to abscisic aldehyde. The unique protein expression pattern of ABA2 compared with other ABA biosynthetic enzymes during drought stress, makes it an interesting candidate to study. Using yeast-two-hybrid screens, we isolated potential interactors of ABA2. Yeast-two-hybrid analyses has revealed a number of proteins whose localization is distinct from ABA2, suggesting that ABA2 may serve as important node of cross-talk among many physiological processes in the plant, such as energy metabolism and regulation between phytohormones. One candidate, IMPA2 (importin alpha isoform 2), has been shown to confer physiological importance in relation to ABA-mediated processes. IMPA2 belongs to the importin alpha family, which functions as nuclear-transport receptors. Germination assays show that loss of function mutant of IMPA2 germinates faster than wildtype, as well as other family members of IMPA, specifically IMPA1, IMPA3 and IMPA7. Taken together, these findings suggest that potential function of IMPA2-ABA2 interaction may be crucial during Arabidopsis germination and should be further investigated.

P11 Functional analyses of the ABA signaling genes at the cellular level.

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Genetic screens based on whole plant phenotypes such as seed germination and early seedling growth have led to the development of a core ABA signalling pathway. However, many of the steps involved in core signalling are genetically redundant and often loss-of-function mutations in these steps result in no obvious whole plant phenotype. To investigate this matter more closely several GFP reporter genes that respond to ABA were introduced into Arabidopsis and crossed to a variety of loss-of-function ABA mutant backgrounds. Based on confocal microscopic analysis, we find that a number of mutant shows clear ABA-dependent cellular based phenotypes although they have no or very weak whole plant ABA related phenotypes. These results will be discussed with respect to the next generation of phenotypic screens that need to be done to uncover genes that regulate ABA signalling.

P12 Evidence for polygenic and regulatory evolution in rice

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P13 Regulation of FUSCA3 accumulation during seed Germination at high temperature in *Arabidopsis thaliana*.

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FUSCA3 (FUS3) is a B3-domain transcription factor required for the proper development of the embryo during seed maturation in *Arabidopsis thaliana*. *FUS3* is expressed at a high level during mid-embryogenesis, where it regulates the accumulation of seed storage compounds, the establishment of dormancy and desiccation tolerance. FUS3 is a central regulator of hormone
levels and it is itself regulated by hormones: *FUS3* expression is induced by auxin, while protein level is negatively regulated by GA and positively regulated by ABA through a C-terminal PEST degradation motif. In turn, FUS3 regulates ABA and GA levels in an opposite way.
Our recent results have shown that *FUS3* mRNA and protein levels increase during seed imbibition at high temperature. However, the accumulation of the FUS3 protein occurs only after long exposure to heat stress, suggesting that it may regulate cellular responses after prolonged
exposure to high temperature. Interestingly, overexpression of FUS3 during germination confers increased seedling survival against high temperature stress by delaying seed germination at high temperature. However, the levels of *FUS3* mRNA and protein do not correlate during germination under heat stress and non-stressed conditions, suggesting translational or
post-translational regulation controlling FUS3 level. Here, we attempt to elucidate the post-translation regulation of FUS3 by heat stress by measuring its degradation in a cell-free environment. Using chemical and genetic approaches, our results show that changes in hormone
levels regulate FUS3 accumulation during heat stress.

P14 A Chemical Screen in *Arabidopsis* and *S. cerevisiae* Identifies Novel Compounds with Potential Strigolactone Activity

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Strigolactones (SLs) are a relatively newly discovered class of plant hormones. SLs were originally identified for their ability to stimulate the germination of parasitic plants of the genera *Striga* and *Orobanche*, but since that discovery it has become clear that SLs are also involved in the promotion of symbiosis with arbuscular mycorrizal fungi, the suppression of axillary branching, and other functions. The α/β hydrolase DAD2 was recently shown to be the likely receptor for SLs in petunia. It was also shown that the synthetic SL GR24 is able to stimulate the interaction between DAD2 and the SL signalling component MAX2. *D14b* is an *Arabidopsis* ortholog of *DAD2* that has been shown to be important in the germination related effects of SLs. 4200 small bioactive molecules were screened for the ability to stimulate the interaction of D14b and *Arabidopsis* MAX2, as measured by a yeast two hybrid system.
The E3 ligase COP1 suppresses photomorphogenesis, and its activity antagonized by SL signalling. In over-expression lines of GUS fused to COP1, a distinctive elongated hypocotyl phenotype is apparent. The chemical library was screened for the ability to suppress the long hypocotyl phenotype of *35S::GUS-COP1 Arabidopsis* seedlings. Several hits were shared between the yeast two hybrid screen and the plant screen. This suggests that these compounds may have SL activity despite lacking an obvious structural similarity to canonical SLs.

Crop Productivity

P15 H_2 oxidization increases soil ACC deaminase activity as part of rotation benefit

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Crop rotation with legumes has been widely used in agriculture practice to promote plant growth and yield. N left over from legumes in the field has often been considered as the main cause of benefit to succeeding crops. Recently, regulation of plant ethylene production by H_2 stimulated rhizobacteria has been proposed as one of the mechanisms to explain the rotational benefit from legume plants. To understand the bacterial activity on plant ethylene control, eight sets of DNA primers for ACC deaminase genes found in different taxonomic groups were designed and used for gene quantification and expression in soil. The results showed that both gene copy number and expression level of ACC deaminase gene increase dramatically after H_2 treatment. In soil adjacent to active nodules of soybeans and fababeans, ACC deaminase genes were higher in both DNA and mRNA levels. Higher gene copy number and expression level were found in rhizospheric soil of barley growing in H_2-treated soil. This suggests that the expression of ACC deaminase gene and population of bacteria carrying the gene has been enhanced by H_2 treatment. ACC deaminase gene expression level will be tested in eight H_2 oxidizing isolates induced by ACC as a nitrogen source. An attempt to obtain entire ACC deaminase gene from H_2-oxidizing isolates is underway by Genomic Walking and will be expressed in *E.coli* to test the gene activity and function on plant growth.

