2014 Canadian Society of Plant Biologists
Eastern Regional Meeting

2014 Délibérations du Congrès de la Société Canadienne de Biologie Végétale
Congrès de l'Est

November 28-29, 2014

University of Guelph, Guelph ON CANADA

Organizing Committee

Tariq Akhtar, Department of Molecular & Cellular Biology, University of Guelph

Gale G. Bozzo (Chair), Department of Plant Agriculture, University of Guelph

Lewis Lukens, Department of Plant Agriculture, University of Guelph

Robert T. Mullen, Department of Molecular & Cellular Biology, University of Guelph

Barry J. Shelp, Department of Plant Agriculture, University of Guelph

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Acknowledgments

The members of the 2014 CSPB/SCBV ERM Organizing Committee gratefully acknowledge Michael Stasiak for assistance with website design. We also thank Kathy Nahwegahbow and Margaret Whiting of the University of Guelph Conference Services for their assistance and guidance during the organization of the meeting.

Program-at-a-Glance

Unless otherwise indicated, all meeting events listed below are scheduled to take place at Rozanski Hall on the University of Guelph campus (refer to map on next page).

Friday, November 28	
6:00 - 8:00 PM	Registration (Rozanski Concourse)
6:30 - 9:30 PM	Mixer (Bullring)
Saturday, November 29	
7:30 - 9:30 AM	Registration (Rozanski Concourse)
7:30 - 8:15 AM	Poster Setup (Rozanski Concourse)
8:15 - 8:30 AM	Conference Welcome
8:30 - 9:00 AM	**Plenary Lecture I** (*Owen Rowland, Carleton University*)
9:00 - 9:30 AM	**Plenary Lecture II** (*Tariq Akhtar, University of Guelph*)
9:30 - 10:30 AM	Refreshment Break & Poster Viewing (Odd Numbered Abstracts)
10:30 - 12:00 PM	Concurrent Session 1 : *"Plant Stress Mechanisms I"*
	Concurrent Session 2 : *"Metabolism I"*
	Concurrent Session 3 : *"Photosynthesis I"*
12:00 - 1:30 PM	**Lunch (Creelman Hall)**
1:30 - 3:00 PM	Concurrent Session 4 : *"Genomics and Biotechnology"*
	Concurrent Session 5 : *"Plant Development"*
	Concurrent Session 6 : *"Metabolism II"*
3:00 - 4:00 PM	Refreshment Break & Poster Viewing (Even Numbered Abstracts)
4:00 - 5:30 PM	Concurrent Session 7 : *"Biotic Interactions"*
	Concurrent Session 8 : *"Plant Stress Mechanisms II"*
	Concurrent Session 9 : *"Photosynthesis II"*
5:30 - 6:00 PM	Eastern Regional Director's Presentation Awards Panel Meeting
6:00 - 6:15 PM	Eastern Regional Director's Presentation Awards Ceremony Closing Remarks

University of Guelph Campus Map

All 2014 SCPB/SCBV ERM events are scheduled to take place at Rozanski Hall. The Bullring and Creelman Hall are venues for the mixer (Friday Nov. 28) and lunch (Saturday Nov. 29), respectively.

Select restaurants within walking distance of the Delta Guelph Hotel, Best Western Royal Brock Hotel and Days Inn are:

Cora's Breakfast & Lunch, 35 Harvard Road, 519-763-2844
Fifty West Restaurant & Bar, 50 Stone Road West (Delta Guelph Hotel)
Milestone's, 185 Stone Road West, 519-836-8882
Montana's, 201 Stone Road West, 519-766-1549
Shakespeare Arms, 35 Harvard Road, 519-767-6003

2014 CSPB/SCBV ERM Program

Friday, November 28

6:30 - 8:30 PM	**Registration** (Rozanski Hall Concourse)
6:30 - 9:00 PM	**Opening Mixer: Bullring** *Delegates are invited to attend the opening mixer at the Bull Ring (across from Rozanski Hall). A variety of hors d'oeuvres and both alcoholic and non-alcoholic refreshments will be served.*

Saturday, November 29

7:30 - 9:30 AM	**Registration** (Rozanski Hall Concourse)
7:30 - 8:15 AM	**Poster Setup** (Rozanski Hall Concourse)
8:15 - 8:30 AM	Conference Welcome (Rozanski Hall, Room 103)
8:30 - 9:00 AM	**Plenary Lecture I - Owen Rowland**, Carleton University (Rozanski Hall, Room 103) *"Plant Surface Lipid Barriers: Biosynthesis, Regulation, and Biotechnology"*
9:00 - 9:30 AM	**Plenary Lecture II - Tariq Akhtar**, University of Guelph (Rozanski Hall, Room 103) *"Plant Polyisoprenoids – Secondary Metabolites or Physiologically Important Superlipids?"*
9:30 - 10:30 AM	**Refreshment Break and Poster Viewing (Odd Numbered Abstracts)** (Rozanski Hall Concourse)

Concurrent Session 1: Plant Stress Mechanisms I (Rozanski Hall, Room 103)

Chair: Keiko Yoshioka, University of Toronto

Session #		
1.1	10:30 - 10:45 AM	**Vanessa Lundsgaard-Nielsen, Dinesh Christendat, Tammy L Sage** *"Investigation of the effect of high temperature stress on reproduction in Arabidopsis thaliana"*
1.2	10:45 - 11:00 AM	**Alex Fortuna*, Jihyun Lee, Huoi Ung, Kimberley Chin, Wolfgang Moeder, Keiko Yoshioka** *"Crossroads of stress responses, development and flowering regulation - the multiple roles of cyclic nucleotide gated ion channel 2"*
1.3	11:00 - 11:15 AM	**Katharina Bräutigam, Stefan Schreiber, Yunchen Gong, Pauline Wang, Barb Thomas, Uwe Hacke, and Malcolm M. Campbell** *"Molecular and physiological responses of hybrid poplars under stress conditions"*
1.4	11:15 - 11:30 AM	**Chevonne E. Carlow*, Annette Nassuth** *"Activity of Vitis CBF/DREB1 and DREB2 transcription factors"*
1.5	11:30 - 11:45 AM	**Devrim Coskun*, Dev T. Britto, Herbert J. Kronzucker** *"A critical evaluation of the two-mechanism model of K+ acquisition in roots of higher plants and the contribution of apoplastic fluxes"*
1.6	11:45 - 12:00 PM	**Julia Nowak, Lining Tian, Frédéric Marsolais** *"Tolerance to the herbicide imazethapyr (Pursuit) in common bean"*

Concurrent Session 2: Metabolism I (Rozanski Hall, Room 102)

Chair: Barry Shelp, University of Guelph

Session #		
2.1	10:30 - 10:45 AM	**Qianru Zhao*, Ian Tetlow, Michael Emes** *"The regulation of transient starch biosynthesis in Arabidopsis thaliana"*
2.2	10:45 - 11:00 AM	**Jose A. Freixas-Coutin*, Seth Munholland, William L. Crosby, K. Peter Pauls, Gale G. Bozzo** *"Transcriptome analysis of seed coats reveals increased expression of proanthocyanidin metabolism genes in darkening cranberry bean (Phaseolus vulgaris)"*
2.3	11:00 - 11:15 AM	**Carolyne J. Brikis*, Adel Zarei, Kristen L. Deyman, Jingyun Liu, Greta Z. Chiu, Christopher P. Trobacher, Gordon J. Hoover, Jennifer R. DeEll, Gale G. Bozzo, Barry J. Shelp** *"Deciphering metabolic pathways for 4-aminobutyrate (GABA) production in 'Empire' apple fruit stored under controlled atmosphere conditions"*
2.4	11:15 - 11:30 AM	**Maye Saechao, Ishari Waduwara-Jayabahu, Shuningbo Ye, A. Nimhani Perera*, Barbara Moffatt** *"Chasing the explanation for the vascular defects in MTN-deficient mutants: is their decreased spermidine content the culprit?"*
2.5	11:30 - 11:45 AM	**Reena Grittle Pinhero, Renuka Waduge, Al Sullivan, Qiang Liu, Rickey Y Yada** *"Screening early potatoes for nutrition – Is there a role for starch?"*
2.6	11:45 - 12:00 PM	**Mehran Dastmalchi*, Sangeeta Dhaubhadel** *"Soybean isoflavonoid metabolon: elements and interaction at the molecular level"*

