



Délibérations du Congrès de la Société Canadienne de Physiologie Végétale & Congrès de Développement Végétale (Congrès Régional de l'Est)



On the front cover:

1. The BioBus, used by the Biodiversity Institute of Ontario in their DNA barcoding efforts and other activities.

Photo provided by John Chenery, Biodiversity Institute of Ontario, Guelph

2. Screening for circadian clock mutants in *Brassica* using cameras to monitor leaf movements over time.

Photo provided by C. Robertson McClung, Dartmouth College, New Hampshire

- 3. Summary of the control of fleshy fruit development and ripening through interactions between developmental cues, hormones, and environmental signals. *Photo provided by Jim Giovannoni, Boyce Thompson Institute, New York*
- 4. Rozanski Hall, a state-of-the-art classroom complex which opened in September 2003 at the University of Guelph. The building is named after Mordechai Rozanski shortly after he left as the University's longest reigning president from 1993-2003. *Photo provided by the University of Guelph*
- 5. The Bullring, built in 1903 as a "Judging Pavilion" for livestock auctions and shows. The University of Guelph converted the Bullring into the "watering" campus pub in the 1970's and this lasted until the 90's. In 2003, the Bullring was fully refurbished and is now solely managed by the Central Student Association. *Photo provided by the University of Guelph*

Welcome to the/ Bienvenue au la 2009

Eastern Regional Meeting of the Canadian Society of Plant Physiologists & Plant Development Workshop

Société Canadienne de Physiologie Végétale Congrès Régional de l'Est & Congrès de Développement Végétale

The University of Guelph Guelph, Ontario, Canada December 4th & 5th, 2009



Local Organizing Committee:

Dr. Barry Micallef Dr. Bernard Grodzinski Dr. Ian Tetlow Dr. Larry Peterson



THANK-YOU to our SPONSORS of the Canadian Society of Plant Physiologists Eastern Regional Meeting 2009 and Plant Development Workshop.



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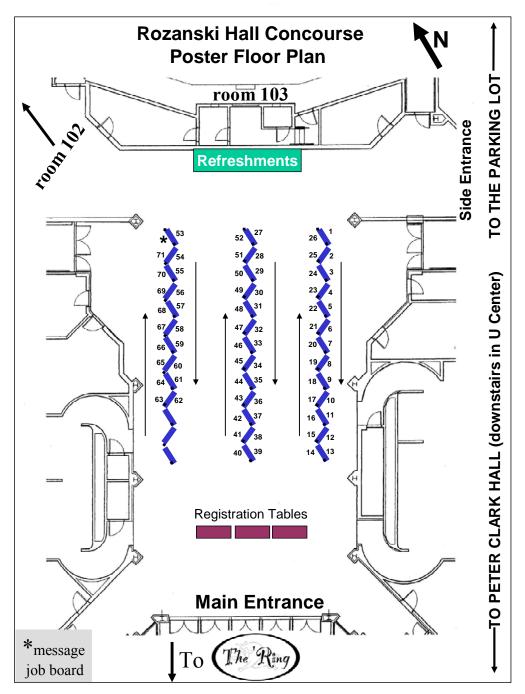


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Program Overview

Friday, December 4, 2009 – Registration and Mixer

Registration and Placing of Posters in Rozanski Hall from 7:00 to 8:30pm Reception at The Bullring starting at 7:00 pm until 10:00 pm (basketprize at 9:00pm)

Saturday, December 5, 2009 – Scientific Program

8:00 AM Registration, Beverages, and Poster Viewing in the Rozanski Concourse

Plenary Session (AM) in Rozanski Hall Room 103

8:45 AM - 9:00 AM Opening Ceremonies

9:00 AM - 9:40 AM 1st Invited Speaker: **Dr. Aron Fazekas**

"DNA barcoding in land plants: challenges, development, and applications"

9:45 AM - 10:25 AM 2nd Invited Speaker: **Dr. C. Robertson McClung**

"Timing is everything: Circadian clock function in Arabidopsis and Brassica"

10:25 AM - 10:45 AM Beverages and Snacks in the Rozanski Concourse, Poster viewing

10:45 AM - 12:00 PM Speaker Sessions A and B run concurrently

Session A (room 103) Metabolism in Plant Development

Session B (room 102) Genomics to Phenomics

12:00 PM - 1:35 PM Lunch at Peter Clark Hall (downstairs in the University Center)

All POSTERS ARE PRESENTED IN ROZANSKI CONCOURSE

including JUDGING OF STUDENT POSTERS

Plenary Session (PM) in Rozanski Hall Room 103

1:40 PM - 2:20 PM 3rd Invited Speaker: **Dr. Jim Giovannoni**

"A genomics systems approach toward identifying regulators of fruit

development and ripening in tomato".

2:25 PM – 3:40 PM Speaker Sessions C and D run concurrently

Session C (room 103) Plant Developmental Mechanisms

Session D (room 102) Plant Biotic Interactions

3:40 PM – 4:00 PM Beverages and Snacks in the Rozanski Concourse, Poster viewing

4:00 PM - 5:15 PM Speaker Sessions E and F run concurrently

Session E (room 103) Plant Cell Walls

Session F (room 102) Plant Abiotic Interactions

5:20 PM **Student Award Presentations**, Concluding Remarks (room 103)

5:35 PM Conclusion of Meeting

Detailed Scientific Program on Saturday, December 5

8:00 AM **Additional Registration** at Rozanski Hall.

Placing of posters in the Rozanski Concourse.

8:15 - 8:45 AM Beverages in the Rozanski Concourse

Poster Viewing in the Rozanski Concourse

Plenary Session (AM) in Rozanski Hall room 103

8:45 - 9:00 AM Opening ceremonies

Welcome from Dr. Barry Micallef, University of Guelph

9:00 - 9:40 AM **Dr. Aron Fazekas -** Invited Speaker

IS-1

DNA barcoding in land plants: challenges, development and applications Aron Fazekas

Biodiversity Institute of Ontario (BIO) and Department of Integrative Biology, University of Guelph, Guelph, Ontario NIG 2W1

The BIO herbarium has been actively engaged in barcoding research since the initial stages of plant DNA barcoding. The concept of DNA barcoding has been designed as a system to facilitate species identification and recognition through the use of nucleotide sequence data derived from a small portion of the genome. In animals, the mitochondrial cytochrome c oxidase (cox1) gene has demonstrated high levels of species resolution in a diverse array of taxa and has facilitated the discovery of new species, provided insight into ecological process, revealed fraud in the food industry, and been used to track invasive species. The primary challenge in barcoding plants has been to identify a similar genomic region to use as a plant DNA barcode. The focus-to-date has been on regions of the plastid genome, with a consensus recently achieved by the international barcoding community. As in animal systems, plant DNA barcoding has wide-ranging applications. In addition to local and international floristic projects, we have used the barcode method in ecological applications. Two projects currently in progress are examining aspects of below ground diversity. The first project explores patterns of below ground plant diversity and its determinants, through an analysis of roots, and compares this with above ground diversity, revealing contrasting patterns of community structure. A separate project uses DNA barcoding to examine the mutualism between plant species and the AMF fungi that colonize their roots, and suggests that the community structure of AMF fungi varies with host plant species.

Further Information

www.biodiversity.uoguelph.ca www.uoguelph.ca/foibis/aron

P.M. Hollingsworth *et al.* (2009) *PNAS* **160**: 12794-12797 A.J. Fazekas et al. (2009) *Mol. Ecol. Resources* 9: 130-139

9:45 - 10:25 AM **Dr. C. Robertson McClung -** Invited Speaker

IS-2

Timing is everything: Circadian clock function in *Arabidopsis* and *Brassica* C. Robertson McClung

Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire, 03755, USA.

Circadian rhythms, biological rhythms with a period of approximately one solar day, are widespread in nature and are driven by endogenous, self-sustaining clocks. Evidence is accumulating in many species that circadian clocks allow organisms to coordinate their biology with their temporal environment and thus enhance fitness. Efforts to elucidate the plant circadian clock mechanism have emphasized Arabidopsis thaliana and show the clock to be composed of multiple interlocked negative feedback loops. I will discuss the roles of a family of Pseudo-Response Regulators in clock responses to temperature, an important environmental time cue. To determine the extent to which the Arabidopsis clock serves as a model for other species, we have investigated the crop plant, Brassica rapa. We have identified Quantitative Trait Loci (QTL) for the period of the circadian rhythm in leaf movement, as well as for a number of morphometric parameters, including flowering time, size of floral organs, and hypocotyl length, in a set of Recombinant Inbred Lines derived from two diverse parents, R500 and IMB211. Efforts to identify the gene responsible for a QTL for period length will be presented. One candidate is GIGANTEA (GI), identified as a clock component in Arabidopsis. GI is polymorphic between R500 and IMB211 and the IMB211 GI allele rescues the short period phenotype of the Arabidopsis gi-201 null mutant, whereas the R500 allele further shortens the period, which is consistent with their relative effects on period in B. rapa. Both alleles rescue the late flowering phenotype of gi-201. We have identified several putative null gi alleles in the B. rapa TILLING collection at the John Innes Institute and will use these to test the effects of the two B. rapa GI alleles on period length in a B. rapa tissue culture system that expresses robust circadian rhythms in gene expression.

Further Information

www.dartmouth.edu/~rmcclung

10:25 – 10:45 AM Beverages and Snacks in Rozanski Concourse **Poster Viewing in the Rozanski Concourse**

10:45 – 12:00noon Speaker Sessions A and B run concurrently (AM)

Speaker Session A. **Metabolism in Plant Development** Chairperson: **Susanne E. Kohalmi**, University of Western Ontario Rozanski Hall room 103

- The role of cytokinins in regulating barley kernel growth and plant yield Adrian F. Powell*¹, H. Olechowski², & R. J. Neil Emery ¹

 Environmental and Life Sciences Graduate Program, Trent University, Peterborough, ON, and ²Hyland Seeds, Blenheim, ON
- 11:00 S-A2 Life without MTN is twisted: MTN deficiency in Arabidopsis leads to abnormal cellular arrangement.

 I. Waduwara *, A. Kong, S. Schoor and B. A. Moffatt.

 Department of Biology, University of Waterloo, Waterloo, Ontario
- 11:15 S-A3 Harnessing high-CO₂: Enhanced and not reduced respiration increases plant and oil productivity

 Sarathi M. Weraduwage¹*, Shezad A. Rauf¹, Malgre C. Micallef¹, Elizabeth

F. Marillia², David C. Taylor², Bernard Grodzinski¹ and Barry J. Micallef¹

Department of Plant Agriculture, University of Guelph, Guelph, Ontario,

National Research Council of Canada, Plant Biotechnology Institute, 110,

Gymnasium Place, Saskatoon, Saskatchewan

- 11:30 S-A4 Can utilization nitrogen use efficiency in rice be improved by overexpressing cytosolic glutamine synthetase?

 Rochon, A.¹, Brauer, E.K¹, Rothstein, S.², Shelp, B.J¹.

 Departments of Plant Agriculture¹ and Molecular and Cellular Biology², University of Guelph, Guelph, Ontario
- 11:45 S-A5

 Determining protein interaction profiles for AROGENATE

 DEHYDRATASES from Arabidopsis thaliana

 Danielle M. Styranko*, Susanne E. Kohalmi

 Department of Biology, University of Western Ontario, London ON

Speaker Sessions for the CSPP-ERM 2009 and PDW

- 1) the presenting author's name is underlined
- 2) student presenters being considered for speaker awards are shown by an asterisk(*)

Speaker Session B. **Genomics to Phenomics** Chairperson: **Annette Nassuth**, University of Guelph

Rozanski Hall room 102

10:45 S-B1	Improving sequence quality from regions with long mononucleotide repeats through the use of a new generation DNA polymerase A.J. Fazekas, R.A.D. Steeves*, and S.G. Newmaster Department of Integrative Biology, University of Guelph, Guelph, ON
11:00 S-B2	Alternative oxidases (AOXs) of Prokaryotes: Evidence for an Endosymbiotic Origin of Eukaryotic AOXs Allison E. McDonald and James F. Staples Department of Biology, The University of Western Ontario, London, ON
11:15 S-B3	Functional analysis of putative adenosine recycling enzymes in <i>Arabidopsis thaliana</i> Katja Engel ^{1*} *, Hanna Blaschke ² , Klaus v. Schwartzenberg ² , Barbara Moffatt ¹ Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, Ontario ¹ ; Biocentre Klein Flottenbek, University of Hamburg, Ohnhorstr. 18, D-22609 Hamburg, Germany ²
11:30 S-B4	Construction of a dual luciferase transient expression analysis system to determine the ICE binding site in Vitaceae CBF genes. Michelle Moody*, Annette Nassuth Molecular and Cellular Biology Department, University of Guelph, Guelph ON
11:45 S-B5	The role of cytokinin oxidase 1 in the pea mutant R50 Scott R. Clemow* and Frédérique C. Guinel Department of Biology, Wilfrid Laurier University, 75 University Avenue West, Waterloo, Ontario

12:00 – 1:35 PM

Lunch at Peter Clark Hall,

located downstairs in the University Center.

ALL POSTERS ARE PRESENTED IN THE ROZANSKI CONCOURSE including JUDGING OF STUDENT POSTERS

Plenary Sessions (PM) at Rozanski Hall room 103

1:40 - 2:20 PM **Dr. Jim Giovannoni** - Invited Speaker

IS-3

A genomic systems approach toward identifying regulators of fruit development and ripening in tomato.

J. Giovannoni, J. Vrebalov, R. J. Lee, R. Alba, M. Chung, Z. Fei Boyce Thompson Institute for Plant Research and USDA-ARS Robert W. Holley Center, Tower Road, Cornell University campus, Ithaca, NY 14853 USA. jjg33@cornell.edu

The ripening and development of fleshy fruits is regulated by environmental, hormonal and developmental cues and influences the accumulation of important nutritional metabolites. Our laboratory uses tomato as a model system to understand ripening regulation and has identified a number of necessary ripening genes via positional cloning of loci underlying ripening mutations and transcriptional profiling studies of ripening associated gene expression. With the advent of modern genomics approaches and accumulation of genomics-scale date sets we are confronted with challenges in data management, analysis and interpretation and also great opportunities to extract novel meaning from these data if analyzed properly. We have attempted to capture data related to genotype, developmental stage, gene expression and nutritional metabolites and have created a public database to facilitate data management and analysis (Tomato Functional Genomics Database, http://ted.bti.cornell.edu/). Emphasis in our group is on the carotenoid, ascorbate and folate biosynthesis pathways during tomato fruit development and ripening. Our goal is to use correlation analysis of gene expression and metabolite levels to identify novel gene candidates associated with ripening and nutrient content. The database and examples of candidate genes and subsequent functional studies will be presented. To date we have identified six transcription factors that we have shown to be necessary for tomato fruit ripening via transgenic studies including two MADS-box, two NAC domain, an Ethylene Response Factor (ERF) and an APETALA2 gene homolog. One of the MADS-box genes, TAGL1, is especially intriguing in that it suggests a molecular link between fleshy fruit development and eventual ripening via a single gene product.

Further Information

www.bti.cornell.edu/JimGiovannoni

2:25 – 3:40 PM **Speaker Sessions C and D run concurrently (PM)**

Speaker Session C. Plant Development Mechanisms
Chairperson: Robin K. Cameron, McMaster University
Rozanski Hall room 103

2:25	S-C1	Antagonistic interaction of BLADE-ON-PETIOLE1 and 2 with BREVIPEDICELLUS and PENNYWISE regulates Arabidopsis leaf and inflorescence architecture Khan, M.*1, Xu, M.1, Hu, T., McKim, S., Murmu, J., Storey, K., Hepworth, S.R. 1 Equal contribution to the paper. Ottawa-Carleton Institute of Biology, Carleton University, Ottawa, ON
2:40	S-C2	The Role of the Exocyst Complex in Female Fertility and the Compatible Pollen-Pistil Response of Arabidopsis thaliana Katrina E. Haasen, Laura A. Chapman*, and Daphne R. Goring. Department of Cell and Systems Biology, University of Toronto, ON
2:55	S-C3	Structure of styles and pollen tubes of distylous Turnera joelii and T. cabra (Turneraceae): Are there different mechanisms of incompatibility between the morphs? D. Safavian*, J.S. Shore Department of Biology, York University, 4700 Keele Street, Toronto, Ontario
3:10	S-C4	Two NAC transcription factors play non-redundant roles in an EIN2-dependent pathway during age-related resistance in Arabidopsis Fadi Al-Daoud*, R.K. Cameron Department of Biology, McMaster University, 1280 Main Street West, Hamilton, ON
3:25	S-C5	Arabidopsis thaliana cyclic nucleotide gated ion channels 11 and 12 contribute to a range of calcium dependent biological responses including gravitropism, senescence, and pathogen defense signaling W.Urquhart ¹ *, K. Chin ¹ , K. Yoshioka ^{1,2} Department of Cell and Systems Biology, University of Toronto, 25 Willcocks St., Toronto, ON and ² Center for the Analysis of Genome Evolution and Function (CAGEF), University of Toronto, 25 Willcocks St., Toronto, ON

3:40 – 4:00 PM Beverages and Snacks in the Rozanski Concourse **Poster Viewing in the Rozanski Concourse**

Speaker Session D. **Plant Biotic Interactions** Chairperson: **Frédérique C. Guinel**, Wilfred Laurier University Rozanski Hall room 102

2:25	S-D1	Conservation of Seed Endophytes During Domestication and Migration of Corn <u>David Johnston-Monje</u> *, Manish N. Raizada Department of Plant Agriculture, University of Guelph, Guelph, ON
2:40	S-D2	Characterization of the <i>Arabidopsis</i> ESCRT machinery: the role of ESCRT in tombusvirus-induced peroxisomal multivesicular body biogenesis Lynn G.L. Richardson* ¹ , Alex S. Howard ¹ , Nick Khuu ¹ , Brett Morphy ¹ and Robert T. Mullen ¹ Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON
2:55	S-D3	Testing efficiency of rhizobial strains on the nodulation of two peamutants E. Macdonald* and F.C. Guinel Department of Biology, Wilfrid Laurier University, Waterloo, ON
3:10	S-D4	Common Bacterial Blight: Development of BiBAC libraries from two sources of CBB resistance in P. vulgaris. G. Perry*, N. Singh, J. Chan, Y. Reinprecht and K.P. Pauls. Department of Plant Agriculture, University of Guelph, Guelph, ON
3:25	S-D5	The Role of the Mitochondrion and Alternative Oxidase during Pseudomonas Syringae Infection of Nicotiana tabacum Leaves Marina Cvetkovska* and Greg C. Vanlerberghe Department of Biological Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, ON

3:40 – 4:00 PM Beverages and Snacks in Rozanski Concourse **Poster Viewing in the Rozanski Concourse**

4:00 – 5:15 PM Speaker Sessions E and F run concurrently (PM)

Speaker Session E. **Plant Cell Walls** Chairperson: **Owen Rowland**, Carleton University Rozanski Hall room 103

4:00	S-E1	Towards coloring soybeans with anthocyanins- Identification of an anthocyanidin/flavonol 3-O-glucosyltransferase cDNA isolated from the seed coat of black soybean Nik Kovinich**a, Ammar Saleem°, John T. Arnason° and Brian Miki* aAgriculture and Agri-Food Canada, Ottawa; Carleton University, Ottawa, University of Ottawa, Ottawa
4:15	S-E2	Three Arabidopsis Fatty Acyl-CoA Reductases, FAR1, FAR4, and FAR5, Generate Fatty Alcohols Associated with Suberin Deposition S. J. Vishwanath* ¹ , F. Domergue ² , J. Joubès ² , J. Ono ¹ , J. Lee ¹ , M. Bourdon ² , R. Alhattab ¹ , C. Lowe ¹ , S. Pascal ² , R. Lessire ² , and O. Rowland ¹ Department of Biology and Institute of Biochemistry, Carleton University, Ottawa, Ontario and ² Laboratoire de Biogenèse Membranaire, Université Victor Ségalen Bordeaux 2, CNRS, UMR 5200, 146 rue Léo Saignat, Case 92, 33076 Bordeaux Cedex, France
4:30	S-E3	Suberin monomer analysis of <i>Iris germanica</i> 's multiseriate exodermis during its maturation and growth under different conditions Chris J. Meyer ¹ *, Carol A. Peterson ¹ , Mark A. Bernards ² Department of Biology, University of Waterloo, Waterloo, ON Department of Biology, University of Western Ontario, London, ON
4:45	S-E4	CASTing for AtMYB61 DNA binding sites Michael B. Prouse ¹ and Malcolm M. Campbell ¹ Centre for the Analysis of Genome Evolution & Function, Department of Cell & Systems Biology, University of Toronto, 25 Willcocks St., Toronto, ON
5:00	S-E5	Plant residues for the auto industry Reinprecht, Y. 1, Riaz, M. 1, Ablett, G. 2, Poysa, V. 3, Rajcan, I. 1, Sahoo, S. 1, Misra, M. 1, Mohanty, A. K. 1, and K. P. Pauls **IUniversity of Guelph, Plant Agriculture, Guelph; 2**University of Guelph, Ridgetown Campus, Ridgetown; 3**Agriculture and Agri-Food Canada, GPCRC, Harrow**

5:20 PM Student Award Presentations, Concluding Remarks (room 103)

5:35 PM Conclusion of Meeting

Speaker Session F. **Plant Abiotic Interactions** Chairperson: **Harold G. Weger**, University of Regina Rozanski Hall room 102