P16 Profiling the root and seed surface lipids of the oilseed crop *Camelina sativa*

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Camelina sativa is an emerging crop with seed oil rich in omega-3 fatty acids and composition suitable for biofuel production. *Camelina* is relatively drought-tolerant and requires much less fertilizer than other oilseed crops, such as Canola (*Brassica napus*). Its seed meal can be used for animal feed and to produce various biodegradable materials. To further develop this alternative oilseed crop, more information is required about its agronomic properties and phytochemical profile. Various lipid- and phenolic-based extracellular barriers of plants help to protect them against biotic and abiotic stresses. One of these barriers is suberin found in the endodermis and peridermis of roots and in the seed coat. The chemical compositions and morphological features of extracellular lipids of *Camelina* seeds and roots were analyzed using GC-MS, GC-FID, and microscopy. Seed coat polyester and root suberin contained *p*-hydroxycinnamic acids, mainly ferulate, and a mixture of aliphatic monomers, with 18-hydroxyoctadecenoic and 1,18-octadecenedioic acids being dominant. Very-long-chain fatty acids and derivatives, including saturated C20:0 and C22:0 ω-hydroxyfatty acids, α,ω-dicarboxylic acids and primary fatty alcohols, were also present. Chloroform-extractable root waxes were primarily composed of alkyl hydroxycinnamates, predominantly C18:0 and C20:0-alkyl esters of caffeic acid and coumaric acid. Compositional data was compared with histochemical and ultrastructural analyses of root sections, which revealed characteristic suberin autofluorescence, Sudan dye-specificity, and suberin lamellae depositions in cell walls of the periderm. This detailed description of the protective surface lipids of *Camelina* may provide insights into its drought-tolerant and pathogen-resistant properties, and also provide an additional source of high-value lipid components that can be extracted from the plant.

P17 The Effect of Long and Short-term CO_2 Enrichment on Gas Exchange, Water Use Efficiency, ^{14}C-Export and C-Partitioning in Iridoid Glycosides in *A. majus*

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Two cultivars, GI and GIV, of *A. majus* were grown and assayed under 40 Pa CO_2 (Control-1 (40=>40)) and 91 Pa CO_2 (Control-2 (91=>91)). Whole-plant and leaf photosynthesis, ^{14}C-export rates and ^{14}C-partitioning patterns among major photo-assimilates were determined. In addition to measuring traits at both growth conditions plants were transferred for a short-term analysis (TS) at the reciprocal CO_2 level (i.e., (TS1 (40=>91)) and at 40 Pa CO_2 (TS2 (91=>40)). In Control-2 (91=>91) leaf and whole-plant gas exchanges were similar indicating minimal mutual shading. Both leaf and whole-plant photosynthetic rates were down-regulated from the TS1 (40=>91) treatments in the GI cultivar but not in the GIV cultivar. However, during acclimation to high CO_2 (Control 2 (91=>91)) export rates of both cultivars were higher than those of all other treatments reflecting the increase in sink demand during long-term high CO_2 acclimation. Also the immediate export rate relative to C-fixation of both cultivars was about 75% for both Control 2 (91=>91) and TS2 (91=>40) in both cultivars. The amount of ^{14}C-partitioned to major IGs was similar under ambient and enriched CO_2 growth; however the sucrose to antirrhinoside ratio was lower under Control 2 (91=>91) and TS 2 (91=>40) treatments due to decreased partitioning of ^{14}C into sucrose.

P18 Introduction of nitrogen fixing bacterium *Gluconacetobacter diazotrophicus* into corn

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Nitrogen is a primary constituent of nucleotides and proteins and thus is essential for all forms of life. Although the atmosphere contains 78% nitrogen, the N_2 diatomic form is inaccessible to most plants. The Green Revolution has seen a huge increase in crop yields due to the massive use of fertilizer. However, recently, the scale of fertilization has come under extensive criticism for two major reasons. First, industrial nitrogen fertilizer production requires huge amounts of energy and is the most expensive part of modern farming. Second, fertilizer uptake by crops is inefficient – often less than 50% of applied nitrogen fertilizer is used by plants. The consequence is that most of the fertilizer leaches from the soil, resulting in environmental problems, including contamination of water systems, eutrophication of lakes and, at the extreme, massive habitat destruction. *Gluconacetobacter diazotrophicus* is an endophytic non-nodule forming nitrogen-fixing bacterium and it can supply its natural host sugarcane with significantly amount of nitrogen. We investigated the possibility of establishment of symbiosis of *G. diazotrophicus* and corn. The resulted showed that *G. diazotrophicus* can be introduced into a large number of corn varieties, including grain corn, sugary-type corn and sweet corn varieties. Detailed analysis showed that the bacterium can move from the inoculated roots to other organs of the plant including stems and leaves. This indicates that the *G. diazotrophicus* can positively adapt to new and related plant species. The research is an important step to use this bacterium for nitrogen fixation in different monocotyledon plants.

P19 CO_2 enrichment of *Arabidopsis thaliana* with partially-suppressed mitochondrial pyruvate dehydrogenase kinase enhances photosynthetic capacity of the inflorescence contributing to increased productivity.

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Sink limitation reduces photosynthesis and productivity particularly under elevated-CO2 (EC). Whole-plant gas analyses of *Arabidopsis thaliana* confirm that the inflorescence contributes over 90% of daily-C-gain (dC) during maturation under ambient (AC) and EC. On a plant-basis rates of photosynthesis (Pn), dark respiration (Rd) and dC were 800-1000% higher when the inflorescence was developed as when rosette leaves alone sustained growth. Although Pn, Rd and dC expressed on a dry-matter-basis were 50% of those at the rosette stage because of biomass allocated to branching structures, when expressed on a surface-area-basis, canopy Pn, Rd and dC remained remarkably constant demonstrating the importance of the inflorescence to carbon-use-efficiency (CUE). Plant water-use-efficiency (WUE) during inflorescence development was double that when the rosette-leaf canopy functioned alone, being further enhanced by CO2 enrichment. The wild-type (WT), plasmid control (pBI121) and two transgenics (10'4 and 3'1) having partially-suppressed expression of mitochondrial-pyruvate-dehydrogenase-kinase (mtPDHK) constitutively, all demonstrated increases in Pn, Rd, dC and WUE at EC. However, differences among lines were observed at particular stages of canopy development. For example, at the rosette stage when laminar structures contributed mainly to canopy Pn, the WT and PBI121 controls were more responsive to EC than were the mutants; however, during a late inflorescence stage, 10'4 and 3'1 responded more. These studies underscore 1) the need for profiling key phenotypic traits such Pn and Rd throughout development as the relative balance among sources and sinks changes, and, 2) the importance of mtPDHK and dR in anabolic processes such as oil synthesis.