Concurrent Session 3: Photosynthesis I (Rozanski Hall, Room 105)

Chair: Allison McDonald, Wilfrid Laurier University

Session #		
3.1	10:30 - 10:45 AM	**Joseph R. Stinziano*, Norman P. A. Hüner, Danielle A. Way** *"Temperature has a stronger effect than photoperiod in regulating seasonal photosynthetic performance in white spruce (Picea glauca)"*
3.2	10:45 - 11:00 AM	**Christopher Y. S. Wong*, John A. Gamon** "*Seasonal photosynthetic activity in evergreen conifer leaves monitored with spectral reflectance"*
3.3	11:00 - 11:15 AM	**Emmanuelle Fréchette*, Christine Chang, Ingo Ensminger** *"Facultative and constitutive changes in pigment composition affect the relationship of the photosynthetic light-use efficiency and the photochemical reflectance index of Eastern white pine (Pinus strobus L.)"*
3.4	11:15 - 11:30 AM	**Allison E. McDonald** *"Teaching photosynthetic electron transport using an active learning exercise in the classroom*
3.5	11:30 - 11:45 AM	**Andrew McKenzie-Gopsill*, Sasan Amirsadeghi, Elizabeth Lee, Lewis Lukens, Clarence Swanton** *"Light, weeds and carbon partitioning - How does a neighbour do it?*
3.6	11:45 - 12:00 PM	**David Hawley, Michael Stasiak, Jamie Lawson, Alan Scott, Per Aage Lysaa, Mike A. Dixon** *"Development of a high-intensity, variable-spectra LED array for optimized plant development in challenging environments."*

12:00 - 1:30 PM **Lunch** (Creelman Hall)

CSBP Executive Meeting & Lunch (Rozanski Hall, Room 109)

Concurrent Session 4: Genomics and Biotechnology (Rozanski Hall, Room 102)

Chair: Peter Pauls, University of Guelph

Session #		
4.1	1:30 - 1:45 PM	**Banyar Aung, Margaret Y. Gruber, Ying Wang, Khaled Omari, Annick Bertrand, Abdelali Hannoufa** *"Ectopic expression of miR156 improves alfalfa yield and quality"*
4.2	1:45 - 2:00 PM	**Megan A. House*, Cortland K. Griswold, Lewis N. Lukens** *"Evidence for lineage-specific selection on gene expression in rice"*
4.3	2:00 - 2:15 PM	**Gregory Perry, Weilong Xie, Esteban Cortez, Denise Cooper, Fawn Turner, Yarmilla Reinprecht, Claudia Dinatale, Seth Munholland, William Crosby, K. Peter Pauls** *"Combining high density SNP analysis to identify introgression regions associated with CBB resistance in Phaseolus species"*
4.4	2:15 - 2:30 PM	**Lily Nasanovsky*, Ian Tetlow** *"Branching enzymes as agents for modifying glucan structure in industrial processing"*
4.5	2:30 - 2:45 PM	**Ann Meyer*, Lewis Lukens** *"Frequency, magnitude, and sources of bias in allele specific transcript abundances' estimates"*
4.6	2:45 - 3:00 PM	**Gang Tian, Qing Lu, Saatian, Behnaz, Lining Tian, Yuhai Cui** *"A new flexible gateway-compatible vector system for plant functional genomics"*

Concurrent Session 5: Plant Development (Rozanski Hall, Room 103)

Chair: Daphne Goring, University of Toronto

Session #		
5.1	1:30 - 1:45 PM	**Behnaz Saatian*, Ryan S. Austin, Susanne Kohalmi, Yuhai Cui** *"GLUCAN SYNTHASE-LIKE8 Is required for early seedling development in Arabidopsis"*
5.2	1:45 - 2:00 PM	**Jerlene Nessia-Halliday*, K. Peter Pauls** *"Characterization of a novel family of GDP-O-fucosyltransferase in Arabidopsis thaliana and Brassica napus microspore culture"*
5.3	2:00 - 2:15 PM	**Trent Faultless*, Annette Nassuth** *"DNA-binding and localization function of the putative nuclear localization signal of Vitis CBF1"*
5.4	2:15 - 2:30 PM	**Jennifer Doucet*, Daphne Goring** *"Investigating the role of stigma specific RLCKs during compatible pollen acceptance signaling"*
5.5	2:30 - 2:45 PM	**M. Atikur Rahman, Thuy Nguyen, Huogen Xiao, Annette Nassuth** *"Morphological and molecular analysis of stomatal development in grape"*
5.6	2:45 - 3:00 PM	**Adrian Hofmann*, Dinesh Christendat** *"Virus induced gene silencing (VIGS) of shikimate kinase like-1 (SKL1) in Solanum lycopersicum (tomato) results in an albino phenotype"*

Concurrent Session 6: Metabolism II (Rozanski Hall, Room 105)

Chair: Michael Emes, University of Guelph

Session #		
6.1	1:30 - 1:45 PM	**Sonia Dorion, Audrey Clendenning, Jean Rivoal** *"The manipulation of cytosolic nucleoside diphosphate kinase in transgenic Solanum tuberosum roots affects growth, respiration and starch metabolism"*
6.2	1:45 - 2:00 PM	**Jonathon Roepke*, Gale G. Bozzo** *"An Arabidopsis thaliana β-glucosidase is involved in flavonol bisglycoside catabolism"*
6.3	2:00 - 2:15 PM	**Fatemeh Mehrpooyan*, Ian J. Tetlow, Michael J. Emes** *"Regulation of maize starch synthase II, a key enzyme of starch biosynthesis"*
6.4	2:15 - 2:30 PM	**Adel Zarei, Christopher P. Trobacher, Barry J. Shelp** *"Polyamines are a potential source of 4-aminobutyrate (GABA): Characterization of apple and Arabidopsis aminoaldehyde dehydrogenases belonging to ALDH10 family"*
6.5	2:30 - 2:45 PM	**Eric Fedosejevs*, Sheng Ying, Yi-Min She, William Plaxton** *"The Ca^{2+}-dependent protein kinase RcCDPK2 in vivo phosphorylates the sucrose synthase isozyme RcSUS1 at serine-11 in developing castor oil seeds"*
6.6	2:45 - 3:00 PM	**Vikramjit S. Bajwa, Mukund R.Shukla, Sherif M. Sherif, Susan J. Murch, Praveen K. Saxena** *"Role of indoleamines in controlling apple and pear browning by inhibiting polyphenol oxidase activity"*
	3:00 - 4:00 PM	**Refreshment Break and Poster Viewing (Even Numbered Abstracts)** (Rozanski Hall Concourse)

Concurrent Session 7: Biotic Interactions (Rozanski Hall, Room 102)