4:00 S-F1	Control of Leaf Sectoring through Photosynthetic Redox Imbalance in Arabidopsis thaliana Variegation Mutants
	Dominic Rosso ^a , <u>Rainer Bode</u> * ^a , Wenze Li ^a , Marianna Krol ^a , Diego Saccon ^a , Shelly Wang ^a , Lori A. Schillaci ^a , Steven R. Rodermel ^b , Denis P. Maxwell ^a , and Norman P.A. Hüner ^a
	^a Department of Biology and the Biotron, University of Western Ontario, London, ON and ^b Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, Iowa
4:15 S-F2	The Long-Term Shoot Regeneration Capacity of Excised Somatic Arabidopsis Tissues is Established During the Initial Hours After Injury, Modulated by a Complex Genetic Network Interacting with Light M. Blair Nameth*, Steven J. Dinka, Steven P. Chatfield, Adam Morris,
	Jenny English, Dorrett Lewis, Rosalinda Oro & Manish N. Raizada Department of Plant Agriculture, University of Guelph, Guelph, ON
4:30 S-F3	Elevated atmospheric CO ₂ and plant allelochemicals: Are there any general responses? Geraldine D. Ryan ¹ *, Susanne Rasmussen ² and Jonathan A. Newman ¹ School of Environmental Sciences, University of Guelph, Guelph, Ontario AgResearch, P.B. 11008, Palmerston North, New Zealand
4:45 S-F4	High stability ferric chelates: interacting mechanisms that affect iron bioavailability Harold G. Weger ¹ , Jackie Lam ² , Nikki L. Wirtz ² , Crystal N. Walker ¹ and Ron G. Treble ² Departments of Biology ¹ and Chemistry & Biochemistry ² , University of
	Regina, Regina, SK
5:00 S-F5	Learning to love arsenic; arsenic tolerance and hyperaccumulation in Pteris vittata. Characterization of PvACR3, a gene necessary for arsenic tolerance in Pteris vittata. Emily Indriolo ^{1,3} , David E. Salt ² and Jo Ann Banks ¹ Department of Botany & Plant Pathology, Purdue University, West Lafayette, IN, ² Department of Horticulture & Landscape Architecture, Purdue University, West Lafayette, IN and ³ Current address: Department of Cell & Systems Biology, University of Toronto, Toronto, ON
5:20 PM	Student Award Presentations, Concluding Remarks (room 103)
5:35 PM	Conclusion of Meeting

Posters for the CSPP-ERM 2009 and PDW

- 1) the presenting author's name is underlined
- 2) student presenters being considered for poster awards are shown by an asterisk(*)

No	Authors	Affiliation	Title
P1	L. Tian, M.E.Orozco-Gaeta*, L.I. D'Silva, M.C. Micallef, J. Robertson, B.J. Micallef	Department of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1	Extended-photoperiod injury in tomato is linked to biological rhythms of nitrogen uptake and assimilation
P2	Bross, C.D.*, Corea, O.R.A., Kohalmi, S.E.	Department of Biology, University of Western Ontario, London, ON	Functional characterization of Arabidopsis arogenate dehydratases in vivo.
P3	C. Long* ¹ , L. Spíchal ² , F.C. Guinel ¹	¹ Department of Biology, Wilfrid Laurier University, Waterloo ² Laboratory of Growth Regulators, Institute of Experimental Botany, AS CR & Palacký University, Olomouc, Czech Republic	Evidence towards the involvement of cytokinin receptor CRE1/AHK4 homologs in pea (Pisum sativum L.) nodulation
P4	Hongwei Hou Toavid Ellis ² , Olivia Wilkins ¹ , Lisa J. Newman ³ , Daniel E. Perazza ⁴ , Shawn D. Mansfield ⁴ , Malcolm M. Campbell ¹	¹ Centre for the Analysis of Genome Evolution & Function, Department of Cell and Systems Biology, University of Toronto, 25 Willcocks St., Toronto, ON ² Plant Genetic Resources Preservation Program, National Center for Genetic Resources Preservation, 1111 South Mason Street, Fort Collins, CO 80526, USA ³ Pioneer Hi-Bred International, Inc., 7250 NW 62nd Avenue, PO Box 552, Johnston, Iowa, 50131-0552, USA 4Laboratoire de Genetique Moleculaire des Plantes, UMR CNRS/UJF 5575, Université Joseph Fourier, BP 53 38041, Grenoble cédex 09, France	Overexpression of an R2R3-MYB DNA-binding domain suppresses fibre differentiation and improves industrial traits of transgenic plants.

P5	Shuhua Zhan*, Lewis Lukens	Department of Plant Agriculture, University of Guelph, Guelph, Ontario	Transcript abundance patterns of Arabidopsis thaliana miRNA biogenesis mutants reveal novel miRNAs and developmental shifts of gene expression
	Harsant J.* ¹ , G. Chiu, L ¹ . Pavlovic ¹ , N. Raghoothaman ¹ , T.L. Sage ¹	Department of Ecology and Evolutionary Biology ^I , University of Toronto, Toronto, ON	Quantitative trait locus analysis in <i>Arabidopsis thaliana</i> reveals a role for <i>KRP7</i> , a negative regulator of cell division, on plant reproduction at high temperature
P7	Sudhakar Pandurangan*, Frédéric Marsolais	Department of Biology, University of Western Ontario, London, Ontario; and Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Ontario	Relationship between asparagine metabolism and protein content in soybean seed.
	K. Dahal* ¹ , K. Kane ² , F. Sarhan ² , B. Grodzinski ³ , N. Hüner ¹	¹ University of Western Ontario, Dept. of Biology and The Biotron, NCB London, ON ² UQAM, Dept. of Biological Sciences Montreal, ³ University of Guelph, Dept. of Plant Ag. Guelph	Cold acclimated winter cereals exhibit an enhanced CO ₂ assimilation under long-term growth at elevated CO ₂
P9	Ibrahim Khalil ^{1,2} , Bahadur Meah ² , Annette Nassuth ¹	¹ Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON and ² Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh	Evaluation of an osmotin gene as a phomopsis resistance marker in eggplant (Solanum melongena L.)
	G. Picard* ¹ , R. Awad ¹ , R.D. Rojas ² , J. Alban ³ , J. T. Arnason ¹ , J. Starr ¹	Department of Biology University of Ottawa ¹ , Universidad Peruana Cayetano Heredia ² , Universidad Nacional Mayor de San Marcos ³	Discovery of novel neurologically active phytochemicals in Neotropical Piperaceae

P11	M. E. Stokes ¹ *, A. Chattopadhyay ¹ , M. M. Campbell ^{1,2}	Department of Cell & Systems Biology ¹ , University of Toronto, Toronto, ON; Centre for the Analysis of Genome Evolution and Function ² , University of Toronto, Toronto, ON	A chemical genetic approach to sugar signaling in Arabidopsis.
	F.Y. Cao ¹ *, W. Moeder ^{1,2} , K.F. Gao ^{1#} , W. Urquhart ¹ , K. Yoshioka ^{1,2}	¹ Department of Cell and Systems Biology, University of Toronto, 25 Willcocks Street, Toronto, ON, ² Center for the Analysis of Genome Evolution and Function (CAGEF), University of Toronto, 25 Willcocks Street, Toronto, ON [#] Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, 900 S. Ashland Ave, Chicago, IL	Characterization of development- and abiotic stress-triggered expression changes in isochorismate synthase 1 in Arabidopsis thaliana
P13	Siobhan Moore*, Lewis Lukens	Department of Plant Agriculture, University of Guelph, Guelph, ON	Do flowering time genes FRIGIDA and Flowering Locus C explain heterosis for multiple traits in <i>Arabidopsis thaliana</i> hybrids?
P14	K. Iglic* ¹	Department of Biology ¹ , University of Western Ontario, London, ON	Iron and Temperature Effects on Photosynthetic and Photoprotective Pigments in Stylophora pistillata Zooxanthellae
P15	Katharina Bräutigam 1,2, Sherosha Raj Olivia Wilkins Malcolm M. Campbell 1,2	Department of Cell & Systems Biology ¹ , and Centre for the Analysis of Genome Evolution & Function ² , University of Toronto, 25 Willcocks St., Toronto, ON	The influence of genotype and clonal history on <i>Populus</i> drought responses
P16	Thomas Canam ^{1,2} , Jacqueline MacDonald ¹ , Alex Tsai ² , Malcolm M. Campbell ^{2,} Emma R. Master ^{1,2}	¹ Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON	In planta expression of cell-wall deconstructing enzymes from fungi in Arabidopsis thaliana

	Riaz*, M. ¹ , Reinprecht, Y. ¹ , Sahoo, S. ² , Misra, M. ² , Mohanty, A. K. ² , Reid, L.M. ³ , P. K. Pauls ¹	¹ Department of Plant Agriculture, University of Guelph, Guelph, ON; ² Bioproduct Discovery and Development Centre, Department of Plant Agriculture, University of Guelph, ON; ³ Eastern Cereals and Oilseeds Research Centre l, Agriculture and Agri-Food Canada, Bldg. 99, Central Experimental Farm Ottawa, ON	Characterization of Corn Cellulose Fiber for Manufacturing Automotive Plastic Parts
P18	Ana Donoso*, Natalie Dunn, Neeta Mathur, John Greenwood, Jaideep Mathur	Department of Molecular and Cellular Biology, University of Guelph. Guelph. ON	ARP2/3 complex mediation in maintaining cell-cell connectivity in plants
P19	Amelie C.M. Gaudin*, Bridget M. Holmes, Manish N. Raizada	Department of Plant Agriculture, University of Guelph, Guelph, ON	Maize (Zea mays L.) Root Architecture in Response to Critical Nitrogen Stress using an Aeroponics System
	J. Murmu ¹ , M. Bush ¹ , M. Khan ¹ , C. Delong ² , C. Malcolmson ¹ , P. Fobert ² , S. R. Hepworth ¹	Department of Biology ¹ , Carleton University, Ottawa, ON K1S 5B6 and NRC-Plant Biotechnology Institute ² , Saskatoon, SK	Arabidopsis bZIP transcription factors TGA9 and TGA10 are redundantly required for glutaredoxin-dependent regulation of anther and pollen development
P21	Ksenija Kokolic*, Resmi Radhamony, Neeta Mathur, Jaideep Mathur	Department of Molecular and Cellular Biology, University of Guelph. Guelph. ON	Development of fluorescent protein tools and strategies for understanding microtubulemicrofilament interactions in plants

P22	Christophe Liseron- Monfils ¹ *, Steven P. Chatfield ¹ , Jan Brazolot ¹ , Stephen J. Dinka ¹ , Erin Hewitt ¹ , Natalie DiMeo ¹ , Salome Ndung'u ¹ , Arani Kajenthira ¹ , Carly A. Wight ¹ , Mimi Tanimoto ² , Adrienne Davidson ¹ , Rosalinda Oro ¹ , Manish N. Raizada ¹	¹ Department of Plant Agriculture, University of Guelph, Guelph, ON ² Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON	Genomic and Physiological Analysis of Two QTLs that Regulate a Critical Stage of Adventitious Shoot Regeneration in Arabidopsis thaliana (L.)
P23	H. B. Massicotte ¹ , L. H. Melville ² , L. E. Tackaberry ¹ , D. L. Luoma ³ , R.L. Peterson ²	¹ Ecosystem Science and Management Program, University of Northern British Columbia, Prince George, BC ² Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON ³ Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA	Mycoheterotrophic plants: the pinnacle of evolutionary plant-fungal specialization
P24	Elyse Roach*, Neeta Mathur, Jaideep Mathur	Laboratory of Plant Development and Interactions, Department of Molecular and Cellular Biology, CBS, University of Guelph. ON	dissection of response hierarchy
P25	Sameh S. Mahmoud ¹ *, Rong Tsao, Manish N. Raizada ¹	¹ Department of Plant Agriculture, University of Guelph, Guelph, ON	Chemical inhibitors suggest endophytic fungal Taxol is derived from both mevalonate and non-mevalonate-like pathways
P26	Satinder K. Gidda ¹ , Jay M. Shockey ² , Stephen H. Vinyard ³ , John M. Dyer ³ , Robert T. Mullen ¹	¹ Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, ² USDA-ARS, Southern Regional Research Center, New Orleans, LA, ³ USDA-ARS, US Arid-Land Agricultural Research Center, Maricopa, AZ	Biogenesis of glycerol 3- phosphate acyltransferase (GPAT): influence of transmembrane domains and protein-protein interactions on the localization of GPAT to ER subdomains

P27	Resmi Radhamony, Elyse Roach, Chris P. Trobacher, Neeta Mathur, John S. Greenwood Jaideep Mathur	Department of Molecular and Cellular Biology, University of Guelph. Guelph. ON.	PtdIns3P-containing tubules facilitate receptor-ligand trafficking during vacuole biogenesis
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P29	Thomas A. DeFalco* ¹ , David Chiasson ² , Brent N. Kaiser ² , Wayne A. Snedden ¹	¹ Department of Biology, Queen's University, Kingston, ON K7L 3N6 ² School of Argriculture, Food, and Wine, University of Adelaide, South Australia 5005, Australia	Biochemical analysis of GmCaMK1, a novel CaM- binding receptor-like protein kinase from soybean root nodules
P30	Roxana Khosravesh ^{1,2} *, Tammy L. Sage ¹ , Hossein Akhani ² , Rowan F. Sage ¹	¹ Department of Ecology and Evolutionary Biology, The University of Toronto, Toronto, ON ² Department of Plant Sciences, School of Biology, College of Science, University of Tehran, Tehran, Iran	Anatomical change leading up to the evolution of C4 Photosynthesis in <i>Anticharis</i> Endl. (Scrophulariaceae)
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P32	Michael Tessaro*, Manish N. Raizada	Department of Plant Agriculture, University of Guelph, Guelph, ON	Glutamine Biosensor as a New Tool for Plant Physiology
P33	Durham, K.M. ¹ *, E.A. Lee ¹ , K.Yu ² , K.P. Pauls ¹ , A. Navabi ^{1,2}	¹ Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada. ² Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food Canada, Harrow, ON	Phenotypic and Genotypic Evaluation of Common Bacterial Blight Resistance in a Resistance Inter-Cross Population of Phaseolus Vulgaris.

P34	Jia Wang, Nirusan Rajakulendran, Sasan Amirsadeghi, Greg C. Vanlerberghe	Department of Biological Sciences and Department of Cell and Systems Biology, University of Toronto Scarborough, 1265 Military Trail, Toronto, ON	Impact of Alternative Oxidase on Plant Response to Temperature
P35	Makoto Yanagisawa* ¹ , Shiu-Cheung Lung ¹ , Simon D.X. Chuong ²	Department of Biology, University of Waterloo, Waterloo, Ontario ¹ These authors contributed equally to the work. ² Author for correspondence	Protoplast isolation and transient gene expression in the single-cell C ₄ species, <i>Bienertia sinuspersici</i>
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P38	Jennifer Drouin ^{1,2} , S. Crossley ¹ , K. Washburn ¹ , E. Cholewa ¹	¹ Department of Biology, Nipissing University, North Bay, ON, Canada, P1B 8L7; ² Department of Biology, Laurentian University, Sudbury, ON	Developmental anatomy of Arabidopsis thaliana: comparing internal development at specific growth stages through a new histochemical technique
P39	D.Q. Liao ¹ , A. Pezzutto ¹ , A. Pajak ¹ , F. Marsolais ¹	¹ Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, ON, Canada.	Biochemical and Transcriptomic Characterization of S-Methyl- Cysteine Metabolism in Developing Seeds of Common Bean (<i>Phaseolus vulgaris</i>)
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P42	Monika Rewers ¹ , <u>Ewa Cholewa²</u> , Elwira Sliwinska ¹	¹ Department of Genetics and Plant Breeding, University of Technology and Life Sciences, Bydgoszcz, Poland, ² Department of Biology, Nipissing University, North Bay, ON	Seed structure and unusual germination of <i>Eriophorum</i> vaginatum L.
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	J. Nisler*, M. Zatloukal, I. Popa, M. Strnad, L. Spíchal	Laboratory of Growth Regulators, Palacky University & IEB AS CR, Olomouc, Czech Republic	Development of cytokinin antagonists acting at the receptor level
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P55	S.C. Farrow, R.J.N. Emery.	Department of Biology, Trent University, 1600 West Bank Drive, Peterborough, ON	A rapid and sensitive method for the detection and quantification of cytokinins using the Qtrap® 5500® triple quadrupole mass spectrometer and Kinetex® HPLC

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P59	Jillian D. Bainard* ¹ , Steven G. Newmaster ¹	Department of Integrative Biology, University of Guelph, Guelph ON ¹	Endopolyploidy in bryophytes: a first account
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	Z. Han* ^{1,2} , J. Zhang ¹ , R. Menassa ² , L. Tian ²	¹ College of Life Science, Northwest A&F University, 22 Xinong Road, Yangling, Shaanxi, P. R. China, 712100; ² Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, ON	Study of histone deacetylase genes for stress tolerance in plants
P62	Hamanishi, E.T. ^{1*} , Wilkins, O. ² , Raj, S. ² , M.M. Campbell ^{2,3}	¹ Faculty of Forestry, ² Department of Cell and Systems Biology, ³ Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto, ON	Intraspecific variation in the <i>Populus balsamifera</i> drought transcriptome
	J. Skaf ^{1,4} *, E. T. Hamanishi ^{2,4} , O. Wilkins ¹ , S. Raj ¹ , M. M. Campbell ^{1,3}	¹ Department of Cell & Systems Biology, ² Faculty of Forestry, ³ Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto, ON ⁴ Contributed equally	The impact of artificial night lighting in an urban environment on plant photosynthesis and gene expression

P64	Zeinab Yadegari*, K.P. Pauls	Department of Plant Agriculture, University of Guelph, Guelph, ON	Molecular characterization of dihydroflavonol 4-reductase (DFR) gene in common bean (Phaseolus vulgaris L.)
	J.L. Carviel* , R. K. Cameron	Department of Biology, McMaster University, 1280 Main St West Hamilton, Ontario	The role of <i>IAP1</i> in Age-Related Resistance in <i>Arabidopsis thaliana</i>
	I. Szucs*, M. Escobar, R. R. Cloutier, D.E. Leonardos, B. Grodzinski	Department of Plant Agriculture, University of Guelph, Guelph, ON	Transport patterns within the Plantaginaceae
P67	Adel Zarei, Gregory Baute, David J Wolyn	Department of Plant Agriculture, University of Guelph, Guelph, ON	A member of Pentatricopeptide repeat family differentially expressed in homeotic cytoplasmic male sterile and fertile carrot flowers.
P68	K. Chin ¹ *, W. Moeder ^{1,2} , H. Abdel-Hamid ¹ , D. Shahinas ¹ , K. Yoshioka ^{1,2}	¹ Department of Cell and Systems Biology, University of Toronto, 25 Willcocks Street, Toronto, ON ² Center for the Analysis of Genome Evolution and Function (CAGEF), University of Toronto, 25 Willcocks Street, Toronto, ON	Importance of the α C-helix in the Cyclic Nucleotide Binding Domain for Stable Channel Regulation and Function of Cyclic Nucleotide Gated Ion Channels in Arabidopsis thaliana
	Jingyun Liu ¹ *, JR DeEll ² , GG Bozzo ¹ , BJ Shelp ¹	¹ Dept of Plant Agriculture, University of Guelph, Guelph, ON; ² Ontario Ministry of Agriculture, Food and Rural Affairs, P.O. Box 587, 1283 Blueline Rd & Hwy #3 Simcoe, ON	Physiological Disorders of Postharvest Apples: A Role for Glutamate Decarboxylase Derived g-Aminobutyrate?
P70	M. Cheung*, GC Vanlerberghe	Department of Biological Sciences and Department of Cell and Systems Biology, University of Toronto, Scarborough	Investigating the Role of Alternative Oxidase in Light Acclimation Response
	S.M.H. Slater, M.C. Micallef, J. Zhang, B.J. Micallef	Department of Plant Agriculture, University of Guelph, Guelph, ON	Identification of the first null- activity mutant for an enzyme in daytime sucrose synthesis

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Speaker Presentations

S-A1

The role of cytokinins in regulating barley kernel growth and plant yield

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The present research aims to determine the relationship of cytokinin (CK) levels in barley kernels to kernel mass, kernel number, and plant yield. Early studies with barley found a positive relationship between CK content and kernel mass. However, recent work with rice suggests a connection between CKs and the number of grains produced, but no correlation of CK content to grain mass. With the advent of more sensitive and selective mass spectrometric techniques and established methods for extracting and purifying CKs, the roles of CKs in barley kernel development can be more precisely re-examined. To this end, CK profiles across early developmental stages have been generated for kernels of high-yield and low-yield barley varieties. Analysis and subsequent quantification was achieved by liquid chromatography—tandem mass spectrometry. Our results indicate that there is a relationship between CK concentration in kernels and increased plant yield. Furthermore, we will also discuss the preliminary results of an investigation into how CK oxidase might regulate CK levels in barley grains through the degradation of CKs.

S-A2

Life without MTN is twisted: MTN deficiency in Arabidopsis leads to abnormal cellular arrangement.