Environmental Stress

P20 Feeding Hungry Plants: the Secreted Purple Acid Phosphatase Isozymes AtPAP12 and AtPAP26 Play a Pivotal Role in Extracellular Phosphate-scavenging by *Arabidopsis thaliana*

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Orthophosphate (Pi) is an essential, but limiting macronutrient for plant growth. Extensive soil P reserves exist in the form of organic-P (Po) which is unavailable for root uptake until hydrolyzed by secretory acid phosphatases (APases). The predominant purple APase (PAP) isozymes secreted by roots of Pi-deficient (-Pi) *Arabidopsis thaliana* were recently identified as AtPAP12 (At2g27190) and AtPAP26 (At5g34850). The present study demonstrates that exogenous Po compounds such as glycerol-3-P or herring sperm DNA: (1) effectively substituted for Pi in supporting the P nutrition of Arabidopsis seedlings, and (2) caused upregulation and secretion of AtPAP12 and AtPAP26 into the growth media. When cultivated under -Pi conditions or supplied with Po as its sole source of P nutrition an *atpap26/atpap1*2 T-DNA double insertion mutant exhibited impaired growth coupled with >60 and >30% decreases in root secretory APase activity and rosette total Pi concentration, respectively. Development of the *atpap12/atpap26* mutant was unaffected during growth on Pi-replete media, but was completely arrested when 7-day-old Pi-sufficient seedlings were transplanted into a -Pi, Po containing soil mix. Both PAPs were also strongly upregulated on root surfaces and in shoot cell wall extracts of -Pi seedlings. We hypothesize that secreted AtPAP12 and AtPAP26 facilitate the acclimation of Arabidopsis to nutritional Pi deficiency by functioning: (1) in the rhizosphere to scavenge Pi from the soil's accessible Po pool, while (2) recycling Pi from endogenous phosphomonoesters that have been leaked into cell walls from the cytoplasm. Thus, AtPAP12 and AtPAP26 are promising targets for improving crop P-use efficiency.

P21 Coping with crowding: a role for ethylene production and response in *Oryza sativa* under density stress

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Intra-specific competition between crop plants is of particular interest to agriculture due to the negative impact of density stress on yield. Several approaches were used to study the response of plants grown in close proximity to elucidate the complex nature of density stress. Genome-wide expression, metabolite profiling and physiological analysis were used to investigate the molecular mechanisms underlying density stress response in rice (*Oryza sativa)*. In addition the relationship between nitrogen limitation and density stress was examined at different developmental stages. Plants grown under high density produced less biomass and chlorophyll, fewer tillers, and had significantly lower yield in comparison with plants grown at low density. Microarray data revealed a number of ethylene related genes that were differentially regulated in plants grown under high density stress. In particular, qRT-PCR analysis confirmed expression of *ACC oxidase 7*, *ACC synthase 1* and *ACC synthase 2* by was higher in high density stress plants. Increased expression of ethylene response genes, such as *ERD1*, and decreased expression of cytokynin production (*IPT10*) and response regulation (*RR2*) genes was also demonstrated in high density grown plants, further implicating a connection to ethylene. When nitrogen limitation was factored in, comparison of molecular changes between high and low density grown plants showed changes in similar processes, but, interestingly, different genes were involved. Our results indicate that rice uses ethylene to regulate various aspects of growth and development under high density growth conditions.

P22 Role of CMLs 38 and 39 in salt and freeze stress tolerance

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Calcium (Ca^{2+}) is an important and versatile second messenger in organisms despite its potential toxicity at high levels. Ca^{2+}-oscillations linked to external stimuli are detected by 'Ca^{2+}-sensors/ Ca^{2+}-signalling proteins', which in turn, initiate stress response pathways. Plants contain a unique group of Ca^{2+}-signalling proteins called calmodulin-like (CML) proteins, whose role in stress response pathways remains unknown. The role of a small subfamily of stress-induced CMLs (CMLs 37, 38, 39) was investigated using reverse-genetic approaches and dehydration-based stress assays. Single knockout lines were phenotypically normal whereas CML38 and 39 double knockouts plants showed increased secondary inflorescence production. Surprisingly, these double mutants displayed increased salt tolerance compared to the wildtype Arabidopsis plants indicating a possible role of these CMLs in negative regulation of salinity response. This data suggests an overlapping role for these CMLs in development and stress response.

P23 Growth Irradiance and Purine Ring Catabolism in *Thellungiella*

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Purine compounds perform essential metabolic roles and are integral components for many cellular processes. Levels of purines and their derivatives, such as ureides, are regulated by the metabolic processes of *de novo* synthesis, salvage and degradation pathways. In non-N_2-fixing species, ureides are important as nitrogen storage and transportation compounds, thought to contribute to the recycling/remobilization of nitrogen from source to sink organs. The remobilization of plant metabolites occurs readily during abiotic and biotic stress conditions, facilitating environmental acclimation and adaptation. These metabolic changes are associated with accelerated catabolic activities and it has been suggested that purine catabolism may be critical for the survival of the plant under stress conditions. The objective of this study was to examine ureide metabolism in two ecotypes of the crucifer *Thellungiella salsuginea* , a halophytic species related to *Arabidopsis* and a model plant for the examination of stress tolerance. Varying growth irradiance as our environmental stress, our results demonstrate increases in total ureide accumulation under both low- and high-light growth conditions. Furthermore, it appeared that key genes in the pathway of purine catabolism were also modulated in response to the growth irradiance based on transcript accumulation. The regulation of this pathway and the accumulation of ureides may be important in the context of metabolic reprogramming as part of plant responses to environmental stress.