Chair: Robin Cameron, McMaster University

Session #		
7.1	4:00 - 4:15 PM	**Jamuna Risal Paudel*, Alberto Prado, Jacqueline C Bede** *"Plant early redox response to caterpillar herbivory"*
7.2	4:15 - 4:30 PM	**Huoi Ung, Wolfgang Moeder, Keiko Yoshioka** *"Arabidopsis triphosphate tunnel metalloenzyme2 (AtTTM2) is a negative regulator of the salicylic acid-mediated feedback amplification loop for defense responses*
7.3	4:30 - 4:45 PM	**James Jones*, Frédérique C. Guinel** *"The arbuscular mycorrhizal symbiosis in pea: insights from a hypermycorrhizal mutant"*
7.4	4:45 - 5:00 PM	**Sneha Challa*, Ian Scott, Luis Caceres, Mark Sumarah, Lining Tian, Abdelali Hannoufa** ***"****Natural plant volatiles: A sustainable pest control strategy****"***
7.5	5:00 - 5:15 PM	**Philip Carella*, Marisa Isaacs, Robin K. Cameron** *"PLASMODESMATA-LOCATED PROTEIN overexpression negatively impacts the manifestation of systemic acquired resistance and the long-distance movement of DEFECTIVE IN INDUCED RESISTANCE1 in Arabidopsis"*
7.6	5:15 - 5:30 PM	**Çağdaş Kera Yücel*, Peter Ryser, Nadia Mykytczuk** *"Revealing the microbial diversity between the rhizospheres of six wetland plants"*

Concurrent Session 8: Plant Stress Mechanisms II (Rozanski Hall, Room 103)

Chair: Greg Vanlerberghe, University of Toronto Scarborough

Session #		
8.1	4:00 - 4:15 PM	**Marina Cvetkovska, Keshav Dahal, Nicole A. Alber, Cathy Jin, Melissa Cheung, Greg C. Vanlerberghe** *"Knockdown of mitochondrial alternative oxidase induces the stress state of signalling molecule pools in Nicotiana tabacum, with implications for stomatal function"*
8.2	4:15 - 4:30 PM	**Prabhakharan Munusamy, Louis-Valentin Meteignier, Yevgen Zolotarov, Peter Moffett, Martina Stromvik** *"Regulatory elements in the untranslated regions (UTRs) of ribosome-bound mRNAs of Arabidopsis thaliana involved in translational control of gene regulation under stress"*
8.3	4:30 - 4:45 PM	**Pearl Pei-Chun Chang*, Daryl Enstone, Susan J Lolle** *"A possible role for the Arabidopsis HOTHEAD protein in plant stress response pathways"*
8.4	4:45 - 5:00 PM	**Nadun Chanaka Karunatilleke*, Balakrishnan Prithiviraj** *"Ascophyllum nodosum (L.) Le Jolis. extract enhances drought stress tolerance in tomato (Solanum lycopersicum L.)"*
8.5	5:00 - 5:15 PM	**Thomas A. DeFalco*, Huda Abdel-Hamid, Christopher B. Marshall, Wolfgang Moeder, Mitsuhiko Ikura, Wayne A. Snedden, Keiko Yoshioka** *"A novel calmodulin-binding site regulates the induction of programmed cell death by Arabidopsis cyclic nucleotide-gated ion channel 12"*
8.6	5:15 - 5:30 PM	**Cody G. Thompson*, Pablo Sobron, Nathalie Cabrol, Serge Caron, Mike Dixon** *"Using ion-selective optrode sensors to characterize water chemistry in hydroponic systems and in extreme environments in the context of space exploration"*

Concurrent Session 9: Photosynthesis II (Rozanski Hall, Room 105)

Chair: Danielle Way, University of Western Ontario

Session #		
9.1	4:00 - 4:15 PM	**Christopher Juliao*, Daniel Marsden, Emmanuelle Fréchette, Ingo Ensminger** *"Effects of elevated temperature on the spring phenology of Eastern white pine (Pinus strobus L.)"*
9.2	4:15 - 4:30 PM	**Avery McCarthy*, Michelle Chung, Alexander G. Ivanov, Marianna Krol, Norman P. A. Hüner** *"Sugar insensitivity in a model Arabidopsis thaliana cell suspension culture"*
9.3	4:30 - 4:45 PM	**Laura Verena Junker*, Ingo Ensminger** *"Influence of senescence-associated pigment changes on leaf spectral properties in Acer saccharum"*
9.4	4:45 - 5:00 PM	**Keshav Dahal, Jia Wang, Greg Martyn, Farkhunda Rahimy, Greg C. Vanlerberghe** *"Mitochondrial alternative oxidase maintains respiration and preserves photosynthetic capacity during moderate drought in Nicotiana tabacum"*
9.5	5:00 - 5:15 PM	**Amritpal S. Singh*, A. Maxwell P. Jones, Praveen K. Saxena** *"In vitro propagation and genetic improvement of sugar maple (Acer saccharum Marsh.)"*

9.6	5:15 - 5:30 PM	**Lauren E. Hollis*, Norman P.A. Hüner** *"Photoacclimation in Chlorella vulgaris UTEX 265 is both redox- and photoperiod-dependent"*
	5:30 - 6:00 PM	**Eastern Regional Director's Presentation Awards Panel Meeting**
	6:00 - 6:15 PM	**Eastern Regional Director's Presentation Awards Ceremony**
		Closing Remarks

List of Posters

P1. Effect of mercury chloride and other toxic substances on root water transport of maize seedlings
Aziz Dustmamatov, Maria Popova, Ekkehard Richter, Michael Fritz, Rudolf Ehwald

P2. Characterization of tomato (*Solanum lycopersicum*) phosphatidylinositol 3-kinase C2 domain
Mohd Sabri Pak-Dek*, Krishnaraj Tiwari, Sheriff Sheriff, Jayasankar Subramanian, Gopinadhan Paliyath

P3. The efficacy of ozone and the advance oxidation process in reclaiming horticultural wastewater
Brenden Bertrand, Mike Dixon, Thomas Graham, Ping Zhang, David Llewellyn

P4. Evaluation of potato varieties for chipping and nutritional qualities
Reena Grittle Pinhero, Qiang Liu, Allan Sullivan, Vanessa Currie, Benoit Bizimungu, Alejandro Marangoni, Rickey Y. Yada

P5. Effects of soil temperature on growth of species with different root turnover strategies
Dominique Gagnon, Adrienne Wilson, Jacob Porter, Peter Ryser

P6. Irrigation management strategies for nursery trees based on plant water status measured with automated stem psychrometers
Newton Tran*, Thomas Graham, Ping Zhang, Polina Bam, Katie Black, Bob Reeves, Alec Downey, Mike Dixon

P7. Molecular and biochemical analysis of *chalcone reductase* genes in soybean
Caroline Sepiol*, Jaeju Yu, Sangeeta Dhaubhadel

P8. Role of melatonin and serotonin in alleviating salt stress in *Arabidopsis thaliana*
Mukund R.Shukla, Vikramjit S. Bajwa, Susan J. Murch, Praveen K. Saxena

P9. *Lotus japonicus STYLISH* gene family and its role during nitrogen fixing symbiosis
Arina Shrestha*, Loretta Ross, Alexandre Tromas, Krzysztof Szczyglowski

P10. Measurement of neutral detergent fibre, acid detergent fibre and acid detergent lignin in corn stalk fibres by Fourier-transform infrared spectroscopy
Muhammad Arif, Muhammad Riaz, K. Peter Pauls

P11. Physiological disorders and oxidative stress metabolism in stored pear fruit
Geoffrey B. Lum, Jennifer R. DeEll, Sanjeena Subedi, Gordon J. Hoover, Barry J. Shelp, Gale G. Bozzo

P12. Identification of the PP2C HAI1 as an interactor of ABA2, an ABA biosynthetic enzyme
Catalina Leoveanu*, Yi Zhang, Shelley Lumba, Eiji Nambara