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S-adenosyl-L-methionine is the precursor for three key biosynthetic pathways: polyamines, nicotianamine and ethylene. The byproduct of these three pathways, methylthioadenosine, is re-cycled via the methionine recycling pathway. In this cycle, methylthioadenosine nucleosidase (MTN) irreversibly hydrolyses methylthioadenosine to methylthioribose which is converted back to methionine. Not surprisingly, the disruption of these critical pathways affects many biological processes. Arabidopsis MTN-deficient mutants have a pleiotropic phenotype including organ twisting and auxin transport defects. Non-fixed-handed twisting is seen in almost all organs of the MTN-deficient mutants: stems, leaves, roots, petioles, pedicels and flowers including the petals, filaments and stigmas. To further characterize this change in morphology the following analyses were performed: (1) microscopic imaging of twisted organs, (2) quantification of skewing using slanted hard agar plates and (3) in situ localization of auxin accumulation using DR5::revGFP. Compound, dissecting, SEM and confocal microscopic imaging of the twisted organs along with analysis of LR white embedded sections confirm that the twisting of *mtn* mutants is consistent with the twisting model described by Furutani et al (2000). Further, the preliminary data of the skewing experiment indicating a 25° skew compared to the 10° skew of the wild-type. Thus, this mutant provides a valuable tool for understanding the link between cellular basis of organ twisting and auxin transport.

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S-A3

Harnessing high-CO₂: Enhanced and not reduced respiration increases plant and oil productivity

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Previous studies have confirmed that transgenic *Arabidopsis* lines with partiallysuppressed mitochondrial pyruvate dehydrogenase kinase (mtPDHK) show elevated mitochondrial pyruvate dehydrogenase (mtPDH) activity, enhanced dark CO₂ evolution, and increased oil and seed weight at ambient CO₂, suggesting that sink activity is enhanced in these transgenics. We hypothesized that Arabidopsis transgenics having suppressed mtPDHK will show enhanced plant and oil productivity under high CO₂ due to greater sink strength. Arabidopsis having either constitutive or seed-specific expression of antisense mtPDHK were grown under either ambient (380ppm) or high (700ppm) CO₂, and growth parameters, oil profile and content, mtPDHK transcript levels, and mtPDH activities were measured. Seeds per silique, inflorescence size, seed number and weight, oil content, and harvest indices (HI) were significantly increased under high CO₂; effects were greatest for constitutively-expressed lines 10⁴ and 3¹. Northern analysis, enzymatic assays, and respiratory activity indicate that constitutive lines 10⁴ and 3¹ show an intermediate suppression of mtPDHK, suggesting a dosage effect. Results also revealed the importance of mtPDH in very long chain fatty acid (>C20) synthesis. Collectively, the activity of anabolic pathways such as oil synthesis that depend on respiratory substrate intermediates were enhanced, leading to increased plant and oil productivity at high CO₂.

S-A4

Can utilization nitrogen use efficiency in rice be improved by overexpressing cytosolic glutamine synthetase?

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Recent research suggests that plant nitrogen use efficiency (NUE) is limited by N assimilation, rather than uptake or reduction. We tested the hypothesis that overexpression of the native *Os*GS1;2 enzyme in rice increases utilization NUE (UtE), as measured by the amount of grain biomass per shoot N content. Three homozygous lines containing a *Os*GS1;2 overexpression (OX) construct were identified with higher GS activity than azygous and wild-type (Wt) controls. Physiological and biochemical characteristics of these OX lines were assessed at vegetative and reproductive stages under various N treatments (continuous supply of adequate or limiting N, or transfer from adequate to limiting N during grain filling) and two growth environments (growth chamber and greenhouse). The OX lines accumulated similar shoot biomass as azygous controls at both stages, and in some cases, higher harvest index (HI) and UtE than the azygous controls; these changes were highly correlated with % spikelets filled, spikelet number and spikelet yield. While these data can be interpreted as support for altered N partitioning and enhanced UtE in OX lines, there was also evidence of a transformation effect. We are currently backcrossing OX lines to the WT in an attempt to overcome the transformation effect and create a line that has improved UtE.

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S-A5

Determining protein interaction profiles for AROGENATE DEHYDRATASES from *Arabidopsis thaliana*

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In plants, phenylalanine serves as a precursor for synthesis of proteins and numerous aromatic compounds required for structural support, pigmentation, and pathogen defence, so the regulation of this pathway is important for plant welfare. In *Arabidopsis thaliana*, a family of six AROGENATE DEHYDRATASES (ADTs) has been identified, based on sequence similarity to bacterial prephenate dehydratases (PDTs). These enzymes catalyze the final steps of phenylalanine biosynthesis in plants and microorganisms, where prephenate can be converted to phenylalanine via either a phenylpyruvate or arogenate intermediate. Plants, however, are believed to preferentially follow the arogenate pathway. Recent crystallization data have shown that bacterial PDTs form dimers, and it was suggested that these dimers may be the functional PDT unit. Being that bacterial PDTs form homodimers, we propose that *Arabidopsis* ADTs are able to form and function as homo- and heterodimers. Furthermore, we hypothesize that different dimers play unique roles and that complex compositions respond to internal and environmental cues. As a first step in the analysis of ADT protein interactions we are investigating the homo- and heterodimerization properties of select *Arabidopsis* ADTs using a yeast-2-hybrid approach.

S-B1

Improving sequence quality from regions with long mononucleotide repeats through the use of a new generation DNA polymerase

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Stutter products are a common artifact in the PCR amplification of frequently used genetic markers that contain mononucleotide simple sequence repeats. Despite the importance of accurate determination of nucleotide sequence and allele size, there has been little progress towards decreasing the formation of stutter products during PCR. In this study we test the effects of lowering extension temperatures, inclusion of co-solutes in PCR, PCR cycle number, and the use of different polymerases on sequence quality for a set of sequences containing mononucleotide A/T repeats of 10–17bp. Our analyses show that sequence quality of mononucleotide repeats of up to 15bp is greatly improved with the use of a proofreading DNA polymerase fused to the non-specific dsDNA binding domain Sso7d. Our findings also suggest that the number of nucleotides the DNA polymerase interacts with may be the most important factor in the reduction of slipped-strand mispairings *in vitro*.

S-B2

Alternative oxidases (AOXs) of Prokaryotes: Evidence for an Endosymbiotic Origin of Eukaryotic AOXs

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Alternative oxidase (AOX) is a ubiquinol oxidase responsible for cyanide-resistant respiration in the mitochondrial electron transport chain. Based on a prior analysis of the taxonomic distribution of AOX in all kingdoms, it was hypothesized that AOX is prokaryotic in origin and entered the eukaryotic lineage via the endosymbiotic event that gave rise to mitochondria. An alternative hypothesis cites protein similarities between prokaryotic and plant AOXs as evidence of horizontal gene transfer (HGT) as an explanation for the existence of prokaryotic AOXs. In order to test these hypotheses we investigated AOXs from sequenced prokaryotic genomes as well as those from metagenome datasets. Within prokaryotes AOX is present only in the alpha-, beta-, and gamma- subdivisions of the proteobacteria. AOXs of proteobacteria share some characteristics with not only plants, but also other eukaryotes, such as the slime mold *Dictyostelium discoideum*. In addition, the limited and specific distribution of AOX in prokaryotes, especially in the alpha-proteobacteria (the proposed progenitor of the mitochondrion) supports the hypothesis for a prokaryotic origin of AOX and its subsequent introduction to eukaryotes via endosymbiosis. We propose that further spread of AOX in eukaryotes occurred via vertical inheritance not HGT.

S-B3

Functional analysis of putative adenosine recycling enzymes in *Arabidopsis thaliana* Katja Engel* ¹, Hanna Blaschke ², Klaus v. Schwartzenberg ², Barbara Moffatt ¹ Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, Ontario N2L 3G1, Canada ¹; Biocentre Klein Flottenbek, University of Hamburg, Ohnhorstr. 18, D-22609 Hamburg, Germany ²

In order to gain further understanding of adenosine (Ado) salvage in plants, putative Ado deaminase (ADA) and Ado nucleosidase (ADN) were studied in *Arabidopsis thaliana*. ADA catalyzes the irreversible deamination of Ado to inosine, and the locus At4g04880 (*AtADA*) is computationally annotated as encoding a putative ADA. However, indirect and direct spectrophotometric activity assays of the recombinant enzyme and complementation experiments in *E. coli* demonstrated *AtADA* to have no ADA activity. Phylogenetic analysis revealed that AtADA actually belongs to the group of ADA-like (ADAL) proteins, whose function is not yet known. No further ADA-related genes exist in the genome, therefore it can be concluded that ADA activity is not present in Arabidopsis.

ADN, catalyzes the conversion of purine and pyrimidine ribosides to their corresponding bases. The genome of Arabidopsis was screened for ADN genes using an inosine-uridine nucleoside hydrolase sequence from the protozoa *Crithidia fasciculata*. Two genes, *ADN1* and *ADN2* were identified and their gene products were studied using a spectrophotometric assay. The substrate spectrum of ADN2 includes both purine and pyrimidine nucleosides but it prefers to utilize uridine. Thus, ADN2 is proposed to be involved in the purine and pyrimidine salvage in Arabidopsis but predominantly in uridine recycling.

S-B4

Construction of a dual luciferase transient expression analysis system to determine the ICE binding site in Vitaceae *CBF* genes.

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The ability to cold acclimate is a quantitative genetic trait resulting from the input of many genes. The freezing tolerance pathway involves the activation of a protein called inducer of *CBF* expression (ICE) by a cold stimulus. The ICE protein is then able to bind and activate the promoter region of a *C-repeat binding factor* (*CBF*) gene. Eight potential *CBF* genes, and 3 potential *ICE* genes have been identified in the Vitaceae family and Vr*CBF1-4* were shown to be cold inducible *in planta* (Xiao et al., 2006, 2008). How ICE interacts, and where it binds to a *CBF* promoter is still unknown. A 5' promoter deletion series for the *VrCBF4* gene was created to determine what promoter elements are necessary to induce *CBF* expression. A dual luciferase binary vector was created to study the deletion series by transient expression analysis. The promoter being studied drives the expression of RiLUC, while 35S::FiLUC acts as a normalizer. Preliminary results indicate the presence of enhancer sequences as the min35S::RiLUC vectors already gave expression and thus modifications were made. Results illustrating the proper function of the dual luciferase construct as well as preliminary results with the *VrCBF4* deletion series will be presented.

S-B5

The role of cytokinin oxidase 1 in the pea mutant R50

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Cytokinins are a class of adenine-based plant hormones that stimulate cell division and are therefore essential for organ development. The pea mutant R50 is characterized by delayed nodule formation and high levels of cytokinins in shoots, roots and nodules. Previous analyses have centred on cytokinin oxidase (CKX), the enzyme that degrades cytokinin. When compared to the wild-type, CKX activity R50 was low. However, the transcript levels of one isoform, *PsCKX1*, were significantly higher in the mutant, especially in its nodules. Our objective is to understand the discrepancy between CKX1 expression and enzymatic activity in R50. To monitor CKX1 expression and protein localization throughout nodule development, I designed a spot-inoculation technique to capture different stages of morphogenesis. To determine the optimal time(s) for sectioning, CKX1 expression profiles of RNA and protein have been created for in-situ hybridization and immunolocalization, respectively. Together, the results will reveal the role of CKX1 in nodulation and whether a translational error is causing low CKX1 activity in R50.

S-C1

Antagonistic interaction of BLADE-ON-PETIOLE1 and 2 with BREVIPEDICELLUS and PENNYWISE regulates Arabidopsis leaf and inflorescence architecture $\underline{\text{Khan, M.*}^1}$, Xu, M.¹, Hu, T., McKim, S., Murmu, J., Storey, K., Hepworth, S.R.

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BLADE-ON-PETIOLE (BOP) 1 and 2 are two NPR1-like growth regulators that function redundantly to control the boundary architecture of Arabidopsis leaves, flowers and fruits. We show that BOP activity controls proximal-distal patterning in leaves in part by excluding the expression of KNOTTED-like homeobox genes from leaf initials in concert with ASYMMETRIC LEAVES (AS) 1 and 2. Spatial regulation of the KNOTTED-like gene *BREVIPEDICELLUS (BP)* is known to control leaf patterning, phyllotaxy, meristem function and inflorescence architecture. In the shoot apical meristem and inflorescences BP functions in a complex together with PENNYWISE (PNY), a BEL1-like homeodomain protein. We show that BOP activity opposes BP/PNY function to regulate inflorescence boundary architecture. Plants overexpressing *BOP1/2* show *bp* and *pny*-like phenotypes. Conversely, *BOP1/2* are misexpressed in *bp* and *pny* mutants causing inflorescence defects which are rescued in a *bop1 bop2* background. Similarly, inflorescence defects in *bp* and *pny* mutants are also caused by *KNAT2/6* misexpression and are rescued in a *knat2 knat6* background. We are investigating how BOP1/2 and KNAT2/6 interact to cause similar *bp* and *pny* inflorescence defects when comisexpressed.

S-C2

The Role of the Exocyst Complex in Female Fertility and the Compatible Pollen-Pistil Response of *Arabidopsis thaliana*

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The exocyst is an octameric protein complex, which functions to tether vesicles to the plasma membrane in the process of directed secretion in both yeast and animal cells. Orthologs of all of the exocyst subunits have been identified in *A. thaliana*, and select exocyst subunits involved in root-hair development and pollen tube morphogenesis have been described. While pistil components required for the early stages of compatible pollinations are largely unknown, we have recently identified Exo70A1, a putative subunit of the exocyst, as an essential component in the stigma for accepting compatible pollen in *A. thaliana* and *Brassica napus*. Loss of Exo70A1 leads to impaired pollen adhesion and hydration as well as pollen tube penetration through the stigmatic surface. Consistent with its role in these processes, Exo70A1 localizes to the plasma membrane in mature *Arabidopsis* flowers prior to pollination where it is predicted to assemble with the other exocyst subunits to tether secretory vesicles. To investigate the involvement of other predicted exocyst subunits in these stigma-pollen interactions, Sec15b RNAi Arabidopsis plants were generated, and preliminary results has revealed the necessity of Sec15b as well in stigmatic papillae to accept compatible pollen.

S-C3

Structure of styles and pollen tubes of distylous Turnera joelii and T. scabra (Turneraceae): Are there different mechanisms of incompatibility between the morphs? D. Safavian*, J.S. Shore

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We investigate the anatomy and fine structure of styles and pollen tubes of two distylous Turnera species, possessing a heteromorphic self-incompatibility system. We use fluorescence and transmission electron microscopy to provide the first description of the cellular aspects of the pollen-pistil interactions and changes to pollen tubes at the ultrastructural level during the self-incompatibility response of the morphs. Relative to compatible pollen tubes, dramatic alterations occur to the incompatible pollen tube cytoplasm and cell wall. Conspicuous differences were observed between the incompatible pollen tubes of the morphs. Swelling and loss of cristea of mitochondria and dilated rough endoplasmic reticulum were observed for incompatible pollen tubes of the long-styled morph, without any rupture of pollen tubes. For incompatible pollen tubes of the short-styled morph, the tube cell wall apex, and plasma membrane often appear to be ruptured and no easily recognizable organelles, such as mitochondria, could be discerned. Accordingly, we postulate that incompatible pollen tubes of the long-styled morph may be undergoing programmed cell death. as opposed to the incompatible tubes of the short-styled morph, where a necrotic form of cell death may occur. Our results clearly show ultrastructural differences between the morphs, supporting the hypothesis that different self-incompatibility mechanisms might operate between them.

S-C4

Two NAC transcription factors play non-redundant roles in an EIN2-dependent pathway during age-related resistance in Arabidopsis¹

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As Arabidopsis thaliana matures it exhibits age-related resistance (ARR) to virulent Pseudomonas syringae pv. tomato. ARR is associated with flowering, it requires intercellular accumulation of salicylic acid (SA), and it is associated with expression of some jasmonic acid/ethylene (JA/ET)-associated genes. Here, the role of two No Apical Meristem Cup-shaped Cotyledon (NAC) transcription factors, ANAC055 and ANAC092, was studied by characterizing a number of ANAC055, ANAC092, and JA/ET signaling mutants. The ARR defect of anac092 is followed one week later by the onset of an enhanced ARR response, and this is associated with delayed flowering. ANAC092-overexpressing plants display reduced ARR that is associated with decreased expression of some JA/ET-associated genes. Taken together, his suggests that ANAC092 contributes to the onset of ARR and flowering and negatively regulates JA/ET signaling during ARR. Conversely, ANAC055 is a positive regulator of ARR, as reduced ARR in anac055 is associated with decreased expression of some JA/ET-associated genes and the ICS1 SA biosynthesis gene. anac055anac092 double mutant analysis supports the idea that ANAC055 and ANAC092 play non-redundant roles during ARR. Moreover, ethylene insensitive 2-1 (ein 2-1) exhibits an ARR defect and attenuated expression of both NACs, suggesting that EIN2 regulates ANAC055 and ANAC092 during ARR.

S-C5

Arabidopsis thaliana cyclic nucleotide gated ion channels 11 and 12 contribute to a range of calcium dependent biological responses including gravitropism, senescence, and pathogen defense signaling

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Calcium ions are a dynamic signaling molecule that contributes to a range of developmental and environmental responses within plants. Cyclic nucleotide gated ion channels (AtCNGCs) are a large family of channels consisting of twenty members in *Arabidopsis thaliana*. To date there is evidence to suggest that members of this gene family contribute to various calcium ion fluxes exhibited in *Arabidopsis*. Several AtCNGCs, including AtCNGC11 and 12, have been shown to channel calcium in heterologous expression studies. Additionally, the chimeric AtCNGC11/12, of the *Arabidopsis* mutant *cpr*22, has been shown to conduct calcium and induce a programmed cell death that is calcium-dependent. Furthermore, *AtCNGC11* and *12* are involved in pathogen defense responses. To test if *AtCNGC11* and *12* have a broader role in generating calcium signals we have investigated the sensitivity of *AtCNGC11* and *12* knockout seedlings to calcium and evaluated the internal calcium concentration of these knockouts. We have also used *AtCNGC11* and *12* promoter Gus expression lines to identify the expression patterns of *AtCNGC11* and *12* in response to calcium and within senescing leaves. Additionally, we have shown that *AtCNGC11* and *12* are involved in gravitropism and senescence, in addition to pathogen defense signaling, all of which are dependent on calcium signaling.

S-D1

Conservation of Seed Endophytes During Domestication and Migration of Corn David Johnston-Monje*, Manish N. Raizada

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Endophytes are non-pathogenic microbes that live inside a host organism. All plants to date have been found to host at least one endophytic species. We asked whether any endophytic species was conserved in the agriculturally important plant *Zea mays* (corn) as it became domesticated and moved from Mexico. Conserved endophytes might represent candidate microbial biofertilizers. Kernels from populations of 13 different *Zea* species were screened for endophytic microbes by culturing, clone sequencing and tRFLP analysis of 16S rDNA. Seeds of the corn ancestor *Zea mays* spp. Parviglumis were found to contain species of *Pseudomonas*, *Strentophomonas*, *Enterobacter*, *Citrobacter*, *Clostridium*, *Dyella*, and *Pantoea*. *Pseudomonas* and *Pantoea* species were found in all genotypes surveyed. A total of 21 different genera were represented amongst the 140 isolates studied. These have been evaluated for their ability to grow on nitrogen-free media, catabolize ACC, antagonize pathogens, solubilize phosphate, sequester iron, produce auxin and acetoin, to stimulate plant growth and for endophytic habit using GFP tagging. In terms of applied research, 23 growth-promoting isolates are being tested as seed inoculants alongside transgenic *Klebsiella pneumonii* 342 expressing the ACC deaminase gene from *Pseudomonas putida* UW4 and the acetoin operon from *Bacillus subtilis*.

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S-D2

Characterization of the *Arabidopsis* ESCRT machinery: the role of ESCRT in tombusvirus-induced peroxisomal multivesicular body biogenesis

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The hallmark of infection of plant cells by Tomato Bushy Stunt Virus (TBSV) is the extensive invagination of the peroxisomal boundary membrane and the eventual transformation of the organelle into a peroxisomal multivesicular body (pMVB). While the molecular mechanisms underlying pMVB formation are unknown, the topology of this process is equivalent to the budding of certain retroviruses from the plasma membrane of mammalian cells, for which the host-cell's ESCRT (endosomal sorting complex required for transport) machinery is exploited. We therefore propose that TBSV also exploits ESCRT to facilitate the formation of pMVBs. ESCRT consists of several multi-protein complexes that, in yeast and mammalian cells, assemble sequentially at late endosomes and function in cargo recognition and sorting, and the formation of internal vesicles. As a first step in investigating the potential role of plant ESCRT in pMVB biogenesis, we identified all of the putative ESCRT homologues in *Arabidopsis* and employed yeast two-hybrid assays, *in vitro* communoprecipitations, and subcellular localization experiments to demonstrate that the functional organization of the ESCRT machinery is conserved in plants. We also show that certain plant ESCRT proteins interact with TBSV replicase proteins and are localized to pMVBs, supporting the premise that TBSV exploits ESCRT for the formation of pMVBs.