P24 Drought response and growth performance strategies in hybrid poplars

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Both genetics and prior environmental conditions can shape an organism's response to the prevailing environment. This is particularly important for plants with long generation times, such as tree species, that must be able to content with fluctuating environmental conditions throughout their long lifetimes. Here we focus on the role played by previous environmental conditions in shaping current responses to environment perturbation using hybrid poplars, trees of economic importance in Canada and worldwide. We study the effect of genotype and the persistent influence of past experiences on plant performance under non-stress and drought-stress conditions. Six different genotypes were compared. For each genotype, vegetatively propagated plants from different geographic locations (i.e. genetically identical individuals with different clone histories) were studied. Under common garden conditions, analyses of growth performance and physiological responses uncovered genotype-specific differences, as well as differences based on geographic origin of poplar clones with identical genotypes. Modifications in resource allocation (e.g. between above-ground and below-ground biomass) and physiological parameters reveal variation in the strategies employed to contend with drought between genotypes. Moreover, differences between genetically identical plants with different clone histories revealed within-genotype plasticity. Comparison of this plasticity supports a classification of the investigated hybrid poplars into "stable" or "unstable" performers (i.e. genotypes with lower and higher location-dependent variability). Analyses of transcriptome patterns and epigenetic marks are currently underway to obtain insights into the mechanism involved. The project will contribute to our understanding of plant-environment interactions, with applications related to nursery source effects in poplar clones and their impacts on future clone performance in plantations.

P25 Cadmium-induced stress in *Arabidopsis thaliana* triggers up-regulation of phosphoenolpyruvate carboxylase (PEPC) isoenzymes and expression of PEPC specific kinase (PPCK)

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One of the main non-occupational sources for human exposure to cadmium (Cd) is through consumption of crops grown in contaminated soil. Understanding the biochemistry of the uptake and sequestration of Cd in plants would give insight into how toxic metal accumulation can be minimized and ensure safer crops. Phosphoenolpyruvate carboxylase (PEPC) catalyzes an anapleurotic pathway feeding into the tricarboxylic acid (TCA) cycle, increasing the production of metal-chelating organic acids. PEPC activity is tightly controlled by PEPC specific kinase (PPCK) phosphorylation, minimizing PEPC's sensitivity to the accumulation of the allosteric inhibitor malate while concurrently enhancing activation by glucose-6-phosphate. We studied the effect of each *Arabidopsis thaliana* plant PEPC isoenzyme (*AtPPC1—AtPPC3*) on the seedling's response to Cd-induced stress using gene knockout mutants. In roots and shoots relative gene expression of *AtPPC1—AtPPC3* and *AtPPCK1—AtPPCK2* as well as PEPC specific activity were up-regulated at least two-fold in response to 1 and 5 µM $CdCl_2$. Gene expression and specific activity were greater in roots than in shoots. Therefore PEPC must play a role in the plant's response to Cd. These changes in isoenzyme amounts and activities will be compared to Cd-induced changes in metal-binding organic acids. This knowledge will be relevant to agricultural bioengineers involved in creating crops with an improved resistance to and reduced uptake of Cd.

P26 Light Pollution Disrupts the Coordination between Circadian Rythms of Nitrate Update by Roots and Nitrate Assimilation in Shoots for Tomato (*Solanum lycopersicum* L.)

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Light pollution in controlled environments including its effects on photoperiod can impact plants and animals. Light entrains the circadian clock in natural environments. Photoperiodic injury (PI) is a physiological disorder characterized by chlorosis and necrosis of leaves when plants are grown under either long photoperiods or non-24 h light-dark cycles. We have shown a correlation between PI and both altered circadian patterns for the nitrate assimilatory enzymes nitrate reductase (NR) and nitrite reductase (NiR) in tomato shoots, and foliar accumulation of the toxic metabolite nitrite, particularly at times of day (TOD) when the NiR/NR activity ratio is low. PI-tolerant and PI-susceptible tomato lines were grown under various photoperiod regimes to determine: (1) if a positive correlation exists between PI and nitrite accumulation; (2) if 24 h light affects the diel pattern of nitrate uptake in a way that favours PI; and (3) if PI can be alleviated by altering nitrate nutrition at two specific TOD when tomato is susceptible to nitrite accumulation. A strong positive correlation was found between leaf nitrite accumulation and PI. Determination of diel patterns of root nitrate uptake showed that nitrate uptake rate per se is not responsible for PI. Instead, maintenance of circadian nitrate uptake patterns by roots even in 24 h light combined with a loss of circadian activities of NR and NiR in shoots causes PI. PI was significantly decreased by reducing nitrate in the nutrient solution by 75% at two 4-h periods in the day when tomato is susceptible to nitrite accumulation.

P27 Is *in vitro* generated *Eriophorum vaginatum* resistant to heavy metals?

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Eriophorum vaginatum (cotton-grass; Cyperaceae), a tussock forming sedge, is ecologically important wide spread species in Boreal peatlands and Arctic tundra in Northern Hemisphere. *E. vaginatum* is flourishing in the phytotoxic wetlands surrounding Sudbury, ON, Canada, where extensive copper/nickel mining and smelting resulted in environmental devastation. Application of lime and tree planting partially revegetaded polluted soils, but contaminated wetlands are regenerating naturally with dominating populations of *E.vaginatum*. Our research is focused on determining how *E. vaginatum* survives in wetlands contaminated with industrial-borne pollutants, especially phytotoxic chemicals and heavy metals. Majority of field-collected seeds are not viable and natural establishment of new cotton-grass colonies is slow. We have used aseptic tissue culture techniques to regenerate this plant in laboratory and a large colony of *in vitro* regenerated plants is now acclimated in the greenhouse. Those plants can be used as propagules in the process of revegetation of polluted wetlands. Using molecular techniques we have determined that *in vitro* regenerated *E. vaginatum* is genetically stable and identical to original field-collected source plants. The current focus is to assess the pollution tolerance fidelity of *in vitro* regenerated *E. vaginatum* by growing it in metal contaminated soils collected from four different sites surrounding Sudbury: Crowley Lake, South East Bypass, Hess Creek and Cartier. Our results indicate that heavy metal tolerance is innate in this plant species as *in vitro* regenerated cotton-grass grew well in the greenhouse in nutrient-poor, highly acidic, and metal-polluted soils from Sudbury. Subsequent studies will aim to determine the mechanism of this resistance at the molecular level.