P13. Fungal endophytes that promote plant growth on saline and dry soil
Kumkum Azad*, Susan Kaminskyj, James Basinger

P14. Environmental parameters influencing clubroot incidence and severity on canola
Travis J. Cranmer*, Bruce D. Gossen, Mary Ruth McDonald

P15. The SUGAR-DEPENDENT1 lipase associates with the peroxisomal surface and interacts with PEROXISOMAL ABC-TRANSPORTER1
Samantha C. Watt*, Satinder K. Gidda, Harrie Van Erp, Smita Kurup, Peter Eastmond, Robert T. Mullen

P16. *Lonicera japonica* and *Catharanthus roseus* produce secologanin via a common pathway.
Alison Edge*, Vonny Salim, Vincenzo De Luca

P17. Inhibiting phenylpropanoid biosynthesis facilitates protoplast isolation and plant regeneration in American elm (*Ulmus americana)*
Max Jones, Mukund Shukla, Gadab Biswas, Praveen Saxena

P18. Repression of lateral organ boundary genes by PENNYWISE and POUND-FOOLISH is essential for meristem maintenance and flowering in *Arabidopsis thaliana*
Madiha Khan, Brenda C. Salasini*, Laura Ragni, Paul Tabb, Raju Datla, Kiahezi Kuai, Charles Déspres, Halima Morin, Véronique Pautot, Shelley R. Hepworth

P19. NLP8 is required for nitrate to promote seed germination in *Arabidopsis thaliana*
Dawei Yan, Vanathy Easwaran, Yunchen Gong, Eiji Nambara

P20. A jasmonate signaling suppressor from peach mediates the transition from outcrossing to self-pollination
Sherif Sherif, Islam El-Sharkawy, Jaideep Mathur, Pratibha Ravindran, Prakash Kumar, Gopinadhan Paliyath, Jayasankar Subramanian

P21. The role of ethylene action on *CYP707A2* in seed germination
Lisza Duermeyer*, Dawei Yan, Eiji Nambara

P22. Differential effects of mycorrhizal inoculation on seedling growth and survival of two *Cuscuta* species with contrasting ecology
Behrang Behdarvandi, Frédérique Guinel, Mihai Costea

P23. Measuring cell wall mechanical properties on single plant cells using microfluidics
Amir Sanati Nezhad, Muthu Packirisamy, Anja Geitmann

P24. Evaluating the role of mitochondrial alternative oxidase during nitrogen-limited growth and growth on ammonium
Greg D. Martyn*, Fallon J. Hayes, Greg C. Vanlerberghe

P25. An ancient African crop hosts a novel bacterial endophyte that suppresses modern crop diseases
Walaa Kamel Mousa*, Charles Shearer, Manish N. Raizada

P26. Do plant growth-promoting rhizobacteria reduce production of ethylene in *Arabidopsis* under cadmium stress?
Joshua J Frank*, Gurpreet Dhami, Sheila M. Macfie

P27. In vivo interactions between starch branching enzymes require phosphorylation at specific serine residues
Noel Mano*, Fushan Liu, Michael J. Emes, Ian J. Tetlow

P28. Investigation into the role of the Arabidopsis calmodulin-like protein, CML39, in hormonal regulation of early seedling development and fruit formation.
Ubaid Midhat*, Wayne Snedden

P29 *De novo* regulatory motif discovery identifies significant motifs in promoters of five classes of plant dehydrin genes
Yevgen Zolotarov*, Martina Strömvik

P30. Post-translational regulation of starch synthase II in *Arabidopsis thaliana* and *Zea mays*
Jenelle Patterson*, Usha Rayirath, Fushan Liu, Qianru Zhao, Ian J. Tetlow, Michael J. Emes

P31. The role of the exocyst complex in the early stages of compatible pollen-pistil interactions in *Arabidopsis thaliana*
Darya Safavian*, Yara Zayed, Emily Indriolo, Laura Chapman, Abdulla Ahmed, Daphne R. Goring

P32. A novel C-terminal motif serves as the targeting signal for a subset of plastid outer envelope tail-anchored proteins
Howard J. Teresinski*, Thuy N.D. Nguyen, Matthew D. Smith, Robert T. Mullen

P33. Subcellular localization of *Zea mays* calcium-dependent protein kinase 11
Steven Trothen*, Amina Makhmudova, Reynald Tremblay, Michael Emes, Ian Tetlow

P34. Expression analysis of the water stress inducible promoter *Wsi18*, in the model monocot *Brachypodium distachyon*
Patrick Langille*, Jim Karagiannis, Lining Tian

P35. Investigating the role of a new class of *Arabidopsis* lipid droplet-associated proteins (LDAPs) in lipid droplet biogenesis
Michal Pyc*, Satinder K. Gidda, Sunjung Park, David W. Andrews, Kent D. Chapman, John M. Dyer, Robert T. Mullen

P36. Epidermal and meristematic cell type-specific gene regulation in soybean, *Glycine max* (L.) Merr.
Haritika Majithia, Yevgen Zolotarov*, Lila Vodkin, Martina Stromvik

P37. Exploring genomic contributions in a common bacterial blight resistant navy bean population
Fawn Turner*, Alireza Navabi, Rene Van Acker, K. Peter Pauls

P38. Transcriptional responses to exogenous asparagine in Arabidopsis roots
Shrikaar Kambhampati*, Sudhakar Pandurangan, Agnieszka Pajak, Ryan Austin, Frédéric Marsolais

P39. Identification of GmMYB176-specific protein kinase in soybean
Pravesh Lama*, Arun Kumaran Anguraj Vadivel, Sangeeta Dhaubhadel

P40. A bacterial biosensor for *in situ* visualization of glutamine dynamics in maize tissue
Travis L. Goron*, Michael J. Tessaro, Sameh S. M. Soliman, Manish N. Raizada

P41. Purification of the *L*-methionine:2-oxoglutarate aminotransferase from *Ulva intestinalis* and identification of gene candidates
Dylan Levac, Alex Whynot, Michelle M. McLauchlan, Jeffrey C. Waller

P42. Chloroplast chaperone HSP90C assists in the targeting of thylakoid lumen protein OE33 in *Arabidopsis thaliana*
Tim Jiang*, Rebecca Babaei-Rad, Saehong E Oh, Rongmin Zhao

P43. Root suberin load and composition change with age and different soil moisture levels in *Arabidopsis thaliana*
Nayana de Silva*, Isabel Molina, Peter Ryser, Owen Rowland

P44. Early Arabidopsis responses to *Tetranychus urticae* feeding: Local vs. systemic responses
Cristina Rioja, Vladimir Zhurov, Miodrag Grbic, Vojislava Grbic

P45. A two-component enzyme complex is required for dolichol biosynthesis in plants
Megan I. Brasher*, Bryan Leong, Liliana Surmacz, Jocelyn Pitcher, Ewa Swiezewska, Eran Pichersky, Tariq A. Akhtar

P46. Using small molecules to prime and explore anthocyanin accumulation response to low temperature exposure
Katrina Hiiback*, Michael Stokes, Malcolm Campbell

P47. Investigating the role of plant mitochondria in NO synthesis and signalling
Nicole A Alber*, Hampavi Sivanesan, Greg C Vanlerberghe

P48. Characterization of the molecular targeting information for the peroxisomal membrane transport protein PXA1
Erin M. Anderson, Nicholas Khuu, Satinder Gidda, Robert T. Mullen

P49. An *in silico* analysis of cell wall related gene families in soybean stem
Yarmilla Reinprecht , K. Peter Pauls

P50. Autophagy's link to self-incompatibility in the Brassicaceae
Daniel C Johnson*, Daphne R Goring

P51. Determining physiological processes underlying HopZ1a recognition in *Arabidopsis thaliana*
Alexandra Menna*, Dang Nguyen, Darrell Desveaux, David S. Guttman