S-D3

Testing efficiency of rhizobial strains on the nodulation of two pea mutants

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Unique structures called nodules form on roots from a mutualistic relationship between legumes and rhizobia, in which the former receives assimilated nitrogen and the latter gains carbohydrates. Because many competent rhizobial strains are present within the rhizosphere, and because a nodulation mutant studied in our lab exhibited a differential response to rhizobial strains, we investigated their potential effect on nodule number. We tested four different rhizobial strains (128C53K and its transformant 128C79, and 8401 and its transformant lacZ8401) on two pea mutant lines (E151 and R50) and the wild-type (WT; Sparkle). Nodule number, nodule dry weight (DW), and nodule morphology were compared. As expected, Sparkle had more nodules than the low nodulating mutants. On a nodule basis, R50 and Sparkle are similar in their nodule DW; however, E151 nodules had a larger DW because of their multi-lobed morphology. What we observed previously may have been due to an improper comparison, whereby WT from one strain was compared to a transformant from another strain. This exercise was, however, useful because we intend on measuring the nitrogen-fixing ability of our mutants using the hydrogen uptake-deficient mutant 128C79. Knowing its efficiency compared to other strains, based on nodule number, will be invaluable.

S-D4

Common Bacterial Blight: Development of BiBAC libraries from two sources of CBB resistance in P. vulgaris.

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Common bacterial blight (CBB) is an aggressive foliar pathogen of common dry bean (*Phaseolus vulgaris*), and is endemic in most regions where dry beans are cultivated. This disease presents itself as brown lesion of seeds, leaves and pods and is caused by *Xanthomonas axonopodis* pv. phaseoli. The development of commercial bean lines resistant to the *X. axonopodis* represents a major step towards reducing the impact of this disease on bean production in North America, and OAC-Rex represents the first CBB resistant cultivar released in North America, however the genes responsible for this resistance not yet been identified. To aid in the identification of CBB-resistance genes, binary-bacterial artificial chromosome (BiBAC) libraries were created from OAC-Rex and HR67, a second CBB-resistance *P. vulgaris* line, and the libraries were screened with CBB resistance-associated molecular markers identified by previous studies, and the identified clones were analyzed using a gelbased restriction fingerprinting method for assembly into contigs. The fragments at the extreme ends of the contigs were used to re-probe the libraries and expand the coverage of the contig, and selected clones have been brought forward for sequencing.

S-D5

The Role of the Mitochondrion and Alternative Oxidase during *Pseudomonas Syringae* Infection of *Nicotiana tabacum* Leaves

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Mitochondria have an important role in cellular signalling during stress, possibly through the production of reactive oxygen species (ROS) by the mitochondrial electron transport chain (ETC). Plant mitochondria have an alternative oxidase (AOX), that may prevent over-reduction of the ETC during stress, and thus modulate ROS production. We are investigating the potential role of AOX during biotic stress in tobacco leaves infected with a virulent pathogen and with two non-host pathogens, one which induces the appearance of hypersensitive response (HR)-like lesions and another which induces defence responses without the HR. We find that AOX expression is induced very early during defence responses, but not in the case of HR appearance. Salicylic acid (SA) is an important signalling molecule during the HR. It is also known to impact mitochondrial function and induce AOX expression. However, we find that SA accumulation is uncoupled from AOX expression during the HR. Moreover, the activity of Mn-superoxide dismutase, which scavenges mitochondrial superoxide, is reduced during this process. This implies that high levels of mitochondrial superoxide are present during the HR. Further, in transgenic lines lacking AOX, the onset of HR is delayed. Based on our results, we hypothesize that the mitochondrion and AOX are involved in the HR.

S-E1

Towards coloring soybeans with anthocyanins- Identification of an anthocyanidin/flavonol 3-O-glucosyltransferase cDNA isolated from the seed coat of black soybean

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As the prevalence and diversity of engineered crops increases, contamination of non-modified grains with transgenics is becoming problematic. Distinct colors for the visible identification and quantitation of transgenic materials could provide increased safety to the consumer and freedom to the producer. We have proposed to engineer pigment biosynthesis in black soybean (Glycine max (L.) Merr.) to generate distinct seed colors for the visible identification of transgenic grains. The seed coats of black soybean are known to accumulate all anthocyanins required for the red, blue, purple, and orange coloration of plant tissues. However, we demonstrate additional factors as being involved in the black coloration of soybeans. Before grain coloring can be considered as a viable solution towards the identification of GE soybeans, it is necessary to identify the anthocyanin biosynthesis gene. We report the identification and cDNA cloning of a UDP-glucose:flavonoid 3-O-glucosyltranferase (named GmUF3GT) from seed coat of black soybean. Of the 28 flavonoid substrates tested, the purified recombinant enzyme glucosylated only anthocyanidins and flavonols, and demonstrated strict 3-OH regiospecificity. Transfer of GmUF3GT into the A. thaliana T-DNA mutant ugt78d2 restored anthocyanin and flavonol biosynthesis, confirming the in vivo function of the enzyme as an anthocyanidin/flavonol 3-O-glucosyltransferase.

S-E2

Three *Arabidopsis* Fatty Acyl-CoA Reductases, FAR1, FAR4, and FAR5, Generate Fatty Alcohols Associated with Suberin Deposition

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Suberin is a cell wall-associated polymer of phenolics, glycerol and a variety of fatty acid derivatives, including C18:0-C24:0 primary fatty alcohols. Suberin is deposited in various tissue layers, such as the root endodermis, and serves a critical role in controlling water and ion transport. It is also deposited in response to wounding and salt stress. An eight-member gene family encoding alcohol-forming fatty acyl-CoA reductases (FARs) has been identified in *Arabidopsis thaliana*. We have found that three of these genes, *FAR1*, *FAR4* and *FAR5*, are specifically expressed at known sites of suberin deposition. Heterologous expression of FAR1, FAR4 and FAR5 in yeast indicated that they are active alcohol-forming FARs with distinct chain length specificities ranging from C18:0 to C24:0. We found that mutants of *FAR1*, *FAR4*, and *FAR5* are each differentially affected in primary alcohol levels in root and wound-induced suberin. Specifically, C18:0-OH was reduced in *far5-1*, C20:0-OH was reduced in *far4-1*, and both C22:0-OH and C24:0-OH was reduced in *far1-1*. However, the total amount of fatty alcohol present in these mutants was maintained at wild-type levels due to compensatory increases in other primary alcohols when one chain length was reduced, suggesting that fatty alcohol levels in suberin is highly controlled.

S-E3

Suberin monomer analysis of *Iris germanica*'s multiseriate exodermis during its maturation and growth under different conditions

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More than 90% of tested angiosperm species have roots with an exodermis and approximately 18% of these develop a multilayered exodermis (MEX), including *Iris germanica*. All of *I*. germanica's mature MEX walls contain suberin lamellae with a poly(aliphatic) domain (SPAD) that is important for regulating radial water and ion transport. Our objective was to analyze SPAD biosynthesis by measuring suberin-specific compounds at particular stages of MEX maturation and under different growth conditions. Roots were grown in hydroponic culture wherein MEX maturation was delayed in submerged root regions but accelerated in regions exposed to a humid air gap. Suberin monomers, including the key 18:1 α , ω -dioic acids and 18:1 ω-hydroxy fatty acids, were isolated chemically from maturing exodermal tissue, then quantified and identified by GC-MS. Resolving the monomer profiles at specific maturation stages revealed spatial and temporal patterns of SPAD synthesis. As more exodermal layers matured, monomer abundance increased. Interestingly in air gap-exposed root regions. amounts of key monomers were significantly greater in the first and second exodermal lavers compared to those in submerged regions. Increases of key suberin monomers can be expected to make the exodermis more efficient at reducing water loss from the root to the soil during drought.

S-E4

CASTing for AtMYB61 DNA binding sites

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AtMYB61, a member of the R2R3-MYB family of transcription factors in *Arabidopsis thaliana*, modulates gene expression in response to diurnal cues to control the major facets of the plant transpiration stream, namely, changes in stomata aperture, xylem formation and root system architecture. A CASTing (cyclic amplification and selection of target sequences) assay has been developed that facilitates the identification of a transcription factor's DNA-binding sites. This system was used to identify the preferred *in vitro* DNA recognition sites for AtMYB61. AtMYB61 dissociation constants, which are used to describe the affinities for binding sites to a protein, were discovered via nitrocellulose filter binding assays. Furthermore, an EMSA (electrophoretic mobility shift assay) and *in silico* structural analyses of the binding of these motifs to *At*MYB61 validated these interactions.

S-E5

Plant residues for the auto industry

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The use of plant fibers in automotive parts is limited by their poor performance in composite materials. Lignin and hemicellulose reduce the value of plant fibers because they degrade at lower temperatures than cellulose. The objectives of this research were to identify genes that contribute to soybean and corn fiber performance in composites, map Quantitative Trait Loci (QTL) for fiber traits and develop fiber gene-specific markers. Databases were searched for the genes involved in cellulose, hemicellulose, lignin and pectin biosynthesis in soybean stem. Gene specific primers (170) were designed and screened with gDNA of parents (RG10 and OX948) of a recombinant inbred line (RIL) soybean mapping population. Several genes in lignin (PAL2, 4CL2, NAD, peroxidase, laccase) and cellulose (COBL4) biosynthetic pathways were isolated and gene specific markers were developed. A soybean oligo microarray will be hybridized with the gDNA and RNA isolated from stem tissue to identify additional fiber genes and develop SFP (single feature polymorphism) markers. Fifty soybean lines, selected on the basis of a height/lodging index and forty corn RILs from the CG62 x CO387 mapping population selected on the basis of seed ferulic acid content, were evaluated under controlled and field conditions in 2008 and 2009. Characterization of their fibre performance in composites is underway. Initial thermogravimetric analysis (TGA) of ground soybean and corn stem tissues indicated that parental genotypes have different lignin and cellulose contents. The marker and performance data will be used to map fiber performance QTLs. This work will allow us to identify key factors in fiber quality and to develop quick, marker-based screening method(s) to allow rapid introgression of genes related to good fiber quality into elite germplasm and agriculturally acceptable varieties/hybrids.

S-F1

Control of Leaf Sectoring through Photosynthetic Redox Imbalance in Arabidopsis thaliana Variegation Mutants

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We hypothesized that chloroplast energy imbalance sensed through alterations in the redox state of the photosynthetic electron transport chain, measured as excitation pressure, governs the extent of variegation in the *immutans* mutant of *Arabidopsis thaliana*. To test this hypothesis, we developed a nondestructive imaging technique and used it to quantify the extent of variegation in vivo as a function of growth temperature and irradiance throughout the plants development. The extent of variegation was positively correlated ($R^2 = 0.750$) with an increase in excitation pressure irrespective of whether high light, low temperature, or continuous illumination was used to induce increased excitation pressure. Similar trends were observed with the variegated mutants spotty, var1, and var2. Measurements of greening of etiolated wild-type and *immutans* cotyledons indicated that the absence of IMMUTANS increased excitation pressure twofold during the first 6 to 12 h of greening, which led to impaired biogenesis of thylakoid membranes. We conclude that mutations involving components of the photosynthetic electron transport chain, such as those present in *immutans*, spotty, var1, and var2, predispose Arabidopsis chloroplasts to photooxidation under high excitation pressure, resulting in incorrect plastid development and hence in the variegated phenotype.

S-F2

The Long-Term Shoot Regeneration Capacity of Excised Somatic Arabidopsis Tissues is Established During the Initial Hours After Injury, Modulated by a Complex Genetic Network Interacting with Light

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The literature suggests that excised plant tissues require days-to-week(s) of exposure to light/darkness to affect regeneration of a new shoot; in Arabidopsis thaliana, we demonstrate these effects occur within only hours after organ (cotyledon) excision. In Ler-0, continuous light during the initial 24-hours post-excision inhibited shoot regeneration 3-5 weeks later, whereas ≥2hours darkness was stimulatory. Surprisingly, light-inhibition of long-term shoot regeneration required concomitant polar auxin transport and ethylene. CRYPTOCHROME1dependent early-stage blue/UVA exposure was inhibitory, in contrast to Red/Far-Red perception. Only hours of moderate fluorescent light (~100µMolm⁻²sec⁻¹) caused damaging reactive oxygen species. Early-stage photoprotection appeared to require PHYTOCHROME-A (PHYA), photomorphogenic transcription factor HY5, chloroplast xanthophylls (PHYTOENE SYNTHASE, NONPHOTOCHEMICAL QUENCHING-1) and vacuolar anthocyanin (Chalcone Synthase); later stages required PHYB. Accumulation of light-shielding anthocyanin was HY5- and PHYB-dependent, suggesting one mechanism mediating their photo-protection. We observed partial epistasis by light on cytokinin-mediated regulation of shoot regeneration. We conclude that long-term shoot regeneration capacity of an excised plant tissue is established during the initial hours post-injury, modulated by a complex genetic network interacting with light. We hypothesize that inadvertent variation in light/dark exposure immediately following tissue injury may significantly contribute to the experimental variability plaguing in vitro plant stem cell research.

S-F3

Elevated atmospheric CO₂ and plant allelochemicals: Are there any general responses? Geraldine D. Ryan¹*, Susanne Rasmussen² and Jonathan A. Newman¹

Increasing atmospheric CO_2 is hypothesized to alter plant physiology and metabolism, which may have important implications for plant-mediated trophic interactions. Widely held ecological theories such as the carbon-nutrient balance hypothesis (CNBH) and growth-differentiation balance hypothesis (GDBH) predict that CO_2 -induced increases in photosynthetic rates and subsequent increases in the C/N ratio result in carbon products in excess of those needed for primary metabolic functions and as such result in increased carbon-based secondary metabolites. We analyzed published results on the effects of elevated atmospheric CO_2 on four classes of plant-derived allelochemicals (phenolics, terpenoids, nitrogen-based compounds and volatiles). The results suggest that the effect of CO_2 on secondary metabolites conform to ecological theory only for certain classes or subclasses of phytochemicals and effects are pathway dependent. We provide a critical assessment of conventional ecological theories used to predict phytochemical responses to CO_2 and we make some suggestions as to how this field may be expanded and improved. We discuss these results in light of the possible effects of CO_2 -mediated changes on higher trophic levels such as herbivores, parasitoids and predators.

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S-F4

High stability ferric chelates: interacting mechanisms that affect iron bioavailability Harold G. Weger¹, Jackie Lam², Nikki L. Wirtz², Crystal N. Walker¹ and Ron G. Treble² Departments of Biology¹ and Chemistry & Biochemistry², University of Regina, Regina, SK S4S 0A2

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

S-F5

Learning to love arsenic; arsenic tolerance and hyperaccumulation in *Pteris vittata*. Characterization of PvACR3, a gene necessary for arsenic tolerance in Pteris vittata. Emily Indriolo^{1,3}, David E. Salt² and Jo Ann Banks¹

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Arsenic, a known toxin and carcinogen, is one of many elements that plants are exposed to in their environment. Pteris vittata (Pteridacae) is unique because it and its close relatives are the only known plant species that are able to tolerate and hyperaccumulate up to 2% of their foliar dry weight as arsenic. We have identified from P. vittata a gene that encodes a vacuolar arsenite effluxer and named PvACR3. Given that PvACR3 complements yeast mutant for ScACR3 and its expression is induced by the addition of arsenic, PvACR3 is likely to involved in arsenic tolerance and hyperaccumulation in P. vittata. To determine if PvACR3 is necessary for arsenic tolerance and hyperaccumulation, we have an established system for RNAi in P. vittata gametophytes and have used it to knock down the expression of the endogenous PvACR3 genes. Targeting the endogenous PvACR3 gene by RNAi results in PvACR3 gametophytes that either die or show diminished growth in the presence of 500mM arsenite compared to gametophytes grown on arsenite free media. These data indicate that PvACR3 is necessary for arsenic tolerance in *P. vittata*. Interestingly, homologues of *ACR3* genes do not exist in angiosperms, which may explain why angiosperms are unable to tolerate or hyperaccumulate such high levels of arsenic.

Poster Presentations

P-1

Extended-photoperiod injury in tomato is linked to biological rhythms of nitrogen uptake and assimilation

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Photoperiodic injury (PI) of vegetative tissues occurs under either extended photoperiods or non-24 h light/dark cycles in many plants including tomato. Effects of extended artificial lighting, often termed light pollution, is a concern in urban areas and in some agricultural systems. We have shown that PI is linked to nitrite accumulation under light/dark cycles that elicit PI. It was hypothesized that PI occurs under extended photoperiods due to alterations in biological rhythms for nitrate uptake and assimilation resulting in nitrite accumulation and toxicity. Analysis of diel activities of the assimilatory enzymes nitrate reductase (NR) and nitrite reductase (NiR) under either a 12 or 24 h photoperiod showed that nitrite accumulation occurred within 3 h after the NR/NiR activity ratio reached low values in a 24-h period for a PI-susceptible line only. The PItolerant line maintained robust circadian rhythms for NR and NiR transcript levels, NR activity state, NiR activity, and levels of amino acids in 24 h light. In contrast, for the PI-susceptible line these parameters became arrhythmic in 24 h light and tyrosine nitration of leaf protein was significantly increased. Semidian rhythms for nitrate uptake occurred in both 12 and 24 h light; only the PI-tolerant line showed altered biological rhythms for nitrate uptake in 24 h light. Our results indicate that PI in tomato occurs in part through altered circadian coordination of nitrate uptake, NR and NiR, resulting in nitrite toxicity mediated by tyrosine nitration of leaf proteins.

P-2

Functional characterization of Arabidopsis arogenate dehydratases in vivo.

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Plants can synthesize phenylalanine via the prephenate or arogenate pathway. In the prephenate pathway, prephenate dehydratases (PDTs) decarboxylate and dehydrate prephenate into phenylpyruvate, which is subsequently transaminated to phenylalanine. Conversely, in the arogenate pathway, prephenate is first transaminated to arogenate and then decarboxylated and dehydrated by an arogenate dehydratase (ADT) to phenylalanine. Six *ADTs* have been identified in the *Arabidopsis thaliana* genome and *in vitro* assays for the encoding proteins revealed they act either exclusively as ADTs or as ADT/PDTs. Each ADT has three domains: an N-terminal transit peptide, an internal catalytic domain and a C-terminal regulatory (ACT) domain. However, the exact junction between the transit peptide and the catalytic domain is not yet functionally defined. Plant ADTs have a highly conserved region consisting of 20 amino acids prior to the RVAY motif, which we have named the intermediate (I) region. We hypothesize that the I-region is essential for the catalytic function of *Arabidopsis* ADTs as previous studies sequencing lacking the I-region are not functional *in vivo*. Three constructs of difference lengths (full length, intermediate and putative mature version lacking the I-region) have been cloned to test their function in an *in vivo* yeast complementation assay.

P-3

Evidence towards the involvement of cytokinin receptor CRE1/AHK4 homologs in pea (*Pisum sativum* L.) nodulation

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The mutualistic relationship between rhizobia and legumes results in the formation of a biological nitrogen-fixation organ, the nodule. Recent research illustrates a direct connection between cytokinin (CK) receptors and nodulation. We are interested in whether or not CRE1/AHK4, one of three known CK receptors, is involved in pea nodulation. Pea seedlings were inoculated with rhizobia and treated with PI-55, a CK antagonist targeting CRE1/AHK4. Nodule number of treated plants was decreased and their root system enlarged, which both support a role of CRE1/AHK4 in pea nodulation. To ensure that PI-55 can also act as a competitor of exogenous CK and not as a CK agonist, seedlings were treated simultaneously with PI-55 and benzyl-aminopurine (BAP). Nodule numbers of plants treated with PI-55 and BAP were higher than those of BAP-treated plants. Lateral root number was practically recovered and shoot height stimulated. Those results add evidence that PI-55 is an antagonist of both endogenous and exogenous CK at CRE1/AHK4 and that this receptor is involved in indeterminate nodule organogenesis. In future studies, we will assess the role of CRE1/AHK4 in nodulation using a mutant R50 (*sym16*), which has few, pale nodules, and which accumulates CK, while displaying low cytokinin oxidase activity.

P-4

Overexpression of an R2R3-MYB DNA-binding domain suppresses fibre differentiation and improves industrial traits of transgenic plants.

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The R2R3-MYB transcription factor, *Pinus taeda* MYB4 (*Pt*MYB4), modulates the expression of genes encoding multiple steps in the lignin biosynthetic pathway. Here we test the hypothesis that overexpression of dominant-negative *PtMYB4* transgene can be used to modify lignin composition with a beneficial effect on the use of the transgenic plants material harbouring this transgene. A dominant-negative transgene was constructed comprising only the PtMYB4 DNA-binding domain. The truncated *PtMYB4* transgene has all the features of a dominant-negative locus as demonstrated using recombinant protein *in vitro* and overexpression in yeast. Overexpression of the dominant-negative *PtMYB4* transgene in plants not only altered lignin biogenesis, but also suppressed the formation of lignified fibre cells. Overexpression of the *PtMYB4* dominant-negative construct in transgenic hybrid *Populus* (*Populus alba x P. grandidentata*) dramatically reduced lignified fibre production, and also lowered stem lignin content with resultant improvements in pulping characteristics, but without any impact on stem growth. The dominant-negative *PtMYB4* transgene may have utility in the directed improvement of plant material intended for use in the production of pulp, paper or biofuel.