Climate Change Impacts

P28 Elevated growth temperatures alter hydraulic characteristics in trembling aspen: implications for tree drought tolerance

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Although climate change will alter both soil water availability and evaporative demand, our understanding of how future climate conditions will alter tree hydraulic architecture is limited. Here, we demonstrate that growth at elevated temperatures (ambient +5°C) affects hydraulic traits in seedlings of the deciduous boreal tree species Populus tremuloides, with the strength of the effect varying with the plant organ studied. Temperature altered the partitioning of hydraulic resistance, with greater resistance attributed to stems and less to roots in warm-grown seedlings ($p<0.02$), and a 46% (but marginally significant, $p=0.08$) increase in whole plant conductance at elevated temperature. Vulnerability to cavitation was greater in leaves grown at high than at ambient temperatures, but vulnerability in stems was similar between treatments. A Soil-Plant-Atmosphere (SPA) model suggests that these coordinated changes in hydraulic physiology would lead to more frequent drought stress and reduced water use efficiency in aspen that develop at warmer temperatures. Tissue-specific trade-offs in hydraulic traits in response to high growth temperatures would be difficult to detect when relying solely on whole plant measurements, but may have large-scale ecological implications for plant water use, carbon cycling, and, possibly, plant drought survival.

P29 Climate change and permafrost degradation in a boreal forest: impacts on ground vegetation

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High latitude regions continue to experience the most rapid rates of climate warming with some areas having already warmed by at least 3ºC [1]. The most immediate and system-altering manifestation of this is the thaw and degradation of permafrost (perennially cryotic ground). Much of the zone of discontinuous permafrost is characterized by boreal forest-wetland mosaics; the forests are underlain by permafrost while the wetlands are permafrost free. These permafrost plateaus impede water movement among adjacent wetlands, thus permafrost thaw may lead to increased wetland homogenization through increases in connectivity [2,3,4]. At Scotty Creek, NWT, a range of wetland types exists from isolated flat bogs to channel fens. Increased connectivity may be altering water chemistry and plant community composition and function. To examine this possibility, we sampled three types of bog: isolated bogs, bogs with ephemeral hydrological connections to other bogs and bogs connected to a fen. 27 sites were chosen and five $1m^2$ quadrats sampled within each site. At each quadrat, vascular and non-vascular plants were identified to species, nutrients and physical soil parameters were measured. Non-metric multidimensional scaling was used to examine patterns of community composition and relate this to water chemistry and other soil factors. Strong community clustering was detected though the patterns suggest that this may be driven by terrestrial or geologic inputs rather than changing hydrological connectivity.1. IPCC Fourth Assessment Report (2007); 2. Shur et al. (2007) Permafrost Periglac 18:7; 3.Beilman et al. (2003) pp.65 4. Camill (1999) Can J Bot 77 :721

P30 Effect of simulated future climate on autumn freezing tolerance of *Pinus strobus*

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Within the next century, temperature and CO_2 are predicted to increase due to anthropogenic climate change. These environmental changes will affect plant phenology, which is driven by temperature and daylength. We use Eastern white pine (*Pinus strobus*) to examine the effects of future climate on autumn cold hardening in North American conifers. 2-year-old seedlings were grown at ambient and elevated CO_2 summer conditions for six weeks and then shifted to normal, warm, and warm elevated CO_2 autumn conditions. Shoots from summer and autumn-acclimated seedlings were assessed for freezing tolerance at 0, -10 and -20°C using chlorophyll fluorescence. Results show that both summer and autumn trees can withstand 0°C exposure; -10°C does not affect autumn plants, but severely impacts summer plants, and also reveals increased susceptibility to freezing in elevated CO_2 summer tissues; -20°C exposure is lethal to summer-acclimated plants. Normal autumn shoots were unaffected by all three levels of freezing and even increased photosynthetic activity ($\Delta Fv/Fm > 0.1$) when measured 1 month after freezing exposure to -20°C. In contrast, warm autumn shoots suffered mild damage at -20°C ($\Delta Fv/Fm < -0.05$) and showed impaired ability to recover at -20°C. Warm elevated CO_2 autumn shoots suffered mild damage at -10°C, moderate damage at -20°C ($\Delta Fv/Fm > -0.1$), and were unable to recover from freezing damage. Visual observations identified warm elevated CO_2 plants by extensive tissue necrosis 1 month after -20°C exposure. We conclude that warming will negatively impact autumn frost tolerance, and that this negative effect will be enhanced by elevated CO_2.

Organelle Biology

P31 The C-terminus of *Bienertia sinuspersici* Toc159 receptor contains essential elements for its targeting and anchorage to the chloroplast outer membrane

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The majority of chloroplast proteins rely on an N-terminal transit peptide (TP) as a sorting signal for translocation into the organelle, which is mediated by the Toc-Tic complex.
Although Toc159 is known to be a preprotein receptor for specific recognition of TPs at the chloroplast surface, the mechanism for its targeting and integration to the chloroplast outer membrane is not known. Previously, we demonstrated that the C-terminus (CT) of *Bienertia sinuspersici* Toc159 possesses physicochemical and structural properties of TPs, capable of targeting green fluorescent protein (GFP) to the chloroplast surface or stroma depending on its orientation and fusion position (Lung S.C. & Chuong S.D.X. 2012. Plant Cell 24: 1560-1578). In this study, we further investigated the essential elements of this sorting signal by examining the ability of various lengths and truncation of the CT to target GFP in onion epidermal cells and *Arabidopsis thaliana* mesophyll protoplasts. We showed that the 56-residue region of BsToc159 CT functioned as a TP-like sorting signal and mediated reversible binding of the fusion proteins to the envelope, whereas the upstream region contains essential motifs for membrane anchorage, which rendered the fusion proteins resistant to alkaline extraction. Compared to the equivalent regions of *A. thaliana* Toc159 isoforms, the CTs of *B. sinuspersici* isoforms are more efficient sorting signals, suggesting some uniqueness of the targeting of Toc159 receptors to the dimorphic chloroplasts in the single-cell C_4 species.