P52. The use of chemical compounds to help *Arabidopsis* acclimate to a nitrogen limiting environment
Eshan Naik*, Malcolm M. Campbell

P53. Characterization of effector genes in common blight bacteria isolates showing differential pathogenicity on common bean
Weilong Xie, Gregory Perry, Alireza Navabi, K. Peter Pauls

P54. RNA-Seq genotyping and its application in constructing a high-density genetic linkage map of *Zea mays*
Shuhua Zhan, Jan Tosh, Cortland Griswold, Lewis Lukens

P55. Dissecting phenylpropanoid pathway in common bean (*Phaseolus vulgaris*) by RNA sequencing
Mahbuba Siddiqua, Seth Munholland, William L. Crosby, K. Peter Pauls

P56. Wild and ancestral members of the corn family may host endophytes that can suppress a fungal disease in their modern turfgrass relatives
Hanan R. Shehata*, Katerina S. Jordan, Eric M. Lyons, Manish N. Raizada

P57. An assessment of cell-penetrating peptide transfection in soybean somatic embryos
Atiyyah Ferouz*, François Eudes, Danielle Way, Lining Tian

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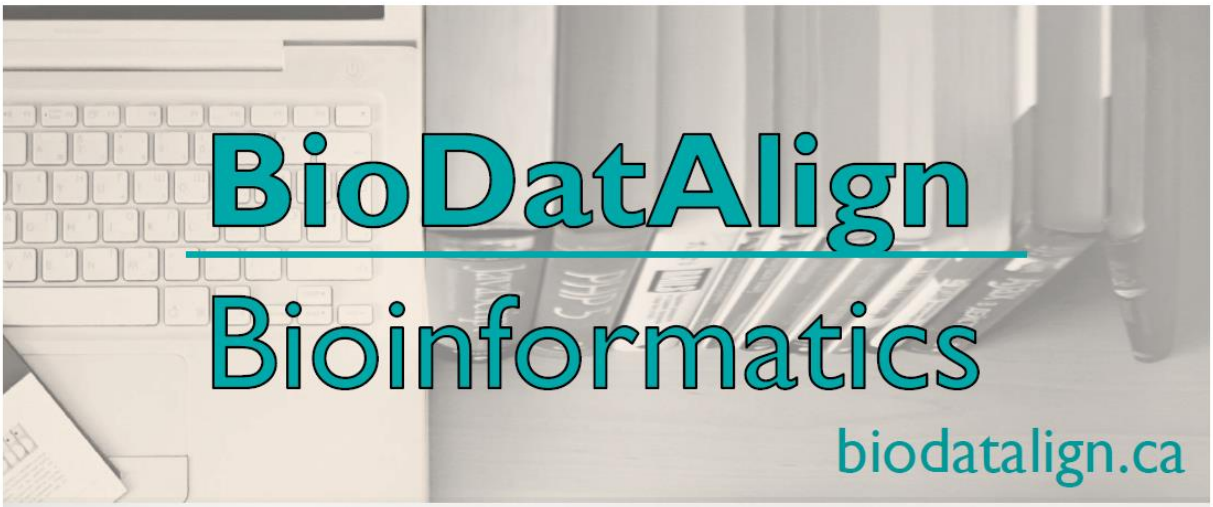

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Plenary Lectures

Plenary Lecture I

Plant Surface Lipid Barriers: Biosynthesis, Regulation, and Biotechnology

Owen Rowland
Department of Biology and Institute of Biochemistry, Carleton University, Ottawa, Canada

Plants have specialized lipid-based barriers that protect them from various environmental stresses such as drought and pathogen attack. Plant aerial surfaces are coated with a cuticle that is composed primarily of polymerized lipids with glycerol (cutin polymer) and associated waxes. Likewise, suberin is a lipid-phenolic heteropolymeric barrier found in the cell walls of various external and internal tissues, including periderms and the root endodermis. The coordinated activities of a large number of enzymes are required to produce the variety of fatty acid derivatives and phenolics that form these surface lipid barriers. My research group is using a combination of genetic and biochemical approaches to study extracellular lipid synthesis and regulation, largely in the model plant *Arabidopsis thaliana* and in the oilseed crop *Camelina sativa*. I will present our recent work on the roles of various enzyme families in generating extracellular lipids and the discovery of a stress-inducible transcription factor governing the regulated deposition of suberin. Our overall goal is to gain important fundamental knowledge regarding the biosynthesis and eco-physiological functions of surface lipid barriers, with the applied aim of providing information and tools for the development of stress-tolerant plants. In addition, plant surface lipids represent renewable hydrocarbons that are chemically suitable for replacement of petroleum products as sources of chemical feedstocks. Detailed knowledge of plant surface lipid biosynthesis is critical for harnessing this renewable chemical resource to its full potential, for example through the metabolic engineering of oilseed crops and microbes.

Plenary Lecture II

Plant Polyisoprenoids –Secondary Metabolites or Physiologically Important Superlipids?

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Since the accidental discovery of polyisoprenoids as contaminants in cellulose pulp extracts, almost 50 years ago, their widespread occurrence in all kingdoms of life has been well documented. In animals and microbes these compounds serve indispensable roles, yet surprisingly the biological relevance of these compounds in plants is almost completely unknown. Moreover, the enzymes that are thought to synthesize plant polyisoprenoids, which are known as *cis*-prenyltransferases (CPTs), remain largely uncharacterized. In this seminar, we discuss the synthesis, occurrence and biological function of this class of plant 'secondary metabolites'.

Concurrent Oral Session Abstracts

1.1. Investigation of the effect of high temperature stress on reproduction in *Arabidopsis*

Vanessa Lundsgaard-Nielsen, Dinesh Christendat and Tammy L Sage

University of Toronto, Department of Ecology and Evolutionary Biology

The negative effect of high temperature (HT) on pollen development is a primary reason for reduced seed set in natural and arable ecosystems. We have identified a gene, *HTT*, for HT tolerance in *Arabidopsis* that encodes a plastid-targeted protein functioning in the removal of reactive carbonyl species (RCS). In the HT tolerant *Arabidopsis Ler*, the gene is induced in the anther, pollen remain viable and HT has little effect on seed set. Cvi pollen is not viable, has low seed set and the gene is not induced at HT. Loss of pollen viability in Cvi and *htt1-1* at HT is associated with abnormal plastid development, lipid accumulation, and autophaged mitochondria. We assessed the degree of protein carbonylation and used mass spectrometry to identify carbonylated proteins to test the hypothesis that *HTT* functions to maintain plastid homeostasis and pollen viability. Pollen plastids in *Arabidopsis* are essential for pollen maturation due to their critical role in fatty acid and carbohydrate metabolism. Consistent with our hypothesis, carbonylated protein levels were higher in Cvi and *htt1-1* than *Ler* at HT. Proteins that were carbonylated in Cvi and *htt1-1* at HT function in lipid and carbohydrate metabolism, as well as detoxification of ROS and other compounds. Protein carbonylation of plastid-targeted genes has been previously demonstrated to adversely affect vegetative development. Our results provide a novel role in *HTT* in ensuring removal of RCS to maintain pollen viability.