P-5

Transcript abundance patterns of *Arabidopsis thaliana* miRNA biogenesis mutants reveal novel miRNAs and developmental shifts of gene expression

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MicroRNAs (miRNAs) are endogenous RNAs of 20~25 nucleotides, derived from stem-loop regions of longer dsRNA precursors, that recognize target mRNAs by base-pairing and thereby regulate their expression. To investigate how proteins involved in miRNA biogenesis affect gene expression, to elucidate the impact of miRNAs on the plant transcriptome, and to identity novel miRNA targets, we analyzed inflorescence transcript populations from the Arabidopsis thaliana miRNA biogenesis mutants dcl1-7, dcl3-1, hen1-1, hst15 and hyl1-2. The canonical 21nt miRNA biogenesis mutants affected the transcript levels of a large number of genes, and transcript changes in both dcl1-7 and hen1-1 as well as hst15 and hyl1-2 greatly overlapped. Among the genes up-regulated in the mutants, known miRNA targets and transcription factors were overrepresented. In contrast, the 24 miRNA biogenesis mutant dcl3 had a little effect on the transcriptome. The miRNA mutants' transcriptomes revealed a developmental shift in gene expression causing the inflorescence transcriptome to resemble the leaf- and meristem-like state and to differ from pollen and seed-like state. Using novel computational approaches, we also identified five new miRNA genes and two complementary miRNAs that target distinct mRNAs. Our results suggest that miRNAs promote developmental progression at the transcript level from a vegetative state.

P-6

Quantitative trait locus analysis in Arabidopsis thaliana reveals a role for KRP7, a negative regulator of cell division, on plant reproduction at high temperature

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Genome wide Quantitative Trait Loci (QTL) mapping, using the Ler x Cvi Recombinant Inbred Line population, was used to evaluate the genetic basis of variation for reproductive traits contributing to reproductive effort under control and high temperature (HT) conditions in *Arabidopsis*. A preliminary analysis performed on 9 floral traits to include ovary/silique length, ovule/seed number, and percent ovule abortion during carpel/silique development has revealed significant QTL. Under control conditions (24C day, 18C night), 4 putative QTL involved in ovary/silique length, ovule/seed number and ovule percent abortion, were detected on Chromosomes 1, 2 and 3. Under HT conditions (32C day, 18C night), 8 putative QTL were localized on Chromosomes 3, 4 and 5. Within the HT QTL, 3 genes encoding Heat Shock Proteins and a gene involved in the negative regulation of cell division (*KRP7*) were identified. The identification of *KRP7* provides direct data to test the hypothesis that high temperature has a negative impact on plant reproduction by down-regulating cell division. There was no overlap between any of the QTL detected under the control and HT conditions, thus supporting a hypothesis that there is a genotype by environment interaction, i.e. different genetic regions are expressed under the different temperature treatments.

P-7

Relationship between asparagine metabolism and protein content in soybean seed. Sudhakar Pandurangan* and Frédéric Marsolais

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Soybean (Glycine max [L.] Merr.) is an important crop grown primarily as a protein and oil source. Asparagine is the main form of organic nitrogen that is transported to developing seeds during seed filling in soybean and other legumes. Asparaginase (ASPG) is considered to be the major enzyme metabolizing transported asparagine in the seed coat and developing cotyledon for the synthesis of other amino acids. Beside ASPG, there is evidence for concurrent expression of asparagine synthetase (AS) enzyme in developing seeds. The determination of free asparagine at mid-maturation in three genetically related cultivars (Maple Arrow, AC-Hercule and AC-Proteus) varying in protein content confirmed its positive correlation with seed protein content (SPC). Further, free amino acid profiles of two parental and eight recombinant inbred lines (RILs) differing at four quantitative trait loci (QTL) determining SPC revealed that the high free asparagine trait is associated with two major QTLs. The relationship between AS and ASPG expression and SPC was investigated in Maple Arrow and AC-Proteus, in developing seed coat and cotyledons by Western blotting and RT-PCR experiments. The results revealed a developmental transition from asparagine biosynthesis to catabolism in the seed coat. This probably ensures a high level of free Asn in the embryo during early development. Higher levels of ASPG protein were observed in the seed coat of the high protein line.

P-8

Cold acclimated winter cereals exhibit an enhanced CO_2 assimilation under long-term growth at elevated CO_2

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Cold acclimation of spring wheat, spring rape and tomato results in decreased photosynthetic capacity estimated as the light saturated rates of carbon assimilation, A_{sat}. In contrast, cold acclimation of winter rye, winter wheat and Arabidopsis thaliana is associated with enhanced A_{sat} due to the global reprogramming of carbon metabolism in response to low growth temperatures. We hypothesized first, that cold acclimated (CA, 5°C) winter cereals (cv Norstar, cv Musketeer) should exhibit higher A_{sat} relative to non acclimated controls (NA, 20°C) under long-term growth and development at elevated CO₂ (700ppm CO₂). Second, CA winter and spring cereals (cv Katepwa, cv SR4A) should exhibit a differential stimulation of A_{sat} at elevated CO₂. Growth at elevated CO₂ stimulated A_{sat} to a greater extent in CA winter cereals (45-60%) relative to NA controls (30-35%) and CA spring cereals (20-25%). Lon-term elevated CO₂ resulted in acclamatory- loss of A_{sat} by about 30% in all NA cereals and by about 15% in CA counterparts relative to maximum A_{sat} observed at short-term exposure to high CO₂. The enhanced A_{sat} of CA winter cereals at long-term elevated CO₂ was reflected into increased biomass (130%) relative to NA controls (95%) and CA spring cereals (55%). We conclude that CA winter cereals exhibit higher A_{sat} under long-term elevated CO₂ relative to NA controls and CA spring cereals.

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P-9

Evaluation of an osmotin gene as a phomopsis resistance marker in eggplant (Solanum melongena L.)

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Eggplant is a major vegetable crop in Bangladesh. Fruit rot caused by *Phomopsis vexans* (Sac & Syd) is reported to cause 15-50% yield loss. Research in Dr. Meah's group has identified the resistant cultivar IPM-31. Recently an osmotin gene was proposed to give resistance against phomopsis in grape (Montteiro et al., 2003). The goal of the presented research is to determine if an osmotin gene in eggplants also correlates with phomopsis resistance. Primers were designed against homologous regions in osmotins, and made to be most similar to sequences in Solanaceae. These primers were used to PCR amplify and clone ~700 bp sequence from both the resistant IPM-31 and the susceptible Laffa S eggplant cultivars. Analysis of the sequences revealed that the sequence from the resistant cultivar encodes some conserved osmotin amino acids including 13 of the characteristic Cys residues (allele A). However, the susceptible cultivar sequence differed such that a presumably inactive osmotin would be translated. Primers to differentiate between the presumably active and inactive alleles will now be used to analyze whether the susceptible Dohazari G cultivar is also lacking allele A, and the resistant progeny of the Laffa S or Dahazari G x resistant IPM-31 have allele A.

P-10

Discovery of novel neurologically active phytochemicals in Neotropical Piperaceae G. Picard*¹, R. Awad¹, R.D. Rojas², J. Alban³, J. T. Arnason¹, J. Starr¹ Department of Biology University of Ottawa¹, Universidad Peruana Cayetano Heredia², Universidad Nacional Mayor de San Marcos³

From recent ethnobotanical studies on the medicinal plants of Belize, Central America, we reported that Q'eqchi' Maya Healers preferentially use a wide variety of Piperaceae species to treat neurological and mental disorders such as anxiety and epilepsy. Subsequent *in vitro* investigations have shown that these species (rich in Piperamide compounds) exhibit potent anxiolytic and antiepileptic properties by interacting within the γ-aminobutyric acid (GABA) system. To expand our knowledge of the traditional use of neuro-active plants across the Neotropics, we conducted a survey of Piper species widely used and collected throughout Peru and the Amazon Basin. As previously, our preliminary results now show that Piper plants are active within the GABA system by both inhibiting the GABA-T enzyme and facilitating GABAergic transmission via the GABA receptors. Future phytochemical profiling of these plants will be determined using High Pressure Liquid Chromatography technologies and bioassay guided isoloation of active principles. From these overall findings, we consider the traditional knowledge of plant medicines in the Neotropics to be an important source of safe and accessible treatments for neuropsychological disorders.

P-11

A chemical genetic approach to sugar signaling in Arabidopsis.

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Sugars play an important role in plant metabolism, acting as structural compounds, metabolic intermediates, and sources of energy. As a result, plants have evolved numerous mechanisms through which they are able to sense and perceive sugars. As a means of revealing novel aspects of sugar signaling, we have employed chemical genetic screen in search of compounds that elicit an altered response when administered in the presence of sucrose, hypothesizing that these compounds would be impinging upon sucrose-dependent processes. The screen revealed the sulfonamide family of compounds, which inhibit one-carbon (C1) metabolism, to exhibit an inhibitory effect onhypocotyl elongation that is augmented by the presence of sucrose. The effect is not related to osmoticum, as equivalent concentrations of palatinose did not enhance the inhibitory effect of the compounds. The interaction between the sulfonamides operates independently of HEXOKINASE1 and is related to the sugar type present in the growth media. Future directions include microarray-based transcriptome analysis as a means of discovering changes in gene expression that may underpin the interplay between C1 metabolism and sugar signaling.

P-12

Characterization of development- and abiotic stress-triggered expression changes in isochorismate synthase 1 in Arabidopsis thaliana

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The importance of salicylic acid (SA) in defence responses has long been recognized. Recent studies also suggested a role of SA in abiotic stress responses. It has been reported that there are two distinct SA biosynthesis pathways; the benzoate pathway that involves phenylalanine ammonia lyase, and a pathway that requires isochorismate synthase (ICS). In this study, in order to characterize the *ICS1*-dependent SA biosynthetic pathway, the expression of *ICS1* upon four different types of abiotic stress was investigated by using transgenic *Arabidopsis* plants that express the *ICS1* promoter -β-glucuronidase (GUS) reporter gene construct. The *ICS1*::GUS gene was up-regulated upon application of the biological SA analog benzo (1,2,3) thiadiazole-7-carbothionic acid S-methyl ester (BTH) and SA itself. The effect of low temperature, dehydration, low humidity, and salinity stress on *ICS1*::GUS expression was examined and it was found that only salt stress induces *ICS1*::GUS expression. Moreover, abscisic acid was found to be capable of suppressing SA-induced *ICS1*::GUS expression. GUS histochemical staining revealed tissue- and developmental stage-specific expression of *GUS* driven by the *ICS1* promoter. The role of the *ICS1*-dependent SA biosynthetic pathway in abiotic stress responses will be discussed.

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P-13

Do flowering time genes FRIGIDA and Flowering Locus C explain heterosis for multiple traits in *Arabidopsis thaliana* hybrids?

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Heterosis, or hybrid vigour, a phenomenon in which the hybrid offspring between two inbred parents shows extreme trait values compared to the parents, is widespread and often utilized in agriculture, but the molecular explanation is still largely unknown. To investigate this, a diallel mating design using 5 ecotypes of *Arabidopsis thaliana* as parents was performed. Twelve traits were measured on the 20 hybrids, and the percentage of heterosis was determined for each genotype for each trait. Heterosis was observed predominantly within crosses containing C24 for 8 of the 12 traits. Traits directly linked to flowering time commonly showed strong heterosis and the same genotypes often showed heterosis for other traits. C24 contains an active FRIGIDA (FRI) allele which in combination with ecotypes containing a functional Flowering Locus C (FLC) allele explains the heterosis for flowering time. To determine if these two genes alone cause all heterotic traits, two ecotypes having every combination of functional and nonfunctional FRI and FLC alleles were evaluated. To test the hypothesis that a significant amount of non-additive gene expression in these hybrids is due to FLC, microarray analysis for hybrids of C24 and Col with (a) functional and (b) non-functional FLC alleles will be performed.

P-14

Iron and Temperature Effects on Photosynthetic and Photoprotective Pigments in *Stylophora pistillata* Zooxanthellae.

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The mass bleaching of reef-building corals under elevated sea surface temperatures and intense sunlight initiates a sharp decrease in photosynthetic efficiency of the symbiotic zooxanthellae. Decreased photosynthetic efficiencies in pelagic phytoplankton can be related to iron limitation, but this effect has not been tested in corals. We show that reducing iron availability to Stylophora pistillata colonies at 27°C by addition of the siderophore desferrioxamine B yields decreased cellular concentrations of photosynthetic pigments (chlorophyll a, c_2 , peridinin, protein complexes) in their freshly isolated zooxanthellae (FIZ). Iron-replete colonies maintained under moderate heat stress (31°C) yield similar pigment concentrations with or without iron limitation. Decreased iron availability also affected diatoxanthin (DT)diadinoxanthin (DD) balance, a photo-protective mechanism that limits over-reduction of PSII by increasing non-photochemical quenching. Under higher temperature and reduced iron availability, the DT/(DD + DT) ratio in FIZ increased 3-fold relative to lower temperature conditions, regardless of the level of available iron, corresponding to decreased Fv/Fm in hospite. Our findings suggest that decreases in iron availability in seawater could exacerbate the declining photosynthetic efficiencies in natural Stylophora pistillata colonies under elevated temperatures.

P-15

The influence of genotype and clonal history on *Populus* drought responses

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Water availability is a key determinant of tree growth and survival. Exposure to episodic drought can impinge significantly on forest health and the establishment of productive tree plantations. There is therefore great interest in understanding the mechanisms underpinning drought responses in ecologically dominant and economically important tree genera, like Populus. To test hypotheses related to the transcriptional regulation of Populus drought responses, genome-wide changes in transcript abundance in response to water limitation were examined in economically important *Populus* clones. To study the interaction between clone history and drought, individuals of the same genotype were obtained from geographically distinct locations and their response to water limitation was studied at the physiological and molecular level under controlled laboratory conditions. Intriguingly, transcriptome analyses using the Affymetrix GeneChip technology uncovered differences in transcript abundance patterns based on differences in geographic origin of clones with identical genotypes. That is, even genotypically identical clones analysed in a common garden experiment could mount different transcriptome-level remodelling in response to water withdrawal if the clones were derived from different sources (i.e., same clone sourced from two different nurseries). The data provide insights into the interplay between genotype and environment, and hint at the potential role played by epigenetic phenomena in this interaction. Moreover, the data provide mechanistic insights into long-standing applied questions related to the nursery source of poplar clones and how that impacts on future clone performance in plantations. Taken together, the results point to the remarkable plasticity that has evolved in this genus to contend with episodic drought.

P-16

In planta expression of cell-wall deconstructing enzymes from fungi in *Arabidopsis thaliana* Thomas Canam 1,2, Jacqueline MacDonald 1, Alex Tsai 2, Malcolm M. Campbell 2 and Emma R. Master 1,2

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The recalcitrance of the plant cell wall represents a significant hurdle to efficient processing of lignocellulosic feedstocks for biofuel and biomaterial production. This is primarily attributed to the complexity of the intra- and intermolecular bonds among the major cell wall components: cellulose, hemicellulose and lignin. For instance, ester linkages between hemicellulose and lignin, as well as the glycosidic bonds within hemicellulose require considerable energy to break during feedstock pre-processing. To this end, plant feedstocks with reduced or modified linkages between or within the cell wall components have the potential to significantly reduce pre-processing expenses associated with the early stages of lignocellulose-based biofuel and biomaterial production. In the present study, previously characterized genes from *Aspergillus nidulans* and novel genes from *Phanerochaete carnosa* encoding cell-wall deconstructing enzymes were expressed *in planta* under the control of the ubiquitous 35S promoter in *Arabidopsis thaliana*. The cell wall chemistry and structural properties of stems from stable homozygous transformants were characterized and compared to those from wild-type plants. The findings from the current study may help to provide the impetus for future experiments designed to enhance the processibility of dedicated biofuel crops.

P-17

Characterization of Corn Cellulose Fiber for Manufacturing Automotive Plastic Parts Riaz*, M.¹, Reinprecht, Y.¹, Sahoo, S.², Misra, M.², Mohanty, A. K.², Reid, L.M.³ and P. K. Pauls ¹

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Corn production in Ontario provides a large source of natural fiber that might be used in automobiles as replacements for glass fibers in plant-based or petroleum-based plastics. However, the use of corn fiber for producing reinforced polymeric composites in automotive parts is limited by the lack of information about its functional properties, especially after exposure to repeated cycles of freezing and thawing. The objective of the current project is to determine the relationships between the genetic makeup of corn inbred lines, their fiber compositions and functional properties. Quantitative trait loci (QTL) for cellulose, lignin, and hemicellulose content as well as QTL for corn stalk fiber composition (especially ferulic acid content) will be determined using a recombinant inbred line (RIL) population that is segregating for ferulic acid content in the seed. Protocols for extraction of phenolics from corn stalks and cobs were standardized. Preliminary results show that the parents differ in the free phenolic contents of their stalks and cobs and the RILs are segregating for free phenolics in their stalks and cobs. The study will provide an understanding of the genetic control of cell wall traits that are important for the use of corn fibers in biocomposite materials. This could lead to the creation of corn lines/hybrids in which the cellulose is more easily extractable as pure filaments and therefore more valuable for biocomposite production.

P-18

ARP2/3 complex mediation in maintaining cell-cell connectivity in plants

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The seven-subunit Actin Related Protein (ARP) 2/3 complex is a highly conserved modulator of the actin cytoskeleton amongst eukaryotes. In plants, mutations in different subunits of the complex result in aberrant actin organization which, in seedling tissues, leads to cell detachment along the longitudinal axis. We hypothesized that during active growth the actin cytoskeleton defects directly impacted cytoplasmic organization and vesicle trafficking in the mutants. Accordingly we sought physiological conditions and growth regulator treatments that would augment the phenotype in different arp2/3 mutants. Concomitantly fluorescent protein aided liveimaging of plasma membrane (YFP-PIP2), ER (ss-RFP-HDEL), Golgi bodies (ERD2-GFP) and endosomal (RFP-2xFYVE) compartments was carried out. Etiolated seedlings displayed increased cell detachment and apparently possessed higher auxin content. Cytokinin treatment augmented the phenotype further and promoted callusing in the mutants. Whereas exogenous application of pectinase to wild-type plants phenocopied the mutants the gaps created through cell detachment were found plugged by callose. Our observations link defects in the ARP2/3 complex to actin cytoskeleton involvement in polarized vesicle mediated trafficking of growth regulators. They also suggest that the complex may play an essential role in maintaining the exocytosis-endocytosis balance between cell wall components for achieving cell-cell connectivity in plants.

P-19

Maize (Zea mays L.) Root Architecture in Response to Critical Nitrogen Stress using an Aeroponics System

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While maize shoot architectural plasticity in response to environmental cues has been well studied, regulation of root architecture and its functional significance remain poorly understood. Yet, root developmental plasticity is an integral part of a plant's strategy to ensure that its resource-capturing organs are optimally positioned to absorb vital soil nutrients, which can be both heterogeneous and seasonably dynamic. Nitrogen (N) is a crucial macronutrient that influences maize yield. Since post-embryonic adventitious roots originating from the stem (crown region) are the major root organs in maize responsible for N capture, this study examined maize root growth and architectural plasticity at post-embryonic developmental stages in response to a critical N supply. To dynamically quantify the maize post-embryonic root system, which is extensive, we engineered an aeroponics system, where the roots are suspended in the air and misted at regular intervals with a nutrient solution. We detected that maize, upon low N stress exposure, cease the elongation of preexisting crown roots, and/or make shorter *de novo* crown roots, in favour of allocating more energy to support lateral root growth and branching. Our results show that root architectural traits that enhance the metabolic efficiency to acquire N may be of greater advantage than root size *per se*.

P-20

Arabidopsis bZIP transcription factors TGA9 and TGA10 are redundantly required for glutaredoxin-dependent regulation of anther and pollen development

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The glutaredoxins encoded by *ROXY1* and *ROXY2* are redundantly required for anther and pollen development in Arabidopsis. Here, we use a reverse genetics approach to show that plants lacking the activities of bZIP transcription factors TGA9 and TGA10 are arrested in anther development with a phenotype that is strikingly similar to that of *roxy1 roxy2* mutants. Loss-of-function *tga9 tga10* double mutants are male-sterile and fail to form viable pollen. As seen for *roxy1 roxy2* mutants, abaxial and adaxial anther lobes are differentially affected, with early lobe differentiation blocked in adaxial lobes, whereas later steps of pollen maturation are disrupted in abaxial lobes. Our data suggest that ROXY-dependent promotion of anther development occurs in part via TGA9 and TGA10. First, *TGA9* is upregulated in the anthers of stage 8-9 flowers coinciding with *ROXY* expression. Second, TGA9/TGA10 positively regulate a subset of anther development genes downstream of *SPOROCYTELESS* that depend on ROXY function for their expression. Third, TGA9/TGA10 and ROXY proteins interact directly in yeast. Our findings suggest that ROXY-mediated reduction of disulphide bonds promotes the transcription factor activities of TGA9 and TGA10, which in turn is essential for completion of anther and pollen development in Arabidopsis.

P-21

Development of fluorescent protein tools and strategies for understanding microtubulemicrofilament interactions in plants

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Microtubules (MT) and microfilaments (MF) play key roles in shaping plant cells. While both cytoskeletal elements have been studied individually their interactions leading to precise localized growth and cell morphogenesis remain unclear. New fluorescent tools and mutantbased strategies have been developed for studying MT- MF interactions in living cells. They include the creation of novel green to red photoconvertible mEosFP::MBD-MAP4 and LIFEACT::mEosFP probes for labeling MF and MT, respectively. Transgenic Arabidopsis plants expressing these probes have been generated and crossed to plants expressing GFP::AtEB1b. The GFP::AtEB1b probe fluorescently highlights MT plus ends and thus facilitates the tracking of cortical microtubule behavior in living cells. Since mEosFP can be excited locally using a thin beam of 405 nm violet-blue light the MT-MF interactions can be studied in well defined regions of cells. Our studies employing these transgenic plants are focused on tip-growing root hair cells, diffuse growing trichomes and jigsaw puzzle shaped pavement cells. The probes have also been introduced in the distorted1 and wurm mutants of the actin modulating ARP2/3 complex and the microtubule organization mutant *mor1*. Our approach combining fluorescent proteins, cytoskeletal inhibitors and mutants is expected to increase our understanding of MT-MF interactions during plant growth and development.