P32 Expression and Purification of a Tic Complex Protein for Functional and Structural Studies

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Tic20 is a protein of the inner envelope membrane of chloroplasts involved in the translocation of proteins into the chloroplast. While there is evidence suggesting it serves as the main protein-conducting pore at the inner membrane, further functional and structural analysis is needed to confirm this hypothesis. To facilitate such analysis, a protocol for the expression and purification of a recombinant version of the protein has been developed and optimized. A variety of expression conditions were tested using traditional IPTG induction and auto-inducing media. A selection of detergents was screened to determine optimal solubilization conditions for isolation. Growth at 20^{o}C in auto-induction media ZYP-5052 provided the highest level of recombinant protein expression. Interestingly, expression under these conditions resulted in the targeting of Tic20 to the bacterial membrane, which necessitated the adoption of a membrane isolation procedure. The zwitterionic detergent Zwittergent 3-14 was the most efficient for solubilizing Tic20 from the bacterial membrane. These findings will serve as a foundation for resolving the crystal structure of Tic20, and studies exploring the interaction between Tic20 and chloroplast-targeting transit peptides. These data may also yield some insight into the behavior of small recombinant proteins in bacterial expression systems.

P33 TOC complex assembly: an examination of the interactions between TOC GTPases

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Chloroplast-destined preproteins are translated in the cytosol, and post-translationally targeted to and translocated across the double envelope membrane of the chloroplast by the coordinated activities of two translocon complexes: the Translocons at the Outer and Inner envelope membrane of the Chloroplast (TOC and TIC, respectively). In the model organism *Arabidopsis thaliana* the core TOC components include two families of GTPase receptors: TOC159 (atToc159, -132, and -120) and TOC34 (atToc33 and -34). These receptor families recognize transit peptides present on the N-terminus of chloroplast-destined preproteins. The GTPase domains of these families are hypothesized to interact in such a way that structurally and functionally distinct TOC complexes are formed. These distinct complexes are thought to have specificity for different subsets of preproteins.

Chloroplasts must differentiate between different subsets of proteins because they are needed in different amounts during various stages of chloroplast biogenesis. We are investigating the propensity for atToc33 and atToc34 to associate with atToc159 or atToc132 using both *in vitro* competitive chloroplast targeting assays, and binding assays in which the GTPase domains of atToc33 or atToc34 are used to test interactions with atToc159 or atToc132. In order to study the influence of the highly divergent A-domain, these associations are also being investigated using A-domain deletion mutants (atToc159GM and atToc132GM), and A-domain swapped mutants (atToc159A132GM and atToc159A132GM). These investigations are giving insight into how key TOC components are assembled into distinct TOC complexes at the chloroplast surface, which are responsible for importing different subsets of preproteins.

P34 A novel C-terminal dibasic motif mediates the targeting of a subset of tail-anchored proteins to the mitochondrial outer membrane

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Tail-anchored (TA) proteins are a unique class of functionally diverse membrane proteins that are defined by their single C-terminal membrane-spanning domain and their ability to insert post-translationally into specific organelles with an $N_{cytosol}$-C_{lumen} topology. The molecular mechanisms by which TA proteins are sorted to different organelles, however, are not well understood. Here we present results indicating that the C-terminal dibasic targeting signal motif -R-R/K/H-X identified previously in the mitochondrial isoform of TA cytochrome b_5, also exists in several other mitochondrial outer membrane TA proteins, but this motif is conspicuously absent in all but one of the TA protein subunits of the translocon of the outer membrane (TOM), suggesting that these two sets of TA proteins utilize different targeting pathways. Consistent with this premise, we show that the C-terminal sequences of the mitochondrial TA proteins that possess a dibasic motif, but not the TA TOM proteins, are both necessary and sufficient for their proper targeting to the mitochondrial outer membrane, and are also interchangeable. We also present results of a mutational analysis of the dibasic motif and adjacent sequences that not only greatly expands the functional definition and context-dependent properties of this targeting signal, but also allowed for the identification of a novel mitochondrial outer membrane TA protein that utilizes the same targeting signal. Collectively, these results provide important insight to the complexity of the targeting pathways involved in TA protein biogenesis and define the consensus dibasic targeting motif utilized by a subset of mitochondrial TA proteins.

P35 A unique N-terminal sequence in the CIRV replicase protein p36 is responsible for recruiting Vps23 during ESCRT-mediated re-modeling of the mitochondrial outer membrane

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Carnation Italian ringspot virus (CIRV) and *Tomato bushy stunt virus* (TBSV) are plus-strand RNA viruses that cause massive structural alterations of mitochondria and peroxisomes, respectively, in infected host cells, including the formation of numerous spherules derived from the organelle's outer boundary membrane that serve as sites for viral replication. Recent studies have indicated that these changes in the peroxisomal membrane in TBSV-infected cells are mediated by the recruitment or 'hijacking' of the host-cell's endosomal sorting complex required for transport (ESCRT) machinery. Specifically, the TBSV membrane-bound replication protein p33 binds to the ESCRT-I component Vps23 via a proline-rich late-domain-like motif and several ubiquitinated lysines in p33, resulting in Vps23 and possibly other components of ESCRT being recruited to peroxisomes from late endosomes where they normally function. Here we show that the CIRV replication protein p36 also recruits Vps23 to mitochondria, and that this recruitment is mediated by a unique sequence located near the N terminus of p36, but which is absent in p33. We show also that the Vps23-recruitment sequence in p36, unlike p33, does not rely on a proline-rich late-domain-like motif or ubiquitinated lysines. However, the Vps23-recruitment sequence in p36 is predicted to form an α-helix that is reminiscent of the secondary structure of the Vps23-binding domain in the ESCRT-I component, Vps28. Taken together, these data suggest that the molecular mechanisms underlying the 'hijacking' of ESCRT by CIRV and TBSV are distinctly different and that the interaction of p36 and Vps23 may mimic that of Vps28 and Vps23.