1.2. Crossroads of stress responses, development and flowering regulation – the multiple roles of cyclic nucleotide gated ion channel 2

Alex Fortuna[1], Jihyun Lee[1], Huoi Ung[1], Kimberley Chin[1], Wolfgang Moeder[1] and Keiko Yoshioka[1,2]

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Cyclic nucleotide-gated ion channels (CNGCs) are non-selective cation channels that were first identified in animals, where they play key roles in light and olfactory signaling. The *Arabidopsis* autoimmune mutant, *defense-no death 1* (*dnd1*) is a null mutant of *CYCLIC NUCLEOTIDE-GATED ION CHANNEL2* (*AtCNGC2*). *dnd1* exhibits constitutive pathogen resistance responses including higher levels of endogenous salicylic acid (SA), which is an important signaling molecule for pathogen defense responses. Recently we have reported that *dnd1* exhibits a significantly delayed flowering phenotype, indicating the involvement of *AtCNGC2* in flowering transition. However, since SA has been known to influence flowering timing as a positive regulator, the delayed flowering phenotype in *dnd1* was unexpected. In this study, we have asked whether SA is involved in the *dnd1*-mediated delayed flowering phenotype. In addition, in order to gain further insight into the involvement of SA and CNGCs in flowering transition, we analyzed the flowering transition of *cpr22*, another CNGC mutant with a similar autoimmune phenotype as *dnd1* (including high SA accumulation), and null mutants of several other CNGCs. Our data suggest that *dnd1* does not require SA or SA signaling for its delayed flowering phenotype, while SA was responsible for the early flowering phenotype of *cpr22*. None of the other CNGC mutants besides *AtCNGC4* displayed an alteration in flowering transition. This indicates that *AtCNGC2* and *AtCNGC4* have a unique role controlling flowering timing and this function is independent from its role in pathogen defense.

1.3. Molecular and physiological responses of hybrid poplars under stress conditions

Katharina Bräutigam[1,3], Stefan Schreiber[2], Yunchen Gong[1], Pauline Wang[1], Barb Thomas[2] , Uwe Hacke[2] and Malcolm M. Campbell[1,3]

[1]*Centre for the Analysis of Genome Evolution & Function, Department of Cell & Systems Biology, University of Toronto, Toronto, ON, Canada*
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[3]*Department of Biological Sciences, University of Toronto Scarborough, Toronto, ON, Canada*

For plants, it is of key importance to respond efficiently to environment perturbation. This is particularly important for plants with long generation times, such as tree species, that must be able to contend with fluctuating environmental conditions throughout their long lifetimes. To further our understanding of key stress responses, it is crucial to link phenotypic responses with underlying molecular patterns. Here we focus on a unique combination of physiological traits, hydraulic characteristics, and transcriptome patterns using NGS in hybrid poplars, trees of economic importance in Canada and worldwide. For five economically important genotypes, vegetatively propagated plants were grown in a common environment and studied under non-stress and stress conditions. The analyses of resource allocation patterns (e.g., between above-ground and below-ground biomass) and physiological parameters (stomatal conductance, vulnerability to cavitation) uncovered different strategies employed by different genotypes to contend with drought, a key abiotic stress. In addition, transcriptome analyses using RNA-seq revealed genotype-specific patterns. More recently, in addition to genetic factors, the importance of epigenetic patterns for plant performance has been reported. Differences in drought transcriptome responses in genetically identical genotypes were found to be paralleled by differences in DNA methylation (currently being investigated using whole-genome bisulfite sequencing) indicating a possible mechanism for adding an additional

layer of plasticity in long-lived organisms. The work contributes to our understanding of plant-environment interactions, with applications related to genotype selection, plasticity of responses, and their impacts on future clone performance in plantations.

1.4. Activity of *Vitis CBF/DREB1* and *DREB2* transcription factors

Chevonne E. Carlow and Annette Nassuth

Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada

The highly conserved CBF pathway is crucial in the regulation of plant responses to low temperatures by CBF/DREB1 proteins, but might be involved in tolerance to other abiotic stresses as well. Closely related DREB2 proteins have been hypothesized to function after stress-induced post translational activation under drought stress, but may be like-wise involved in other abiotic stress responses. We cloned and sequenced seven *CBF* (CRT binding factor) and two *DREB2* (DRE binding factor) genes from *V. riparia* and *V. vinifera.* Transient transactivation assays showed that all but one tested CBF can activate via the core DRE/CRT sequence A/GCCGAC. When longer and more specific DRE/CRT sequences were used, both CBF and DREB2 proteins showed their preferences for certain sequence patterns, however this was not necessarily based on their status as a CBF or DREB2. In addition, a mutation to PEST sequences in both VrCBF4 and VrDREB2-3 was tested, and shown to be important for the stability of VrCBF4. VrCBF5, the only member of this family which was shown to not activate via the DRE/CRT element, is similar to VrCBF6 but lacks the C-terminal domain which contains several hydrophobic groups. Mutations within these groups will be tested to determine if they are indeed important for the activation of VrCBF6. We hypothesize that most, if not all DREB1/CBF proteins have different functions; therefore current studies continue to focus on further unravelling the overlapping regulons of these transcription factors. This work was supported by NSERC operating and ORF grants to AN.

1.5. A critical evaluation of the two-mechanism model of K^+ acquisition in roots of higher plants and the contribution of apoplastic fluxes

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Department of Biological Sciences, University of Toronto, Toronto, Ontario, Canada

Potassium (K^+) acquisition in plant roots is generally described as the sum of activities of two distinct transport systems: (1) the high-affinity transport system (HATS), a saturable system that catalyzes the active uptake of K^+ from external concentrations ($[K^+]_{ext}$) <1 mM, and (2) the low-affinity transport system (LATS), a linear, passive, system operating at $[K^+]_{ext}$ >1 mM. It is generally viewed that the HATS is governed by transporters of the HAK/KUP/KT family (*e.g.*, AtHAK5 in *Arabidopsis*), and the LATS is governed by Shaker-like K^+ channels (*e.g.*, AtAKT1 in *Arabidopsis*); however, studies on the double-knock-out mutant *athak5 atakt1* have recently revealed a genetically unidentified "back-up system" that mostly operates under LATS conditions. In this study, we revisit the fundamental question of what the relative contribution is of the various transport systems in relation to $[K^+]_{ext}$. Using the short-lived radiotracer $^{42}K^+$, we examined K^+ fluxes in roots of intact barley (*Hordeum vulgare*) and *Arabidopsis thaliana* under various $[K^+]_{ext}$, while employing knock-out mutants and pharmacological blockers. We observed that the contribution of transmembrane systems to K^+ uptake in fact *decreases* with rising $[K^+]_{ext}$. Moreover, we demonstrate that the apparent rise in fluxes with increased $[K^+]_{ext}$ is increasingly apoplastic (extracellular) in nature. This is supported by flux analyses of the apoplastic tracer dye 8-hydroxy-1,3,6-pyrenetrisulphonic acid (PTS). This work challenges the classic two-mechanism model of K^+ transport by revealing a declining cellular flux and an increasing apoplastic contribution with rising $[K^+]_{ext}$, with the latter becoming particularly dominant under saline conditions.

1.6. Tolerance to the herbicide imazethapyr (Pursuit) in common bean.

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Herbicide tolerance is a key trait for crop management. For *Phaseolus vulgaris* or common bean, the herbicide Pursuit is generally utilized pre-emergence or pre-plant incorporated to control broadleaf weeds. Although common beans have good tolerance to this herbicide, a major disadvantage is a narrow margin of crop safety which can result in crop injury and reduced yield. Pursuit is part of the imidazolinone or group 2 herbicides which act as inhibitors of acetolactate synthase (ALS). Other crop species with resistance to this herbicide have been developed and marketed in parts of Canada. These cultivars integrate variants of the ALS enzyme incorporating mutations rendering the enzyme insensitive to the imidazolinone inhibitor acting as the herbicide. The initial aim of this study is to determine the mutation that confers resistance to Pursuit in common bean cultivars and whether variation among varieties can be detected. Mutagenesis screens have produced candidates that have been further tested in the field and spray chamber for tolerance. For approaches using genetic transformation, improvement of tissue culture would be crucial to implement cell-penetrating peptide (CPP) technology. This technology may be key to developing herbicide resistant cultivars for Canadian agriculture in combination with genome editing.