P-22

Genomic and Physiological Analysis of Two QTLs that Regulate a Critical Stage of Adventitious Shoot Regeneration in *Arabidopsis thaliana* (L.)

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Plant tissues demonstrate a remarkable ability to regenerate organs after being excised. *In vitro*, these explants often require a hormone pretreatment consisting of high auxin (2.4-D)/low cytokinin (Callus Induction Media - CIM) for a few days following excision. This pretreatment is hypothesized to promote tissue dedifferentiation, making excised tissues more competent to regenerate. Following CIM pretreatment, subsequent exposure to a different high auxin (NAA)/low cytokinin media promotes root organogenesis, whereas exposure to high cytokinin/low auxin promotes shoot organogenesis. In order to understand the critical high auxin (2,4-D) dedifferentiation step, we undertook a natural variation screen in Arabidopsis thaliana to identify ecotype(s) that could bypass this requirement yet regenerate shoots efficiently. We screened for additional ecotypes that were strongly dependent on this hormone pre-treatment. Using near isogenic lines we generated following F1 crosses between these extreme parents, we demonstrate that two OTL regions, first identified by Lall et al. (2004) as promoting shoot regeneration, in fact can also bypass the CIM competency requirement. Interestingly, we demonstrate that these QTLs alter hormone responses in intact seedlings. We used global gene expression analysis to identify candidate hormone pathways involved in the regulation of shoot regeneration and present a summary model of these results.

P-23

Mycoheterotrophic plants: the pinnacle of evolutionary plant-fungal specialization H. B. Massicotte¹, L. H. Melville², L. E. Tackaberry¹, D. L. Luoma³, R.L. Peterson²

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Mycoheterotrophic plants lack chlorophyll and therefore are dependent on associated symbiotic fungi and third party autotrophic plants for their carbon needs. There are 400 mycoheterotrophic species in 87 genera and 10 families of angiosperms. Among these are 10 genera in the subfamily, Monotropoideae (family Ericaceae). In this subfamily, there is considerable specificity between plant host and fungal symbionts; however, all fungal species involved form ectomycorrhizas with a wide variety of autotrophic trees and shrubs. Based on our studies of 5 of the 10 genera in the Monotropoideae, as well as published literature, consistent structural features characterize these mycoheterotrophs: a fungal mantle, a Hartig net confined to the host epidermis, and 'fungal pegs', hyphae that enter epidermal cells but are then enclosed in host-derived finger-like wall projections. These wall modifications are similar to those of transfer cells involved in short-distance transport of nutrients in other systems. The unique structural characteristics of the host-fungus interaction have been used to describe this association as a separate mycorrhiza category known as 'monotropoid'.

P-24

Fluorescent protein aided dissection of response hierarchy between mitochondria and peroxisomes exposed to oxidative stress

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Plants combat diverse environmental stresses while remaining rooted to a spot suggesting that plant cells possess the molecular cell biological machinery essential for responding rapidly to stress and minimizing its negative effects. Mitochondria and peroxisomes are major organelles involved in the production and scavenging of reactive oxygen species (ROS). Both organelles produce tubular protrusions called matrixules and peroxules, respectively, in response to oxidative stress. These extensions have been observed separately and it is unclear whether a stress response hierarchy exists between the two organelles or their responses occur independently. Experiments using transgenic Arabidopsis plants simultaneously expressing GFP targeted to mitochondria and YFP targeted to peroxisomes showed that exogenous application of H₂O₂ results in an earlier response from mitochondria than from peroxisomes. Similar results were obtained with high intensity light (200 µmol/m²/sec) and ABA treatments. A hierarchy of organelle response is suggested during which mitochondria start extending earlier and at low levels of oxidative stress as compared to peroxisomes. Both organelles undergo fission following stress-induced elongation leading to their increased numbers within a cell. These studies are crucial in understanding the rapidity of intercellular responses in plants and pave the way for subsequent molecular and biophysical dissection of the phenomenon.

P-25

Chemical inhibitors suggest endophytic fungal Taxol is derived from both mevalonate and non-mevalonate-like pathways

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Yew trees (*Taxus*) produce the anti-cancer drug, Taxol, but surprisingly host fungi that also produce Taxol in vitro. A key question is whether the biosynthetic pathway for fungal Taxol is similar to that of plants. To answer this question, we applied chemical inhibitors shown to block early steps in plant Taxol biosynthesis to a Taxol-producing fungus. Previous reports suggest that the backbone of plant Taxol is derived from the plastidic non-mevalonate terpenoid pathway. Fosmidomycin, an inhibitor of DXR, an early enzyme in the plastidic/bacterial terpenoid pathway, inhibited fungal Taxol production by >99%, suggesting the fungus may have enzymes in common with the plastid terpenoid pathway, a surprising result. At low concentrations, compactin, an inhibitor of HMGR, an enzyme in the (eukaryotic) cytosolic mevalonate terpenoid pathway, inhibited Taxol production by >99.9%. The compactin result suggests that fungi may utilize the mevalonate pathway to generate precursors for Taxol to a much greater extent than in plants. TLC results suggest that the mevalonate versus non-mevalonate enzymes contribute different terpenoid components of the fungal Taxol molecule. We conclude that enzymes from two major terpenoid pathways are individually required for fungal Taxol biosynthesis: the normal yeast mevalonate-like pathway and the plastidic or bacterial non-mevalonate-like pathway.

P-26

Biogenesis of glycerol 3-phosphate acyltransferase (GPAT): influence of transmembrane domains and protein-protein interactions on the localization of GPAT to ER subdomains Satinder K. Gidda¹, Jay M. Shockey², Stephen H. Vinyard³, John M. Dyer³, and Robert T. Mullen¹

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Glycerolipids are the major components of cellular membranes in all plant cells, storage oils in developing seeds, and the cuticular surface of plant organs. Using the tung (Vernicia fordii) triacylglycerol (TAG) biosynthetic enzymes as model system, we previously showed that the type 1 and 2 diacylglycerol acyl transferase (DGAT1 and DGAT2) involved in the committed step of TAG biosynthesis are located in distinct subdomains of the ER [Shockey et al. 2006. Plant Cell 18:2294]. Here, we describe the cellular properties of the enzymes that mediate the initial step in the production of glycerolipids, glycerol-3-phosphate acyltransferases (GPATs). We show that both GPAT8 and a newly-identified GPAT with homology to mammalian GPAT3, GPAT9, are localized to the same ER subdomain as DGAT2. Split-ubiquitin yeast-two hybrid assays revealed that GPAT8, GPAT9 and DGAT2, but not DGAT1, physically interact and comprehensive mutational analysis of GPAT8 revealed that the protein's two transmembrane domains and intervening loop region are both necessary and sufficient for ER subdomain localization. The implications of these results in terms of our understanding of the regulation and organization of glycerolipid biosynthesis and the formation of ER subdomains via high-ordered, protein homo- and hetero-oligomeric complexes are discussed.

P-27

PtdIns3P-containing tubules facilitate receptor-ligand trafficking during vacuole biogenesis Resmi Radhamony, Elyse Roach, Chris P. Trobacher, Neeta Mathur, John S. Greenwood and Jaideep Mathur

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A variety of proteins are trafficked via the endoplasmic reticulum (ER), Golgi stacks and trans-Golgi-network (TGN) into pre-vacuolar compartments (PVCs) and onwards to the lytic vacuole for degradation. Cortex-directed tubules have been observed on the vacuolar surface but their role in the vacuolar pathway or their relationship to other endocytic compartments remains unclear. Using GFP/RFP/EosFP fusions with a 2xFYVE domain we demonstrate that a subset of the tubules on the vacuolar surface are enriched in PtdIns3P and can be clearly distinguished from GFP::delta TIP labeled tonoplast extensions. The tubules exhibit complete co-localization with the SNARE AtPep12/SYP21, an established marker for PVCs. Like other PVCs the tubules dilate rapidly in response to the PtdIns3P-kinase inhibitors wortmannin and LY-294002 while release from inhibitor treatments results in tubule pinching and budding of multi-vesicular bodies. In triple labeling experiments the FYVE-labeled tubules were found to sequester the vacuolar-sorting receptor BP-80 and its ligand sporamin. Our live-imaging observations suggest that PtdIns3P-containing tubules on the vacuolar surface are PVCs whose elongated shape gets created through frequent fusions with tonoplast extensions. These tubular PVCs appear to have a direct role in channeling proteins to the lytic vacuole.

P-28

MUM ENHANCER 4 is required for mucilage production in the seed coat of Arabidopsis thaliana

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Pollination triggers the differentiation of the ovule integuments to form a specialized seed coat. One specialization is the production of pectinaceous mucilage in the epidermal cells of the seed coat. This mucilage is released upon wetting and forms a gel capsule that is believed to aid seed hydration and germination. The mucilage secretory cells undergo a complex differentiation process in which cell growth is followed by synthesis and secretion of a large amount of mucilage. Mucilage secretion to the apoplast in the outer tangential portion of the cell is accompanied by constriction of the vacuole and formation of a volcano-shaped columnella beneath the mucilage pocket.

A number of genes have been identified affecting mucilage secretory cell differentiation, including MUCILAGE-MODIFIED4 (MUM4). mum4 mutants produce a reduced amount of mucilage and have a flattened columnella. Cloning of MUM4 revealed that it encodes a rhamnose synthase that is developmentally upregulated to provide rhamnose for the backbone of the primary pectin found in Arabidopsis mucilage. In order to identify further genes acting in mucilage secretory cell differentiation, pectin synthesis and secretion, a screen for enhancers and suppressors of the mum4 phenotype was performed. Here we discuss the isolation and initial characterization of mum enhancer4 (men4).

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Biochemical analysis of GmCaMK1, a novel CaM-binding receptor-like protein kinase from soybean root nodules

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Transient Ca²⁺ fluxes are used as a second messenger in many eukaryotic signaling pathways. and are transduced by Ca²⁺ sensor proteins such as calmodulin (CaM). Upon binding Ca²⁺ CaM modulates the activity of numerous target proteins, including protein kinases, though such CaMkinases have been largely unstudied in plants. Here we present the isolation and biochemical characterization of GmCaMK1, a novel Ca²⁺/CaM-binding kinase from sovbean root nodules. GmCaMK1 is a 47 kDa receptor-like protein kinase, which interacts with CaM in a Ca²⁺dependent manner. An N-terminal transmembrane domain appears to associate GmCaMK1 with an unspecified cellular membrane. We have delineated a 20 residue CaMBD adjacent to the conserved ser/thr kinase domain, and have shown that this motif binds the globular domains of CaM with high affinities via isothermal titration calorimetry. Analysis of substitution mutants has identified critical residues of the CaMBD. Autophosphorylation activity of recombinant GmCaMK1 has appears to be independent of Ca²⁺/CaM, suggesting CaM regulates GmCaMK1 by other means, such as substrate interaction. Analysis by qPCR shows that GmCaMK1 is expressed primarily in lateral roots, as well as developing nodules. This expression pattern is corroborated by GUS reporter expression, and as such we are examining the effect of RNAimediated *GmCaMK1* knockdown on nodule formation and morphology.

P-30

Anatomical change leading up to the evolution of C4 Photosynthesis in *Anticharis* Endl. (Scrophulariaceae)

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C4 photosynthesis has independently evolved over 50 times, with the majority of origins occurring within the eudicots. One origin occurs in the genus *Anticharis* (Schrophulariaceae). which consists of ten species from arid regions of southwest Asia and eastern and southern Africa, In Anticharis, C4 photosynthesis has never been studied. In this study, we mapped the results of a carbon isotope screen for C4 photosynthesis onto a preliminary ITS phylogeny of Anticharis. Herbarium specimens from seven Anticharis species spanning the C3 to C4 transition were sampled for leaf anatomical analysis. Results showed that one Anticharis species (A. ebracteata) has a C3 carbon isotope ratio. It branched in the phylogeny basal to the C4 species, demonstrating that C3 photosynthesis is the ancestral condition in *Anticharis*. Six of the 10 Anticharis species are shown to be C4 and all examined exhibited the Atriplicoid type of Kranz anatomy. One species that also branched basal to the C4 species, A. scoparia, has a carbon isotopic value of -20.1, indicating C3-C4 intermediacy. This species also had an intermediate leaf anatomy, with large bundle sheath cells and close vein spacing, which we term proto-Kranz anatomy. Notably, the C3 species A. ebracteata also exhibited proto-Kranz anatomy, indicating that enlargement of the bundle sheath cells may be an important precondition for the evolution of C4 photosynthesis in *Anticharis*. Understanding the genetic control over Kranz leaf development may therefore have to address developmental changes in the immediate C3 ancestors of the C4 lineages.

P-31

A Novel *in vivo* Antioxidant assay utilizing *Drosphila melanogaster* SOD null mutants confirms *in vitro* determined high antioxidant properties of Sweet Fern (*Comptonia perigrina*).

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Sweet Fern (*Comptonia peregrina*), an abundant shrub in northern Ontario, was traditionally used by First Nations people for its medicinal attributes. In present study, the antioxidant capacity of Sweet Fern ethyl acetate extract was determined (53.9 \pm 0.8% μ M Trolox equivalents per 0.01 g/L of extract) using the oxygen radical absorbing capacity (ORAC). Furthermore, we have developed a screen for in vivo antioxidant efficacy. We are utilizing a Drosophila stock with a mutation in superoxide dismutase (SOD-null) with reduced metabolic capacity to detoxify highly reactive superoxide radicals. In our assay, adult flies laid eggs on sweet fern supplemented media and developing larvae were exposed to acute oxidative stress by paraguat treatment which causes 95% mortality. Our results using this screen confirm the *in vivo* efficacy Sweet fern extracts in protection against oxidative stress where 5 mg/mL Sweet Fern extract eliminated the developmental delay induced by paraguat in SOD-null homozygous larvae and were essentially 100% effective in protecting against acute exogenous oxidative stress during development. HPLC analysis of ethyl acetate revealed presence of 21 peaks indicating the presence of at least 21 different substances in this extract. The isolation and characterization of these substances could lead to the identification of specific compounds with potent antioxidant and antibacterial properties in Sweet Fern.

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Glutamine Biosensor as a New Tool for Plant Physiology

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Glutamine is the first amino acid to be synthesized following the uptake of a nitrogen source and is then used in the biosynthesis of other amino acids, and also serves as a signalling molecule to regulate the nitrogen status of the plant. There are several ways in which to measure glutamine including HPLC and GC-MS, however most of the currently available methods have the disadvantages of being expensive, time consuming and requiring some level of technical expertise. To overcome these disadvantages, we have created a whole-cell glutamine biosensor. This biosensor was generated by transforming a glutamine auxotrophic strain of E. coli with a plasmid which contains a constitutively active Lux operon. Using this biosensor the detection limit for glutamine in solution has been pushed to levels < 0.5 pmol using a simple procedure. The use of this biosensor has the potential to allow for glutamine quantification in multiple plant tissues and other sources quickly and at low cost. The ability to visualize glutamine concentrations in planta is also of interest. The biosensor has also been used to visualize spatial differences in glutamine concentrations within maize plant tissues.

P-33

Phenotypic and Genotypic Evaluation of Common Bacterial Blight Resistance in a Resistance Inter-Cross Population of *Phaseolus Vulgaris*.

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Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is a damaging widespread disease of common bean (*Phaseolus vulgaris L*.). Genetic resistance to CBB in common bean is limited, but has been introgressed through inter-specific crosses with tepary bean (*P. acutifolius*). A recombinant inbred line population of a cross between the resistant genotypes OAC Rex and HR45, known to carry different resistance QTL, was evaluated for resistance to CBB and genotyped with molecular markers associated with the two CBB QTL. Segregation of CBB response in the population, the effect of each QTL and the interaction effect of the two QTL will be discussed.

P-34

Impact of Alternative Oxidase on Plant Response to Temperature

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The plant mitochondrial electron transport chain (ETC) includes a non-energy conserving alternative oxidase (AOX) hypothesized to dampen reactive oxygen species (ROS) generation by the ETC and / or facilitate respiratory carbon metabolism. In *Nicotiana tabacum*, AOX levels increase dramatically at low temperature. To investigate the role of AOX, we compared wildtype (Wt) tobacco plants to transgenic plants with enhanced or silenced AOX expression. Plants were initially grown at a day / night temperature of 28°C / 22°C and then shifted to 12°C / 5°C for up to 72 h. Following the cold shift, there was a dramatic accumulation of glucose and fructose in the leaf. Interestingly, plants overexpressing AOX accumulated significantly more of these sugars that Wt while plants with silenced AOX expression accumulated significantly less than Wt. These results are inconsistent with a widely held hypothesis that AOX may act to burn excess carbohydrate, but rather suggests a role for AOX to aid sugar accumulation, at least during cold stress. We measured levels of leaf lipid peroxidation as one indicator of the level of oxidative damage in plants prior to and after the cold shift. Under control growth conditions (ie. prior to cold shift), plants overexpressing AOX displayed significantly lower levels of lipid peroxidation than did the Wt while plants with silenced AOX expression displayed consistently higher levels than WT. These results are consistent with the hypothesis that AOX can dampen ROS generation. However, at 24 h post cold shift, all plants showed an increase in lipid peroxidation, with the exception of the most strongly silenced plant line (RI29), in which levels of lipid peroxidation declined significantly. This decline correlated with a more rapid induction of ROS-scavenging enzymes (CuZn-superoxide dismutase, Fe-superoxide dismutase, glutathione peroxidase, ascorbate peroxidase) in RI29 than the Wt. These data suggest that the lack of AOX during the cold shift enhanced a stress-signaling pathway (likely mitochondrial in origin and perhaps ROS-based) able to increase the ROS-scavenging capacity of the cell.

P-35

Protoplast isolation and transient gene expression in the single-cell C₄ species, *Bienertia* sinuspersici

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Transient gene expression with reporters such as green fluorescent protein (GFP) is powerful for gene expression and protein localization studies. However the establishment of a highly efficient and reliable transient gene expression remains a challenge in many plant species. A reliable transient transformation protocol has not been established in the single-cell C₄ species, *Bienertia sinuspersici*, even though it has the potential of serving as a model system in C₄ photosynthesis. Here we report the first protocol to isolate a large number of viable protoplasts from leaves of this unique single-cell C₄ plant. Moreover, we have also established and achieved high transient expression of PEG-mediated transformation of the isolated protoplasts. Protoplasts transformed with various GFP fusion constructs were used to examine organelle localization and dynamics. Overall, our efficient protoplast isolation and transient transformation system can be utilized to examine functional gene analysis and biochemical manipulation in *Bienertia sinuspersici*.

P-36

The alleviation of low temperature sweetening in potatoes through transformation Reena Pinhero^a, Rinu Pazhekattu^b, A.G.Marangoni^a, Qiang Liu^c, Rickey Y Yada^a Dept of Food Science, ^bHuman Health and Nutritional Sciences, University of Guelph, ^cGuelph Food Research Centre, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada N1G2W1

Potato tubers stored at temperatures below 9-10°C result in high concentrations of reducing sugars such as glucose and fructose known as low temperature sweetening (LTS). These reducing sugars participate in the Maillard browning reaction with free amino acids during frying resulting in dark-brown colored fries and chips, which are unacceptable to consumers. These reducing sugars also result in the possible production of acrylamide which has been linked to many cancers. Our earlier research with a LTS-tolerant and LTS-susceptible variety has shown that, ethanol and lactate levels are higher in LTS-tolerant variety and were strongly correlated to improved chip color. As well, a positive correlation was observed between reducing sugar concentration and the K_M of pyruvate decarboxylase (PDC), with the LTS-tolerant ND 860-2 possessing a lower K_M and reducing sugar content than the LTS-susceptible Monona. These results suggest a role for PDC in LTS-tolerance. To examine the role of PDC, the potato variety Snowden was transformed with Arabidopsis cold-inducible pyruvate decarboxylase gene 1 (APDC1) under the control of cold-inducible promoter rd29A. The insertion of APDC1 gene and its expression in the transgenic potato plants selected were confirmed by Southern and Northern analyses, respectively. Storage studies were conducted with transgenic tubers. The results from these experiments will be discussed in relation to LTS-tolerance.

P-37

Protective role of polyphenols against photooxidative stress in maple leaves

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Polyphenols accumulate in plants exposed to oxidative stresses (UV, high light, heavy metals, etc.) but their significance as antioxidant *in planta* remains unclear. In this study, we aimed to characterize and correlate the oxidation of polyphenols with the occurrence of oxidative damages during the exposure to high light of maple leaves infiltrated with and without methylviologen (MV). The presence of MV enhanced the decrease of the maximum photochemical efficiency of photosystem II (estimated by the fluorescence parameter Fv/Fm). MV also caused significant losses of chlorophyll *a+b* leaf contents that accelerated after two hrs of photoinhibitory treatment. Photoinhibition affected polyphenols only in MV-treated leaves. This was first indicated by significant decreases in MV-treated leaves of absorbance at 262 nm and more directly by cyclic voltammetry, showing oxidation of polyphenols having redox potentials between 0 and 400 mV but not of those with potentials between 400 and 800 mV. Differences between voltammograms of untreated- and treated leaves show distinct current waves near 170 and 350 mV. Interestingly, the oxidation of polyphenols was completed after two hrs of photoinhibitory treatment, and coincided with the acceleration of chlorophyll losses. These results indicate that polyphenols could protect maple leaves against photo-oxidative damages.