P36 Phosphatydylglycerol – Putative key component of chloroplast biogenesis at low temperature

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Previous studies have shown that the activity of plastidic PGP (phosphatidylglycerol phosphate) synthase is reduced by 80% in the *pgp1* mutant of *Arabidopsis thaliana*, causing an approximate 30% reduction in PG content compared to wild-type (WT) plants. The mutant exhibited similar growth rates and phenotype to that of WT plants when grown under optimal growth conditions. However, transferring the mutant plants from 20 to 5°C caused reduced growth and a pale green phenotype compared to WT. This was accompanied by impaired chloroplast biogenesis, decreased biosynthesis of pigments and major chlorophyll-protein complexes as well as decreased photochemical activities in thylakoid membranes of *pgp1* plants grown at low temperatures (5°C). A shift of cold-acclimated mutant plants from 5°C to 20°C was sufficient for re-greening of the *pgp1* leaves. Interestingly, the transfer of WT and *pgp1* mutant from 5 to 20°C demonstrated two different patterns of pigment and protein accumulation. This could be explained by two different biological processes. While WT plants had already developed chloroplast and the shift from 5°C to 20°C involves acclimation mechanism(s) to different growth temperature, the shift of *pgp1* mutant from 5°C to 20°C involves biogenesis of functional chloroplast. The results revealed that PG is not critical for the maintenance of chloroplast structure and functions at optimal temperatures. However, PG is indispensable for biogenesis of functional chloroplast under low temperatures by influencing (directly or indirectly) the synthesis, maturation, transport, stability and degradation of photosynthetic proteins.

Plant Defenses

P37 Mineral oil inhibits the movement of *Potato virus Y* in potato plants and reduces defense gene expression in tubers

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Potato virus Y (PVY) is an aphid-transmitted virus that causes one of the most devastating diseases threatening potato producers worldwide. Spraying mineral oil on potato fields reduces PVY infection, but its mode of action is not fully understood. The purpose of this research was to investigate how mineral oil affects the movement of PVY in potato plants. Plants were mechanically inoculated and inoculated with aphids in the greenhouse and in field trials, and PVY movement in the plants was analyzed using real time PCR. Less viral RNA was detected in systemic non-inoculated plant tissue, including tubers and leaves, of mineral oil-treated plants compared to the control. This was associated with reduced expression of defense genes, such as *Pathogenesis-Related 1* (*PR1*), *PR10*, and *Plant Defensin 2.1*, in tubers of mineral oil-treated greenhouse plants as compared to tubers of control plants. Collectively, this data suggests that mineral oil inhibits PVY movement in potato plants, and this is associated with a diminished defense response in systemic tissue.

P38 PEP peptides and associated receptors in *Arabidopsis* defence response to spider mite feeding

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A growing human population necessitates new and adaptive agricultural technologies to produce large quantities of healthy crops. New approaches to agriculture must provide alternatives to the current use of pesticides to control for herbivorous pests and will undoubtedly be rooted in the field of plant resistance to herbivory. To that end, I am involved in characterizing the participation of several endogenous plant peptides (PEPs) and their receptors (PEPRs) in plant defence response following two-spotted spider mite (*Tetranychus urticae)* attack on the host plant *Arabidopsis thaliana*. PEP peptides are recognized by cell surface receptors and represent a mechanism for the plant to perceive damaged tissue. My hypothesis is that PEPs play a role in defence against spider mites, acting as damage associated molecular patterns. My objectives include determining the significance of PEPRs in the defence response to spider mites using plant damage assays and mite developmental tests on *pepr* mutants as a quantitative measure of plant and mite performance respectively and to check if downstream defence responses are compromised in *pepr* mutants using qRT-PCR of selected defence marker genes. Another objective is to determine the kinetics of PEP precursor and receptor transcripts following spider mite attack using qRT-PCR on wild type plants submitted to a time course treatment of spider mite feeding. Initial results indicate that PEPs and PEPRs play a role in the defence response following spider mite attack, hinting at the possibility of modulating these peptides or their receptors *in planta* to offer a means of enhanced crop protection.

Plant Development Workshop

P39 Functional characterization of flowering time orthologues in sugarcane

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Sugarcane is an important tropical grass that is mostly used for the production of sugar and ethanol in Brazil. Understanding the regulatory mechanisms of flowering in this species is crucial as it impacts commercial production systems as well as breeding programs. Floral induction is responsible for sucrose remobilization from the stalks to initiate reproductive growth. As a result, the stalks start drying and sugar accumulation is lost. Nevertheless, little is known about the regulatory mechanisms in sugarcane. Photoperiod is the primary signal of flowering induction. Genes related to photoperiodic control of flowering in Arabidopsis include *FT*, which is expressed under inductive conditions in the leaves and transported to the shoot apex to induce the floral transition. *ID1* is a monocot-specific zinc finger transcription factor that is predominantly expressed in immature leaves of maize, regardless of the day-length. Aiming to understand the genetic control of the floral induction in sugarcane, we have isolated putative *FT*, *TFL1* and *ID1* orthologues (*ScFT*, *ScTFL1* and *ScID1*, respectively). Quantitative and semi-quantitative expression analyses demonstrate that these genes share the same expression patterns as their putative orthologs. Functional activity of these genes is being tested by over-expression of *ScFT* and *ScTFL1* into Arabidopsis flowering time mutants. In addition, the closely related C4 model plant *Setaria italica* is being transformed with sugarcane flowering time genes to test for function. So far, Arabidopsis T1 seeds have been selected and genotyped to confirm the presence of the transgenes. Future directions include screening independent Arabidopsis and *Setaria italica* lines.

P40 Functional analysis of a unique *β-glucosidase* gene linked to the maize floral transition

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The plant shoot apical meristem (SAM) gives rise to all aerial organs, including leaves and flowers. During the floral transition the SAM becomes competent to receive a florigenic signal, which causes a switch from vegetative to reproductive growth. This is a nonreversible developmental change in many higher plants, such as maize (*Zea mays*). The maize *indeterminate1* (*id1*) gene is a key regulator of the floral transition that encodes a zinc finger transcription factor localized to nuclei of immature leaf cells. *id1* mutant plants exhibit a prolonged vegetative growth phase; i.e. they produce many more leaves than normal maize and flower very late. Three novel β-glucosidase genes, termed *Zea mays dhurrinase (ZmDHR) 1*, *2* & *3*, were identified in an expression profile comparison between normal and *id1* mutant plants. Plant β-glucosidases have been implicated in diverse roles, such as cell wall remodeling, hormone homeostasis and pathogen defense. The ZmDHR genes are downstream targets of *id1* and share its unique immature leaf expression pattern. This study aims to elucidate the function of *ZmDHR2* through phylogenetic analysis, sub-cellular localization and transgenic over-expression in Arabidopsis. In a preliminary analysis, over-expressing 35S::ZmDHR2 transgenic lines flowered earlier than wild-type Arabidopsis and exhibited structural defects in the hypocotyl and roots that suggest altered cell wall integrity. Characterization of these β-glucosidase genes could establish a link between plant development, metabolism and endogenous floral inductive signals.