2.1. The regulation of transient starch biosynthesis in *Arabidopsis thaliana*

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Starch is an important carbohydrate in higher plants and is widely used in food and non-food industries. In plastids,

starch is synthesized through the actions of starch synthases (SS), starch branching enzymes (SBE), and starch debranching enzymes (DBE), each with multiple isoforms. Starch biosynthetic enzymes can form phosphorylation-dependent protein complexes. In cereal amyloplasts, a heteromeric protein complex involving SSI, SSIIa, and SBEIIb is formed in the stroma and also localized in the starch granule as a function of the glucan-affinity of SSIIa. SBEIIb is phosphorylated within this complex, and three phosphorylation sites have been identified on maize SBEIIb. It is currently unknown whether regulation of starch biosynthesis by phosphorylation-dependent protein complex assembly is a universal mechanism, used in both transient and storage starch biosynthesis. This work investigated the regulation of starch biosynthesis in *Arabidopsis* leaf chloroplasts. The sub-organelle distribution of starch biosynthetic enzymes was determined; SS2 and SBE2.2 were detected in the wildtype starch granules at the end of a 16/8h light/dark photoperiod. However, starch granules of *ss2*- mutant also lack SBE2.2, suggesting an interaction between SS2 and SBE2.2. Recombinant SBE2.2 was shown to be phosphorylated by chloroplast protein kinases, and two phosphoserine residues that are conserved with SBEII were identified by site-directed mutagenesis. A phosphorylation dependent protein complex could be formed *in vitro* between recombinant *Arabidopsis* SBE and maize SSI and SSII, suggesting that the interaction mechanism of starch biosynthetic enzymes is common across species. The results from this study provide new insight into transient starch biosynthesis regulation in chloroplast.

2.2. Transcriptome analysis of seed coats reveals increased expression of proanthocyanidin metabolism genes in darkening cranberry bean (*Phaseolus vulgaris*)

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Cranberry bean (*Phaseolus vulgaris*) seed coat darkening occurs during postharvest storage and culminates in reduced visual quality, effectively decreasing shelf life and economic value. Seed coat darkening is associated with the biosynthesis of proanthocyanidins during seed maturation, and their subsequent oxidation during commercial postharvest handling. We hypothesize that expression of proanthocyanidin metabolism genes is involved in cranberry bean seed coat darkening. Here, recombinant inbred lines from an 'Etna' (darkening) by 'Wit-Rood' (non-darkening) cranberry bean cross were greenhouse cultivated; RNAseq analysis was performed on seed coats representing early, intermediate and mature developmental stages using a next generation sequencing (Illumina TruSeq) platform. Assembled darkening and non-darkening seed coat transcripts representing approximately 27,000 protein coding genes were aligned to the *P. vulgaris* reference genome. Analysis of fragments per kilobase of transcript per million mapped reads (FPKM) revealed as many as 430 genes were up-regulated in darkening seed coats relative to the non-darkening RIL. At early and intermediate stages of bean development in the darkening RIL, approximately 55% of highly expressed genes (FPKM >50 fold relative to non-darkening seed coats) belong to the flavonoid pathway leading to proanthocyanidins, including genes encoding for the biosynthetic enzymes, anthocyanidin reductase (ANR) and leucoanthocyanidin reductase, as well as their putative transcription factors. Biochemical characterization of a bacterially expressed recombinant ANR, PvANR1, is underway. This project may provide important information for breeding efforts aimed at preserving the aesthetic appeal and marketability of cranberry beans.

2.3. Deciphering metabolic pathways for 4-aminobutyrate (GABA) production in 'Empire' apple fruit stored under controlled atmosphere conditions

Carolyne J. Brikis[1], Adel Zarei[1], Kristen L. Deyman[1], Jingyun Liu[1], Greta Z. Chiu[1], Christopher P. Trobacher[1], Gordon J. Hoover[1], Jennifer R. DeEll[2], Gale G. Bozzo[1] and Barry J. Shelp[1]

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Apples are highly perishable at ambient temperature and CO_2. Controlled atmosphere (CA) storage is commonly used to delay fruit ripening; but can result in physiological injury and accumulation of 4-aminobutyrate (GABA), a well-known abiotic stress signature. In the present study, profiling of metabolites and transcripts associated with pathways for GABA production was conducted in 'Empire' apples to determine their relationship with CA storage time and conditions (0 & 3°C, 0.03 & 5 kPa CO_2, 2.5 kPa O_2). Dramatic changes in the levels of glutamate, GABA, alanine, succinate, NADH/NAD^+ and NADPH/$NADP^+$ ratios, 4-hydroxybutyrate, putrescine, spermidine and spermine were observed. The responses to CA storage were both short and long term. GABA levels were more associated with glutamate than polyamines during the initial stages of storage, and were negatively associated with both metabolites over the longer term. NAD(P)(H)/$NAD(P)^+$ ratios increased at the onset of storage, followed by decreasing NADH/NAD^+ and increasing NADPH/$NADP^+$ ratios. The second most abundant *glutamate decarboxylase (GAD)* and *amine oxidase (AO)*, as well as two *aldehyde dehydrogenases* genes, were rapidly induced by CA storage and likely associated with early GABA accumulation. The most abundant *GAD* and *AO*, as well as *GABA-transaminase1,2* and *succinic semialdehyde dehydrogenase1* were induced more slowly with storage time, and this accumulation might be associated with maintenance of long-term GABA levels. Fruit stored under elevated CO_2 were more prone to external CO_2 injury. These findings provide a relatively complete picture of gene-dependent and independent processes involved in GABA accumulation and their association with CA-related physiological disorders.

2.4. Chasing the explanation for the vascular defects in MTN-deficient mutants: is their decreased spermidine content the culprit?

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Methylthioadenosine (MTA) is the common by-product of nicotianamine, ethylene and polyamine bio-synthesis. *Arabidopsis thaliana* mutants deficient in MTA nucleosidase (MTN) activity have a complex phenotype, the most fundamental of which appears to be disruption of vascular development. We hypothesize that the underlying basis of the vascular defects of MTN-deficient plants is their altered polyamine profiles, particularly a decrease in spermidine content. Although polyamines have been implicated in plant development and stress responses, their specific mechanisms of action remain generally unclear. Fortunately a direct target of spermidine has been documented in eukaryotes. It is required as a cofactor for the activation of the essential eukaryotic initiation factor, eIF5A. In fact, the modification of eIF5A is the major sink for spermidine in yeast cells and possibly plants too. However, examining the involvement of eIF5A in plants is complicated by its phloem mobility. One goal of our current research is to determine whether any of the phenotypic changes of MTN-deficient mutants are the consequence of altered eIF5A activation. Surprisingly, random secondary branches of some mutant seedlings grown for 14 days on spermidine-supplemented media have WT vascular architecture at maturity. We are now developing mutants deficient in activated eIF5A in order to compare them with MTN-deficient mutants. We specifically want to test the effect of spermidine exposure on the expression of auxin and vascular markers in each mutant background relative to WT. These experiments should determine whether there is a direct relationship between spermidine-dependent activation of eIF5A and the vascular defects in MTN mutants.