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Developmental anatomy of *Arabidopsis thaliana*: comparing internal development at specific growth stages through a new histochemical technique

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The principle growth stages based on phenotypic characteristics of *Arabidopsis* have become a framework for reporting data from various laboratories on a uniform plant material. Thirty growth stages were identified based on the BBCH scale (Plant Cell, 2001, 13:1499). Our research complements this growth stage model by defining the internal anatomy at each developmental landmark. Specifically, we defined the development of tissue layers in the root, hypocotyl and inflorescence of *Arabidopsis*. To visualize the anatomy under the microscope, we developed a new contrasting histochemical technique to be applicable to free-hand sections. This technique involved using TBO in 20% calcium chloride to stain lignified cell walls blue and counterstained primary cell walls with ruthenium red. When mounted in 100% glycerol, hand sections are preserved semi-permanently without losing the intensity and contrast of staining for several months, allowing for future observations for further analysis. Using this technique, the differentiation of primary tissues and the secondary growth in Arabidopsis was recorded. Specifically, changes in the development of the vascular and dermal tissue systems were observed from germination through to senescence. This research builds a foundation for plant researchers working with Arabidopsis, allowing for utilization of uniform plant material in future research.

P-39

Biochemical and Transcriptomic Characterization of S-Methyl-Cysteine Metabolism in Developing Seeds of Common Bean (*Phaseolus vulgaris*)

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The levels of sulphur amino acids in seeds of common bean are suboptimal for human nutrition, and sulphur amino acid deficiency is prevalent in protein-energy malnutrition. Phaseolus and several Vigna species are characterized by the accumulation in seeds of high levels of the nonprotein amino acid S-methyl-Cys, which cannot substitute for Cys or Met in the diet. It was found that the content of Met and Cys is improved in mature seeds of SMARC1N-PN1 lacking phaseolin, phytohemagglutinin and arcelin, with S-methyl-Cys and γ-Glu-S-methyl-Cys correspondingly decreased as compared with the genetically related wild-type line SARC1. These lines are advantageous to study the molecular mechanisms controlling the metabolism of sulphur amino acids, especially S-methyl-Cys, in seeds of common bean. Our objectives are 1) to profile key metabolites of the S-methyl-Cys metabolic pathway, e.g. S-methyl-Cys, γ-Glu-Smethyl-Cys and Met during seed development in the reference genotype BAT93, and determine the key sampling stages to generate seed-specific EST data and perform transcript profiling analysis; 2) to reveal transcript profiles and regulation of S-methyl-Cys metabolism and related sulphur amino acid pathways via high-throughput microarray analysis on four seed developmental stages of SARC1 and SMARC1N-PN1; 3) to compare gene expression differences in BAT93 and Vigna mungo, lacking S-methyl-Cys and accumulating γ-Glu-Met, using 454 cDNA sequencing technology.

P-40

Functional characterization of a subfamily of developmentally regulated, stress-responsive CMLs in *Arabidopsis*

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In the paradigm of calcium (Ca²⁺) signaling, perception of a stimulus at the cell surface leads to a rapid and transient influx of Ca²⁺ into the cytosol. These stimulus-specific fluxes are termed Ca²⁺ signatures. Once generated, the Ca²⁺ signature is detected by a suite of proteins collectively known as Ca²⁺ sensors, whose function is to decode and transmit the signal to the downstream effectors responsible for mediating the physiological response. The most well characterized Ca²⁺ sensor in both plant and animal systems is calmodulin (CaM). In addition to conserved CaM, plant genomes are predicted to encode greatly expanded families of CaM-like proteins (CMLs), the majority of which remain unstudied. Previous work in our lab has begun to characterize the expression patterns of a subfamily of CMLs, CML37, CML38 and CML39, revealing that these Ca²⁺ sensors are regulated differentially throughout development and in response to a variety of environmental stresses. Currently, we are using an array of approaches to i) determine the parameters of Ca²⁺-binding, ii) define the spatial domains of CML function within the cell, and iii) isolate the binding partners of these CMLs in order to gain insight into their physiological function.

P-41

Somatic embryogenesis and plant regeneration of Eriophorum vaginatum L.

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Sexual reproduction of *E. vaginatum* varies in success from year to year and is strongly dependent on environmental conditions. Micropropagation offers a promising alternative to produce large numbers of plants in a short time for wetland creation and restoration. Furthermore, somatic embryos are amenable to *in vitro* cryopreservation and thus provide long-term *E. vaginatum* germplasm storage. Efficient tissue culture is also a first step in using genetic engineering for *E. vaginatum* phytoremediation. A variety of explants (roots, leaves, seeds) were assessed for callus formation and seeds proved to be the best source. Sterile seeds were placed on a callus induction medium containing different concentrations of 2,4-D and kinetin. After callus development explants was placed onto shoot-regeneration medium supplemented with 6-benzylaminopurine (2 mg l⁻¹, 4 mg l⁻¹) in the low light conditions. A month later callus with distinctly differentiated shoots of somatic embryos was obtained. Regenerated shoots were transferred onto root regeneration media without growth regulators and after root formation acclimatized in a greenhouse. Thus *E. vaginatum* has callus-forming capability and its seeds are the best starting material for the plant regeneration via somatic embryogenesis.

P-42

Seed structure and unusual germination of Eriophorum vaginatum L.

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Eriophorum vaginatum L. is an invasive sedge, widely distributed in the wet boreal and circumpolar regions. Although vegetative propagation by rhizomes is considered to be the major reproductive mechanism of this plant, sexual reproduction and seed dispersal are very important for the establishment of new colonies to guarantee genetic variability. The endosperm constitutes the majority of E. vaginatum seed volume and the small cone-shaped embryo is located at the micropylar end. The cells of the endosperm contain numerous protein bodies in the center and spherical starch grains at the periphery. Both the endosperm and the embryo are surrounded by one layer of living, lipid-containing aleurone cells. In the mature embryo, the scutellum occupies the largest volume and is attached to the curved plumule-radicle axis. Seeds collected from contaminated wetlands are dormant and only application of gibberellins or seed placement on MS medium overcome dormancy. E. vaginatum seeds display an "unusual" type of germination where the coleoptile emerges first through the pericarp.

P-43

Does nutrient supply affect growth and toxicity of a Harmful Algal Bloom dinoflagellate, *Gymnodinium mikimotoi*?

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A recent algal red-tide event occurred off the coast of United Arab Emirates (UAE) in September 2008 and has caused massive fish kills and ecological devastation along the coastal waters. Not only is the underlying fish-killing mechanism poorly understood but factors that induce algal toxicity are also unclear. Some fish-killing mechanisms include the production of reactive oxygen species (ROS), haemolytic activity or mucus. Each mechanism leads to agitation and suffocation of fish. *Gymnodinium mikimotoi* has been isolated from this red-tide along the UAE coast and its growth response has been determined at varying nitrate to phosphate ratios (N:P). Algal cells grown under different N:P ratios, will also be analyzed for different forms of algal toxicity, such as extracellular hydrogen peroxide, haemolytic activity and mucus production. The nutrient use and toxicity of this specific isolate taken from the UAE coast can be used to retrospectively analyze the causes of novel blooms in the area.

P-44

Cadmium uptake in plants under high and low transpiration rates

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Cadmium (Cd) may accumulate in plants to levels that are of concern in human diets. A hydroponic experiment was carried out to investigate the effects of transpiration rate on Cd uptake in lettuce (*Lactuca sativa*), barley (*Hordeum vulgare*), and radish (*Raphanus sativus*). Plants were grown in nutrient solution containing a range of concentrations (0 to 2.0 μM CdCl₂ for lettuce and barley; 0 to 0.5 μM CdCl₂ for radish based on dose response) of Cd at either 60% or 80% relative humidity in order to manipulate transpiration rate for 28 days. The effect of transpiration on total Cd uptake varied among species and Cd concentration. The results showed a positive relationship between transpiration rate and total Cd uptake in barley grown at both 0.5 and 1.0 μM CdCl₂ concentrations. Plants grown in 0.1 μM CdCl₂ depleted Cd from solution prior to harvest, which may explain why increased transpiration did not result in increased Cd uptake. The lack of relationship in barley grown at 2.0 μM CdCl₂ may be due to Cd toxicity. In the case of lettuce and radish, the relationships were insignificant. These results suggested that the effect of transpiration on Cd uptake is species dependent.

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P-45

Distinct Pathways Mediate the Sorting of Tail-Anchored Mitochondrial Outer Membrane Proteins

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Little is known about the biogenesis of tail-anchored (TA) proteins localized to the mitochondrial outer membrane in plant cells. To address this issue, we screened all of the (>500) known and predicted TA proteins in *Arabidopsis* for those annotated, based on Gene Ontology, to possess mitochondrial-related function(s) and/or contain a C-terminal sequence resembling the targeting signal motif characterized previously for mitochondrial isoforms of the TA protein cytochrome b₅ (Cb₅). Surprisingly, only two of the ~30 TA proteins annotated as mitochondrial possessed a Cb₅-like targeting signal. Using tobacco BY-2 cells as a model *in vivo* targeting system we confirmed the mitochondrial localization and TA topology for several novel members of each group of proteins, and subsequent mutagenic analyses revealed that their C termini, while distinctly different at the primary sequence level, were both necessary and sufficient for mitochondrial targeting. We also employed split-YFP assays to show that while both groups of TA proteins interact with certain components of the mitochondrial outer membrane translocase (TOM), only a few TA proteins interact also with the sorting and assembly machinery (SAM). Collectively, these results in combination with other findings suggest that mitochondrial TA proteins rely on several different and possibly parallel sorting pathways.

P-46

Localization of DIR1 during Systemic Acquired Resistance in Arabidopsis

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Systemic Acquired Resistance (SAR) is a plant defense response induced by an initial infection that leads to production of a long distance signal(s) that moves to and is perceived by distant leaves resulting in resistance to normally virulent pathogens. DIR1 is involved in SAR long distance signaling as demonstrated by the presence of DIR1 in phloem sap of SAR-induced wild type, but not mock-induced or *dir1-1* plants. Expression of DIR1 in one *dir1-1* leaf, using *Agrobacterium*-mediated transient transformation, followed by SAR induction, rescued the *dir1-1* SAR defect. Additionally, western blot analysis demonstrated that DIR1 protein was found in distant leaf phloem exudates of induced plants suggesting that DIR1 moves to distant leaves and may be a SAR long distance signal. Homology modeling of DIR1-like (88% amino acid similarity with DIR1), revealed clues to explain why DIR1-like rarely compensates for the SAR defect in *dir1-1*. We also developed a Cucumber-Arabidopsis SAR model to further our SAR studies. Phloem exudates from SAR-induced cucumbers contain a DIR1-like protein that rescues the Arabidopsis *dir1-1* SAR defect suggesting that SAR long distance signaling mechanisms are conserved in both the Cucurbit and Brassica plant families.

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P-47

Functional analysis of grape CBF genes

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The CBF pathway functions in the acquisition of freezing tolerance in plants. Eight *CBF* genes are present in *Vitis riparia* (Vr). It is likely that these genes have different functions because they are present on different chromosomes and place on different clades in a dendogram produced with all CBF protein sequences known to date. VrCBF1 and VrCBF4 were overexpressed in *Arabidopsis* to detect such a difference in function. Whole plant, electrolyte leakage and chlorophyll imaging experiments showed that both CBF1 and CBF4 transgenics had increased freezing and drought tolerance, whereby CBF4 affected freezing tolerance and CBF1 affected drought tolerance to a larger degree. Phenotypic changes included dwarfing, production of more leaves prior to flowering and smaller and thicker leaves. Microscopy revealed that this was correlated with features that could explain the increase in tolerance, such as larger palisade mesophyll cells, a higher number spongy mesophyll cells with fewer airspaces, and a higher number of trichomes. Our hypothesis is that the difference between VrCBF1 and VrCBF4 expressing plants is due to a difference in the preferred promoter binding site for these transcription factors. The activation of *COR* genes with different promoter elements in VrCBF1 vs VrCBF4 expressing plants supports this hypothesis.

P-48

Traits related to water use efficiency in soybean (*Glycine max* L. Merr.) – do greenhouse screens predict field results?

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Dark-adapted leaf conductance (g_{dark}) is a trait shown to be negatively correlated with water use efficiency (WUE, amount of crop dry matter produced per unit soil water transpired) in soybean. The basis of this correlation is that g_{dark} is positively correlated with leaf internal CO_2 concentration (C_i) in leaves photosynthesizing at steady state. The objective of this study was to determine if genotypic differences in g_{dark} in field-grown plants could be predicted from greenhouse screening. Seven commercial soybean varieties were compared for WUE, g_{dark} and C_i in the greenhouse, and for g_{dark} and C_i in the field. Field g_{dark} was strongly correlated with greenhouse g_{dark} (r = 0.89, p = 0.008). WUE measured in the greenhouse was negatively correlated with g_{dark} measured either in the greenhouse (r = -0.82, p = 0.03) or the field (r = -0.82, p = 0.02). Genotypic variation for C_i was found only in the greenhouse (p = 0.006), and C_i was correlated with both g_{dark} (r = 0.83, p = 0.02) and WUE (r = -0.76, p = 0.05) as expected. We conclude that greenhouse measurements of g_{dark} can predict genotypic variation for this trait in the field.

P-49

Linkage mapping of genes associated with dehydrodiferulic acid mediated cross-linking in maize cell walls and resistance to *Fusarium graminearum* (Schwabe)

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The fungus Fusarium graminearum Schwabe [sexual state: Gibberella zeae (Schweinitz) Petch] causes Gibberella ear rot in maize and Fusarium head blight in wheat. The most devastating effect of these diseases is the deposition of mycotoxins in the grain. Molecular markers may allow rapid introgression of stable genetic resistance. Previous work has shown that there is a negative correlation between severity of disease and the concentration of cell wall bound dehydrodiferulic acid (DFA) in the pericarp of the grain, and some chromosomal regions that are important for resistance are also important for DFA content. Since DFA is a derivative of the phenylpropanoid pathway, we have adopted a candidate gene approach to identify the genes that are responsible for the resistance/DFA QTL. Polymorphisms have been discovered in putative phenylalanine ammonia lyase (PAL), caffeoyl-coenzyme A 3-O-methyltransferase (CCoAOMT), and hydroxycinnamoyl-coenzyme A shikimate/quinate hydroxycinnamovltransferase (HCT) genes. Mapping of these loci in a recombinant inbred line population has indicated that they are located between the flanking markers of various resistance/DFA QTL. Statistical associations between markers, resistance, and pericarp DFA content will be presented. This research should provide a foundation for gene-derived marker assisted resistance breeding in elite maize adapted to the northern United States and Canadian corn growing areas.

P-50

Regulation of fibre development and secondary cell wall deposition: Not the usual suspects Heather L. Wheeler*¹, Michael E. Stokes¹, Malcolm M. Campbell^{1,2}

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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 therefore been intensely studied. However, certain aspects of secondary cell wall development, such as the transport of monolignols across the plasma membrane, remain poorly understood. We hypothesize that genes that are co-regulated with those of the lignin biosynthetic pathway play a role in secondary cell wall development. To test this, we identified such genes in *Arabidopsis thaliana* using the BAR Expression Angler and acquired *Arabidopsis* with T-DNA insertions within the selected genes. Inflorescence stems were sectioned and the vascular tissues were analyzed using toluidine blue and phloroglucinol staining. Genes for which T-DNA insertion generated an altered vascular phenotype include genes which have key roles in lignin biosynthesis as well as a transcription factor that was recently shown to be expressed in vascular tissues during secondary cell wall development. Other genes identified encode proteins that are localized to the plasma membrane. We hypothesize that such proteins are involved in the transport of monolignols across the plasma membrane for incorporation in the secondary cell wall.

P-51

Time-of-day shapes the Arabidopsis transcriptome

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Under natural conditions, plants experience episodes of drought for periods of days or longer. Plants respond to drought stress by reconfiguring their transcriptome activity. Transcriptome changes in response to drought are dynamic, and are likely to be shaped by mitigating factors such as diel signals. To gain insights into the dynamics of transcriptome reconfiguration in response to gradual soil drying, the drought-induced transcriptomes of *Arabidopsis thaliana* were examined at four time points over a single diel period – midday, late day, midnight, and predawn. A core set of genes was identified that was responsive to drought, independent of the time of day at which they were measured. Strikingly, the magnitude of the drought-induced changes for these genes varied in a time-of-day-dependent manner. An additional set of time-of-day-specific drought-responsive genes were also identified. The diurnal patterns of transcript accumulation for these genes was strongly influenced by drought stress. This study indicates that analysis of a single time point would miss suites of drought-responsive genes that are revealed through assessment of the dynamics of diurnal changes, emphasizing the value of characterizing multiple time-of-day-specific drought transcriptomes.

P-52

Plant metal uptake & soil metal bioavailability in the AM mycorrhizosphere

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The arbuscular mycorrhizal (AM) symbiosis is a mutually beneficial association between the roots of most herbaceous plants and the Glomeromycota fungi recognized for enhancing plant acquisition of nutrients and water. Recent reports also acknowledge a role in regulating soil metal bioavailablity via mycorrhizal-induced processes. We designed a compartmental pot greenhouse experiment involving a sunflower cultivar and an arbuscular mycorrhizal fungus aimed at comparing the impact of roots (rhizosphere), roots and hyphae (mycorrhizosphere), or strictly hyphae (hyphosphere) on plant growth, metal uptake, and edaphic conditions using the micronutrient zinc (Zn) as a typical metal contaminant. We demonstrated that mycorrhizosphere plants had lower Zn uptake (especially in shoots and flowers) and a lower incidence of leaf chlorosis than rhizosphere plants at the highest soil-Zn concentrations, which we associated with mycorrhizal-induced biosorption processes that reduce metal bioavailability to delay the onset of metal toxicity. We also observed that mycorrhizosphere plants cause a slight alkinization of the soil whereas rhizosphere plants tend to acidify it, then having important consequences toward the soil-Zn bioavailability and nutrient leaching. Altogether, we consider the AM mycorrhizosphere to be a key factor in environmental remediation by buffering plant growth conditions and enhancing soil resiliency via these mycorrhizal-induced processes.

P-53

Development of cytokinin antagonists acting at the receptor level

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Recently we published 6-(2-hydroxy-3-methylbenzylamino)purine (PI-55) as the first molecule antagonizing cytokinin activity at the receptor level. Here we present synthesis and in vitro biological testing of six PI-55 derivatives and five new benzylaminopurine analogues substituted in the benzyl ring. The ability of the compounds to interact with *Arabidopsis* cytokinin receptors AHK3 and CRE1/AHK4 was tested in bacterial receptor and live cell binding assays, and in Arabidopsis ARR5:GUS reporter gene assay, respectively. The cytokinin activity was further assayed in classical cytokinin bioassays (tobacco callus, wheat leaf senescence and Amaranthus bioassay). None of the PI-55 modifications led to the improvement of antagonistic properties; however, 6-(2,5-dihydroxybenzylamino)purine (LGR-991) was identified as a new cytokinin perception antagonist. At the molecular level, LGR-991 blocks cytokinin receptor CRE1/AHK4 with the same potency as PI-55. Moreover, LGR-991 acts as a competitive inhibitor of receptor AHK3, and importantly it shows reduced agonistic effects in comparison to PI-55 in ARR5:GUS and cytokinin bioassays. LGR-991 causes more rapid germination of Arabidopsis seeds which is a characteristic of seeds from plants with reduced cytokinin content. To conclude, LGR-991 shows a new structural motif that can lead to preparation of cytokinin antagonists with broader activity and reduced agonistic properties.

P-54

Genetic analysis of BLADE-ON-PETIOLE interactions with auxin in control of phyllotaxy, leaf initiation, and leaf patterning in *Arabidopsis thaliana*

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The Arabidopsis *BLADE-ON-PETIOLE* genes encode a pair of growth regulators that redundantly control the architecture of boundaries in leaves, inflorescences, fruits, and flowers. We show that leaf initiation in *bop1 bop2* mutants is slower relative to wild-type and that response to auxin is enhanced in leaf petioles, consistent with the ectopic formation of leaflets and midveins in this region. Leaf initiation and leaflet formation require the formation of auxin maxima, which result from the polar localization of PIN-FORMED1 (PIN1) auxin efflux carriers, dependent in part on the kinase activity of PINOID (PID). Genetic analysis of *bop1 bop2 pin1-1* and *bop1 bop2 pid-3* triple mutants shows a partial rescue of the "pin" inflorescence phenotype. Also, phyllotaxy defects in *pid-3* seedlings are enhanced by loss-of-function *bop1 bop2*. These interactions suggest that auxin accumulation in the triple mutants may be stabilized, enhanced, or support organ formation at a lower threshold. This may result from a broader pattern of *PIN1* expression, reduced recycling of PIN proteins, or the ectopic activation of auxin biosynthetic genes in *bop1 bop2* mutants. We are currently examining PIN1::GFP expression patterns to determine how BOP and PIN/PID activities might overlap to determine patterns of organ initiation in the plant.

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P-55

A rapid and sensitive method for the detection and quantification of cytokinins using the Qtrap $^{\text{@}}$ 5500 $^{\text{@}}$ triple quadrupole mass spectrometer and Kinetex $^{\text{@}}$ HPLC.