P41 Chemical screen uncovers link between one-carbon metabolism and sucrose signalling during Arabidopsis seedling development.

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Sugars play an important role in plant biology, acting as structural components, metabolic intermediates, and sources of energy. As a result, plants have evolved numerous mechanisms through which they are able to sense and perceive sugars. To uncover signalling pathways involved in sugar detection, forward-genetic screens have been widely implemented to isolate mutants with altered sugar responsiveness. This approach has proven successful in uncovering multiple sugar-signalling pathways, but is not without limitations related to seedling lethality and genetic redundancy. As a means of circumventing these issues, a chemical genetic approach was undertaken to uncover novel aspects of sugar sensing and signalling. Over 2100 compounds from the Library of Active Compounds in Arabidopsis (LATCA) were screened for the ability to perturb seedling responses to sucrose. This screen revealed that the sulfonamide family of compounds, known inhibit one-carbon (C1) metabolism in plants, restrict hypocotyl elongation in a sugar-dependent fashion. Transcriptome analysis uncovered a small set of transcripts that respond differently to the compound when it is administered in the presence of sucrose, including a number of transcripts involved in auxin signal transduction. Complementary forward- and reverse-genetic approaches are being pursued to elucidate the genetic basis for crosstalk between C1-metabolism and sucrose signalling during seedling development.

List of Registrants

Last Name	First Name	Institution/Organization	Abstracts
Ahmed	Zaheer	University of Guelph	1C
Akhtar	Tariq	University of Michigan	2A
Al-Daoud	Fadi	Agriculture and Agri-Food Canada	P37
Amyot	Lisa	AAFC	1C, 2C, P8
Aung	Banyar	The University of Western Ontario	P8
Baltzer	Jennifer	Wilfrid Laurier University	P29
Barton	Kiah	University of Guelph	1C, 2C
Bitzos	Stephanie	University of Guelph	
Bou Daher	Firas	University of Guelph	2B
Braeutigam	Katharina	University of Toronto	1A, P24
Bruinsma	Kristie	University of Western Ontario	2A, P38
Cameron	Robin	McMaster University	1B
Campbell	James	Wilfrid Laurier University	P32
Carella	Philip	McMaster University	1B
Chacon	Micaela	Carleton University	3A
Champigny	Marc	McMaster University	1A
Chang	Christine	University of Toronto	P30
Chen	Chen	Saint Mary's University	P15
Chen	Cherry	University of Waterloo	
Chin	Kimberley	University of Toronto	1B
Chiu	Rex	University of Toronto	P13
Cholewa	Ewa	Nipissing University	P27
Clendening	Eric	University of Guelph	2B, P34, P35
Coelho	Carla	University of Guelph	P39
Colasanti	Joseph	University of Guelph	1C, P21, P39, P40
Columbus	Melanie	Western University	1B
Coneva	Viktoriya	University of Guelph	P40
de Araujo	Charlotte	University of Toronto	3A
De Luca	Vincenzo	Brock University	
DeFalco	Thomas	University of Toronto	3A
Dong	Shaowei	University of Toronto	3B, P5
Dong	Zhongmin	Saint Mary's University	P15
Doucet	Jennifer	University of Toronto	
Downs	Gregory	University of Guelph	
Duermeyer	Lisza	The University of Toronto	
Ecclestone	Mark	Wilfrid Laurier	
Elliott	Drew	Nipissing University	
Emami Meybodi	Davood	Agriculture and Agri-Food Canada	P9

Espie	George	University of Toronto	3A
Fafard	Melissa	Wilfrid Laurier University	P29
Fréchette	Emmanuelle	University of Toronto	P30
Geitmann	Anja	Université de Montréal	2A
Gray	Gordon	University of Saskatchewan	P23
Grodzinski	Bernard	University of Guelph	1B, P17, P19
Guinel	Frederique	Wilfrid Laurier University	1B
Hamanishi	Erin	University of Toronto	P6
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Hoang	Tuan (Patrick)	University of Guelph	
Holbrook-Smith	Duncan	University of Toronto	P14
House	Megan	University of Guelph	P12
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Indriolo	Emily	University of Toronto	3C
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Janakirama	Preetam	Western University	3C
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Khan	Madiha	Carleton University	3C
Kong	Zhaoyu	University of Waterloo	
Krol	Marianna	University of Western Ontario	P36
Lakshminarayan	Shailu	Western University	2C
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Liu	Fushan	University of Guelph	3A
Lukens	Lewis	University of Guelph	3B, P12
Lung	Terry Shiu Cheung	University of Waterloo	P31
Lunman	Corey	Hoskin Scientific	
Macfie	Sheila	Western	1B, P25
Macleod	Mitchell	McMaster	1A
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Massey	Sarah	University of Guelph	3A
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Minow	Mark	University of Guelph	
Miri	Mandana	University of Western Ontario	1B
Misyura	Maksym	University of Guelph	P21
Moeder	Wolfgang	University of Toronto	1B, 3A
Moffatt	Barb	University of Waterloo	3B
Molinaro	Alyssa	University of Toronto at Mississauga	P3
Muhammad Razeq	Fakhria	Carleton University	P16
Mullen	Robert	Univeristy of Guelph	2B, P34, P35
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Nam	Eric	University of Toronto	P11
Natarajan	Purushothaman	University Toronto at Mississauga	1A
Nowak	Julia	University of Toronto	1C
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Peddle	Dylan	Nipissing University	P27
Plaxton	Bill	Queen's	P20
Prouse	Michael	University of Toronto	
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Rayirath	Usha	University of Guelph	P4
Richardson	Lynn	University of Guelph	2B, P35
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Sareen	Madhulika	University of guelph	1C
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Skaf	Joseph	University of Toronto	P6
Smith	Matt	Wilfrid Laurier University	P31, P32, P33
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Teresinski	Howard	University of Guelph	P34
Thompson	Cody	University of Guelph	P2

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Wang	You	University of Guelph	
Way	Danielle	Western University	1A, P28
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Weretilnyk	Elizabeth	McMaster University	1A, 1B
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Willick	Ian	Western University	P25
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Notes