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Cytokinins are an important group of plant growth regulators that control several developmental events in a plants ontogeny. To date, there have been several analytical methods for the determination of CKs in plant samples including radioimmunoassay, ELISA, GC-MS, LC-MS and LC-MS/MS. However, all of these methods have drawbacks ranging from the required use of radiolabeled compounds, or hazardous derivatization compounds, poor sensitivity, lack of resolution between cytokinin isomers, expensive UHPLC systems or lengthy run-times. Recently, we have developed a more rapid and improved analytical method for several cytokinins using a standard HPLC system equipped with a new type of HPLC column (Kinetex®) and coupled to a triple quadrupole mass spectrometer (Sciex, QTRAP 5500®). By using this new method, we are able to resolve the cytokinins with high sensitivity in under 6minutes, which is roughly four times faster than our previous method that used conventional C18 columns. This method allows us to analyze 100-samples in one day without a costly UHPLC system. The sensitivity is about 100-times over our previous method and the chromatographic resolution of the nucleobases is far superior. This method will ensure that future investigation can accurately quantify a more comprehensive set of cytokinins from just a few mg of fresh plant tissue.

P-56

Investigating the Three Novel Phosphorylation Sites of Bacterial-type Phosphoenolpyruvate Carboxylase from Developing Castor Oil Seeds

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Phosphoenolpyruyate carboxylase (PEPC) is a tightly controlled cytosolic enzyme situated at a major branch point in plant carbon metabolism. Plant genomes encode several closely related plant-type PEPCs (PTPC) and a single enigmatic bacterial-type PEPC (BTPC) that exhibits low sequence identity with PTPCs. The unusual 'allosterically-desensitized' 910-kDa Class-2 PEPC hetero-octameric complex of developing castor oil seeds (COS) arises from a tight interaction between 107-kDa PTPC and 118-kDa BTPC polypeptides, and was hypothesized to support carbon flux to malate required for storage lipid synthesis. Although BTPCs lack the conserved N-terminal seryl phosphorylation site characteristic of PTPCs, Pro-Q Diamond staining and phosphate-affinity PAGE established that native COS BTPC is in vivo phosphorylated at multiple sites. LC MS/MS and LTQ-FT MS have identified pThr4, pSer425, and pSer451 as novel COS BTPC phosphorylation sites (93% sequence coverage). Phosphorylation-site specific antibodies have been produced to address the phosphorylation status of each site during various temporal and physiological states of COS development. The physical and kinetic effects of sitespecific COS BTPC phosphorylation are being assessed via phospho-mimetic mutagenesis of recombinant BTPC (by incorporating Asp at each phospho-site). Protein phosphorylation analyses using these powerful tools will to help elucidate the function and control of this important metabolic enzyme.

P-57

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

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Mild heat stress of *Brassica napus* microspore cultures causes some of the cells to develop into embryos. Embryo development in microspore cultures closely resembles zygotic embryo development. In a microarray screen of cDNA from sorted embryogenic versus nonembryogenic cells a gene that was uregulated 8-fold was identified that is homologous to the Arabidopsis gene AT1G73740, of unknown function. The objectives of the current work are to functionally characterize the Brassica homolog(s) of AT1G73740. Three genes (82% similar to AT1G73740) were isolated from B. napus and named BnMicEmUP (for B. napus Microspore Embryogenesis Up regulated) genes. BnMicEmUP1 is expressed in embryogenic microspore cultures but not in noninduced cultures. BnMicEmUP2 is expressed in leaf, root, stem and pollen cells. Overexpression of BnMicEmUP1 in Brassica reduced or prevented seed production. Long-lasting flowering and juvenile stages were also observed in Brassica plants transformed with 35S:: BnMicEmUP1. The overexpression and suppression of BnMicEmUP1exhibited delayed *flowering*-time phenotype in *Arabidopsis*. Information from studies of microspore embryogenesis is applicable to improving this system for producing homozygous plants for plant breeding and basic information on embryogenic processes leads to a greater understanding of totipotency in plants.

P-58

Molecular characterization of key genes for folate synthesis in common bean W. Xie, Y-S. Shim, F. Garabagi, A. Navabi, K.P. Pauls Department of Plant Agriculture, University of Guelph, Guelph, ON N1G2W1

Previous results showed that high levels of folate content in bean (*Phaseolus vulgaris*) varieties are correlated with high levels of expression of aminodeoxychorismate synthase (ADCS) and dihydroneopterin aldolase (DHNA) in the folate synthesis pathway. Positive clones of DHNA were identified from genomic BAC libraries of cultivars OAC Rex and G19833, which represent Mesoamerican gene pool and Andean gene pool, respectively. Full-length DHNA sequences were obtained after sequencing these positive clones. There is only one single-nucleotide polymorphism (SNP) in the 393 bp coding region between OAC Rex and G19833. The translated sequences (130 amino acids) are identical between the two cultivars. The deduced amino acid sequence of DHNA in common bean is closest to a DHNA sequence (ACU16784) from soybean with 86% identity. Based on a SNP between the two core map parents Bat 93 and Jalo EEP558, a SNP marker was developed and used to genotype a recombinant inbred population containing 70 individuals derived from a cross between Bat 93 and Jalo EEP558. ADCS gene was then mapped on the long arm of chromosome 7.

P-59

Endopolyploidy in bryophytes: a first account

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Endopolyploidy occurs in an individual when DNA replication is not followed by mitosis (also known as endoreduplication), resulting in cells with varying DNA contents. Among land plants, endopolyploidy is common in angiosperms, but appears to be rare in gymnosperms and ferns. The presence and role of endopolyploidy in bryophytes has not been previously explored. The present study surveyed thirty moss genera and six liverwort genera for the presence and prevalence of endopolyploidy. Stained nuclei were analyzed using flow cytometry to determine the number of nuclei at each ploidy level present in the individual. The results revealed that all of the moss genera were highly endopolyploid, except for the genus *Sphagnum*. All endopolyploid mosses had DNA contents corresponding with 1C, 2C, and 4C nuclei, and some also had 8C and 16C nuclei present. In contrast, the liverwort genera tested did not exhibit any endopolyploidy. Changes in DNA content will have an effect on the physiology and function of plant cells. As bryophytes represent the earliest plants to inhabit terrestrial ecosystems, the role of endopolyploidy is relevant to increase our understanding of the ecology and evolution of plants.

P-60

Characterization and Regulation of Starch Synthase IV in Maize Endosperm

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The storage starches are synthesized in sub-cellular organelles called amyloplsts in higher plants. The synthesis of the starch granule is a result of the coordinated activity of several groups of starch biosynthetic enzymes. There are four major groups of these enzymes, ADP-glucose pyrophosphorylase (AGPase), starch synthases (SS), starch branching enzymes (SBE), and starch debranching enzymes. Growing evidence indicates that these enzymes are regulated post translationally by several mechanisms including allosteric modulation, redox modulation, and protein phosphorylation. Recent evidence indicates that some of these enzymes (SS and SBE classes and starch phosphorylase) form complexes during starch synthesis and that formation of these complexes is phosphorylation-dependent.

SSIV is a recently discovered isoform of SS which may play a selective role in the priming of starch granule formation, although it its exact function in starch synthesis is still not clear. This research work aims to characterize and investigate the role of SSIV in maize endosperm. The SDS-PAGE followed by immunoblot analysis using peptide-specific anti-maize SSIV antibody revealed that SSIV is exclusively localized in amyloplast stroma in maize endosperm and is likely involved in starch biosynthesis at later stage of endosperm development. Coimmunoprecipitation studies using ATP-treated and untreated amyloplast lysate showed SSIV does not show any direct interaction with other starch biosynthetic enzymes. *In vitro* phosphorylation experiments with isolated amyloplasts and γ - 32 P-ATP analysis suggest SSIV is not phosphorylated, nor is the catalytic activity of SSIV affected by treatment with ATP.

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P-61

Study of histone deacetylase genes for stress tolerance in plants

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Histone deacetylases (HDACs) can be divided into four families, namely, RPD3, HDA1, SIR2 and HD2. The HD2 family is specific to plants. HDACs involve in chromosome remodeling and can lead to gene expression repression. Recent studies indicate that HDACs may also involve in other biological processes in plant. HD2 genes were studied in Arabidopsis thaliana with respect to plant stress tolerance. HD2A, B, C and D genes were transferred into in Arabidopsis. Results showed that over expression of HD2 genes inhibited the main root elongation and growth but promoted the lateral root development. The expression of HD2 genes also delayed the flower bud formation. Biochemical analysis of transgenic plants showed that the levels of malonaldehyde (MDA), electrical conductivity and glutathione reductase decreased compared to that in the control plants. The contents of proline, betain and total antioxidant capacity (TAC), on the other hand, were increased in over expressing seedlings. The plants expressed HD2 genes exhibited increased tolerance to stress conditions, including drought, salt and low temperature. The results indicate that over expression of HD2 genes altered plant physiology status and improved the plant's tolerance to stresses.

P-62

Intraspecific variation in the *Populus balsamifera* drought transcriptome

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Drought is a major limitation to the growth and productivity of trees in the genus *Populus*. Physiological and morphological responses to drought vary considerably both between and within *Populus* species, suggesting that *Populus* is a useful genus with which to examine the molecular underpinnings of the drought response. The ability of *Populus* trees to contend with drought is a function of genome responsiveness to water limitation, involving alterations in transcript abundance to appropriately remodel growth, development, and metabolism. The *Populus* drought transcriptome is influenced by many factors including genotype. However, the extent to which intra-specific variation shapes this response is not well understood. The transcriptome responses of six genotypes of *Populus balsamifera* were surveyed to test hypotheses related to how intra-specific variation in growth and productivity are influenced by water availability, and how these differences are underpinned by variation in the transcriptome. Analysis of the *P. balsamifera* drought transcriptomes revealed many of the genes that change in response to drought were common to all the genotypes; however, understanding the drought response is not limited to examining the commonalities and differences in the drought transcriptome, but also taking into consideration the magnitude of these changes in gene expression. The data provide insights into the interplay between genotype and environment, and point to the remarkable plasticity that has evolved in the genus *Populus* to contend with episodic drought.

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The impact of artificial night lighting in an urban environment on plant photosynthesis and gene expression

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Urban regions of the planet have a great amount of illumination at night due to artificial nighttime lighting (ANL), yet little is known how this may impact plants. The aim of the present research is to test the hypothesis that ANL affects plant photosynthesis and gene expression patterns. To this end, trees of two genotypes of *Populus balsamifera* were planted in a stereotypical urban setting, where one half of the plants were exposed to ANL and, as a control, the other half were not. Net leaf carbon assimilation rate (A_N) of the trees was examined at four hour intervals throughout a 40 h time period. During the day, trees exposed to ANL showed lower levels of A_N than the control trees (t-test, P < 0.05). Conversely, trees exposed to ANL showed higher levels of A_N at night than the control trees (t-test, P < 0.05). This may suggest ANL acts as a pollutant to plants by affecting both day and night A_N levels. Current experiments are in place to see if artificial nighttime lighting also affects trends of transcript abundance for diel-regulated genes. These findings will provide insights into the impact of light pollution on plants in an urban environment.

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Molecular characterization of dihydroflavonol 4-reductase (DFR) gene in common bean (Phaseolus vulgaris L.)

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Anthocyanins are the most conspicuous class of flavonoids, widespread plant secondary metabolites, as they are the main pigments in flowers and seed coat of common bean. Dihydroflavonol 4-reductase (*DFR*) is regarded as central enzyme of the flavonoid pathway and the first enzyme that leads to the anthocyanin production. This enzyme catalyzes the reduction of dihydroflavonols to leucoanthocyanidins using NADPH as a cofactor. The DFR gene has been amplified from genomic DNA of common bean, cloned and partially sequenced. The expression pattern of this gene was studied in cDNA obtained from the seed coat of three different tester lines of common bean with black and white seed coat. PCR primer pairs were designed to amplify polymorphic region of the gene in the Core recombinant inbred line population derived from cross between JaloEEP558 and Bat93. Although its segregation ratio was skewed toward Bat93, DFR gene was mapped to the linkage group seven of common bean. The cloned fragment was used as a probe to screen genomic library of common bean and the BAC clone that contained this gene was sequenced using next generation sequencing method. Our results show that this gene has six exons based on its similarity to DFR1 of soybean.

P-65

The role of IAP1 in Age-Related Resistance in Arabidopsis thaliana

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Age-Related Resistance (ARR) has been observed in numerous plant species, resulting in increased disease resistance as the plant matures. Evidence from our lab suggests that ARR in Arabidopsis to *Pseudomonas syringae* pv *tomato* involves the accumulation of salicylic acid (SA) in the intercellular space where it may act as an anti-microbial agent. EDS1 and PAD4, key regulators of SA accumulation during basal resistance in young plants are also required for the ARR response. Additionally, *IAP1* (important in the ARR pathway) was identified in an ARR mutant screen and shown to be required for ARR as well. Both intercellular and intracellular SA accumulation was reduced in *iap1-1* compared to wild type, suggesting that *IAP1* lies upstream of SA accumulation during ARR. The *iap1-1* mutation does not affect basal resistance in young plants, implying that it is specific to the ARR response, although R-gene mediated resistance to *Pst* (*AvrRps4*) and *Pst* (*AvrRpt2*) is partially compromised suggesting that the two pathways share common elements. Mapping of *IAP1* is ongoing. Identification and characterization of *IAP1* will provide important insights into the ARR defense pathway.

P-66

Transport patterns within the Plantaginaceae

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The Plantaginaceae are an interesting model family to use to examine the theory that higher photosynthesis and export rates can be maintained under stress conditions if the leaf can manufacture auxiliary sugars and/or photo-assimilates (iridoids and sucrose) that are mobile. The newly re-classified Plantaginaceae now includes the ornamental Antirrhinum majus L. and the common weed, *Plantago lanceolata* L. In addition to starch and sucrose, both species produce alcohol sugars. While manitol in A. majus is not heavily ¹⁴C labeled, the alcohol sugar, sorbitol, in *P. lanceolata* is heavily labeled during ¹⁴CO₂ feeding and appears to accumulate in sink tissues. A. majus contains two monoterpenes (iridoids): the phloem mobile antirrhinoside that accounts for 15 to 24% of total carbohydrates transported and the less-mobile antirrhide, which is also heavily labeled and accumulates in the laminar tissue. Interestingly, P. lanceolata contains two structurally similar iridoids, catalpol and aucubin that could function analogous to those of antirrhinoside and antirrhide, respectively. In P. lanceolata these intermediates are heavily labeled in addition to sucrose and sorbitol. This study examines the effects of temperature stress and elevated CO₂ using steady-state ¹⁴CO₂ labeling in Plantaginaceae. Results will provide insight into how environmental challenges alter the synthesis and turnover of major assimilates during photosynthesis and what roles iridoids play in maintaining leaf homeostasis and plant growth.

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A member of Pentatricopeptide repeat family differentially expressed in homeotic cytoplasmic male sterile and fertile carrot flowers.

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Cytoplasmic male sterility (CMS) can occur in carrot as a homoetic mutation, where both petals and stamens are replaced by green bract-like organs. Because CMS is thought to be controlled by the mitochondrion, and floral development is regulated by nuclear genes, a mitochondrial-nuclear signaling pathway can be hypothesized. To identify the nuclear components of homeotic CMS in carrot, stamen primordia from CMS line and its isonuclear fertile line were harvested through laser capture microdissection (LCM) and used to construct forward and reverse suppression subtractive hybridization (SSH) cDNA library. Five percent of 1600 SSH clones surveyed were differentially expressed, and five were upregulted in the sterile line. One clone had homology to members of the pentatricopeptide repeat gene family (PPR). In situ hybridization confirmed higher PPR gene expression in both stamen- and petal-whorls of early stage flowers of the sterile genotype compared to the isonuclear fertile line. Recovery of the full length gene, subcellular localization and gene functional analysis are currently under investigation.

P-68

Importance of the αC-helix in the Cyclic Nucleotide Binding Domain for Stable Channel Regulation and Function of Cyclic Nucleotide Gated Ion Channels in *Arabidopsis thaliana* K. Chin^{1*}, W. Moeder^{1,2}, H. Abdel-Hamid¹, D. Shahinas¹, and K. Yoshioka^{1,2}

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The involvement of cyclic nucleotide gated ion channels (CNGCs) in signal transduction of light and odorant perception in animals has been well documented. Although CNGCs have been identified to mediate multiple physiological responses in plants, studies that aim to elucidate their structural and regulatory properties are still in their infancy. Here, we investigated the structure-function relationship of plant CNGCs by using the chimeric AtCNGC11/12 gene, which induces multiple defense responses in the Arabidopsis mutant cpr22, to identify functionally essential residues. A genetic screen for mutants that suppress cpr22-conferred phenotypes identified over 20 novel mutant alleles in AtCNGC11/12. One of these mutants, S58, possesses a single amino acid substitution (R557C) in the αC-helix of the cyclic nucleotide binding domain (CNBD). S58 had lost all *cpr*22-related phenotypes, including spontaneous lesion formation under ambient temperature. However, these phenotypes were recovered at 16°C suggesting that channel function is temperature-dependent. In silico modeling and site-directed mutagenesis analyses suggest that R557 is important for channel regulation, but not for basic function. Furthermore, another suppressor (S136) that lacks the entire α C-helix due to a premature stop codon lost complete channel function. Our data presented here indicate that the α C-helix of the CNBD is functionally important in plant CNGCs.

P-69

Physiological Disorders of Postharvest Apples: A Role for Glutamate Decarboxylase Derived γ -Aminobutyrate?

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In controlled atmosphere (CA) storage, the metabolic activity of apple fruit is decreased by controlling the O_2 and CO_2 partial pressures in combination with a reduction in temperature. Optimal conditions are a compromise to minimize the incidence of physiological storage disorders (e.g. internal browning). Here, we tested the impact of elevated CO_2 (5% versus 0.03%) and/or low temperature (5°C versus 0°C) in the presence of 2.5% O_2 on the incidence of browning in 'Honeycrisp', 'McIntosh' and 'Empire' apples. More browning (up to 18 fold) was apparent in Honeycrisp and Empire under elevated CO_2 at both low temperatures after 2 months of storage. McIntosh was unaffected by CA. At low temperature, elevated CO_2 can result in the accumulation of γ -aminobutyrate (GABA). While glutamate decarboxylase (GAD) derived GABA accumulation is a universal stress indicator in photosynthetic tissues, its role and the mechanisms associated with its accumulation in fruits are unknown. A putative GAD, which was cloned from apple fruit, is 75% identical and 86% similar to Arabidopsis Ca^{2+} /calmodulin-dependent GAD1. Recombinant apple GAD will be assayed for Ca^{2+} /calmodulin-dependent activity, and HPLC analyses will establish whether GABA accumulation is associated with the onset of browning disorders in CA stored apple.

P-70

Investigating the Role of Alternative Oxidase in Light Acclimation Response M. Cheung*, GC Vanlerberghe.

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Photosynthesis in the chloroplast produces ATP and NADPH, which are used for carbohydrate production via the Calvin Cycle. To avoid over-reduction of the photosynthetic electron transport chain, plants must balance light absorption and energy consumption. This can potentially be achieved by exporting excess reductant to the mitochondrion, the location of a unique terminal oxidase called alternative oxidase (AOX). Because it is non-energy-conserving, AOX is an ideal candidate for facilitating oxidation of excess reductant, and hence could contribute to regulating the chloroplast redox status in response to changes in irradiance. To clarify the role of AOX in light acclimation, transgenic *Nicotiana tabacum* lines RNAi-silenced or over-expressing AOX are being analyzed in response to both short and long-term high-light treatment. Plants are grown at 120 µmol m⁻² s⁻¹ and then shifted to 700 µmol m⁻² s⁻¹ (short-term acclimation) or grown at 700 µmol m⁻² s⁻¹ (long-term acclimation). Preliminary measurements of chlorophyll fluorescence are being conducted under these different light regimes. In addition, soluble sugar content, chlorophyll content, and chlorophyll a/b ratios are currently being assayed. This project aims to clarify the intriguing relationship between chloroplast and mitochondrion during light acclimation, and the possible role of AOX in this process.

P-71

Identification of the first null-activity mutant for an enzyme in daytime sucrose synthesis S.M.H. Slater, M.C. Micallef, J. Zhang, <u>B.J. Micallef</u>

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Sucrose is the primary transport compound in most plants. To date, no null-activity mutants in daytime sucrose synthesis have been identified for any plant species, suggesting that such mutations are lethal. Research has focused on C3 species. Here we report the identification of a natural null-activity mutant for cytosolic fructose bisphosphatase (cytFBPase) from the C3-C4 photosynthetic intermediate Flaveria linearis (F. linearis). CytFBPase catalyzes the first irreversible reaction of daytime sucrose synthesis. Enzymatic activities and RFLP patterns for F2 progeny show one gene copy of cytFBPase in F. linearis; null activity is linked to the structural gene for cytFBPase. A point mutation in the structural gene results in a cryptic premRNA splice site and a corresponding 23 amino acid deletion spanning the enzyme active site. CytFBPase protein could not be detected in the mutant. Surprisingly, null F2 progeny segregated for daytime leaf starch accumulation whereas high starch was expected for all null lines. All null lines showed CO₂-insensitivity of photosynthesis indicating a reduced capacity for end-product synthesis. Genetic variation was found for a potential compensatory enzyme PPi-dependent phosphofructokinase Collectively, the data indicate alternative pathways for daytime sucrose synthesis in F. linearis. Implications for improving productivity in high biomass C3-C4 and C4 species will be discussed.

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