2.5. Screening early potatoes for nutrition – Is there a role for starch?

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Diabetes, obesity and cardiovascular diseases are inter-related with diet being a major factor. In this light, increased carbohydrate intake has often been implicated, with potatoes often perceived as a prime contributor being a carbohydrate rich food and its association with glycemic index (GI). Potatoes, however, are an important and affordable source of protein, minerals, vitamins, phytochemicals and antioxidants. The GI of food (especially potatoes) is affected by the digestibility of starch. Early potatoes usually have a different starch structure from fully matured counterparts. Nutritionally, starch is classified based on its rate of digestion into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). Starch digestion rate and its consequent glycemic impact are influenced by the food composition such as RS, phytonutrients, dietary fibre, protein, and fat content as well as cultivar and cooking methods. As part of a larger study investigating the nutritional benefits of colored early potatoes and its role in glycemic load, the effect of cultivars and cooking method (boiling) on starch composition (total starch and amylose content) and digestibility are being investigated. Significant differences were noted in total starch and amylose contents between varieties. Highest total starch was obtained in Adora, Smart, F10090 and Norland whereas lowest contents in French Fingerlings and Ciklamen. Amylose content contents also varied with highest in Smart and lowest in Ciklamen. Varieties also showed significant differences in RDS, SDS, RS and estimated glycemic index (eGI). Yellow star showed the highest RS, lowest RDS and eGI.

2.6. Soybean isoflavonoid metabolon: elements and interaction at the molecular level

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Isoflavonoids are plant natural products, almost exclusive to legumes, synthesized by the phenylpropanoid pathway. They are actors in symbiosis with nitrogen-fixing bacteria and in plant stress response. Isoflavonoids are noted for their human health benefits. The current model of isoflavonoid biosynthesis is of soluble cytoplasmic enzymes forming a 'metabolon' at the surface of the endoplasmic reticulum (ER), anchored by cytochrome P450s, including isoflavone synthase (IFS), the key branch-point enzyme in this pathway. Evidence for an isoflavonoid metabolon has been scant and largely circumstantial. We have identified the key players in this pathway, established *in planta* subcellular localization, and investigated their protein-protein interactions. Some members of the metabolon have shown localization to the nucleus, in addition to the cytoplasm, raising questions as to alternative functions, outside of synthesis. IFS, however, is localized to the ER. Results from our Bimolecular Fluorescence Complementation (BiFC) assays have shown for the first time that IFS works as a nucleating metabolic center at the surface of the ER, interacting with upstream enzymes, including chalcone isomerase, chalcone synthase and chalcone reductase. We have also widened the scope of the known interactome of IFS through co-immuno-precipitation (Co-IP) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Our results indicate that IFS and cinnamate 4-hydroxylase (C4H) form adjacent metabolic centers shuttling metabolites from the general phenylpropanoid pathway into the isoflavonoid branch. Understanding the mechanics of this metabolon: the elements and their interactions, adds a layer to our knowledge of the pathway at the protein level that has been hitherto lacking.

3.1. Temperature has a stronger effect than photoperiod in regulating seasonal photosynthetic performance in white spruce (*Picea glauca*)

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Elevated temperatures associated with global climate change are expected to extend the growing season in high latitude forests, potential increasing seasonal carbon uptake and helping offset anthropogenic CO_2 emissions. However, trees use not only late season declines in temperature to sense when to prepare for winter dormancy, but also declines in day length. Because photoperiod is not affected by climate change, day length signals might constrain the ability of forests to extend their growing season in a warmer climate. I hypothesized that photoperiod is more important than temperature in regulating seasonal photosynthetic capacity in a dominant boreal conifer, white spruce (*Picea glauca*), as changes in photo-period are a more reliable cue of seasonality than changes in temperature. White spruce was grown under combinations of either naturally declining or constant temperature and photoperiod treatments, to simulate autumn environmental cues. Two different warming treatments both delayed autumn photo-synthetic declines, while photoperiod treatments had little effect on patterns of seasonal carbon uptake capacity. Weekly variation in leaf nitrogen (reflecting leaf protein concentration) was closely coupled to changes in photosynthetic pigments, although photosynthetic nitrogen use efficiency (carbon fixation per unit leaf nitrogen) increased under warming. In contrast, water use efficiency (water loss per unit carbon fixed) remained constant across all treatments. These data suggest that temperature is more important than photoperiod in regulating seasonal changes in photosynthetic parameters in white spruce, implying that as the climate warms, the growing season length for boreal conifers may be extended, enhancing carbon sequestration in forest ecosystems.

3.2. Seasonal photosynthetic activity in evergreen conifer leaves monitored with spectral reflectance

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In boreal conifers, the seasonal downregulation and recovery of photosynthesis is largely invisible and difficult to assess with remote sensing. To better understand the mechanism and timing of these hidden physiological transitions, we explored the seasonal dynamics of several assays and optical indicators of photosynthesis in lodgepole (*Pinus contorta*) and ponderosa pine (*Pinus ponderosa*) in Edmonton, Canada over one year. Spectral reflectance was measured weekly at the leaf-scale and the Photochemical Reflectance Index (PRI), often used as a proxy for xanthophyll cycle activity, carotenoid:chlorophyll pigment pools and photosynthetic light-use efficiency (LUE), was used to track the seasonal dynamics of photosynthetic activity. Additional physiological measurements included leaf pigment content, chlorophyll fluorescence, and gas exchange. All the metrics indicate large seasonal changes in photosynthetic activity, with a sharp transition during spring recovery and a more gradual winter downregulation. The PRI was a good indicator of several parameters including seasonally changing photosynthetic activity, chlorophyll fluorescence, and pigment pool sizes (carotenoid:chlorophyll). Leaf- and canopy-scale PRI measurements exhibited parallel responses during the winter-spring transition. Together, our findings indicate that evergreen conifers photosynthetic system possesses a remarkable degree of resilience in response to large temperature changes across seasons, and that optical remote sensing can be used to observe the seasonal effects on photosynthetic activity. Furthermore, the seasonal transitions between photosynthetically active and inactive states can be clearly detected by the PRI. These findings have implications for using remote sensing to detect dynamics in photosynthetic activity in response to changing growing season length in northern latitudes.

3.3. Facultative and constitutive changes in pigment composition affect the relationship of the photosynthetic light-use efficiency and the photochemical reflectance index of Eastern white pine (*Pinus strobus* L.)

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The photochemical reflectance index (PRI) is a proxy for xanthophyll-cycle activity and the light-use efficiency of photosynthesis (LUE) in plants. In autumn, when evergreen conifers downregulate photosynthesis, dynamic xanthophyll-cycle-mediated nonphotochemical quenching (NPQ) is replaced by sustained NPQ. This shift in energy-partitioning coincides with adjustments of pigment pools and affects the PRI-LUE relationship. We aimed to characterize changes in energy-partitioning and pigment composition during the summer-autumn transition in an evergreen conifer to reveal mechanisms affecting the strength of the PRI-LUE relationship. Photosynthetic downregulation was studied in *Pinus strobus* seedlings exposed to cold (low temperature/short photoperiod) or warm (elevated temperature/short photoperiod) controlled autumn conditions. Shifts in energy-partitioning during simulated autumn occurred with constitutive pigment pool adjustments, including decreased chlorophyll and increased photoprotective carotenoids. On a seasonal scale, PRI was controlled by carotenoid pool sizes rather than xanthophyll-cycle activity. The magnitude of variation in PRI due to constitutive pigment pool adjustments was three times higher than the magnitude of variation in PRI caused by diurnal changes in xanthophyll cycle pigments. Furthermore, exposure to cold weakened the PRI-LUE relationship, likely due to alternative NPQ mechanisms undetected by the PRI. We conclude that facultative and constitutive pigment effects

on PRI operate at different timescales, and must be considered together to effectively integrate PRI into models assessing photosynthesis in evergreens.

3.4. Teaching photosynthetic electron transport using an active learning exercise in the classroom

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Each year I teach 30-65 upper year students in a course entitled "Environmental Stress Biology of Plants". In one course module I demonstrate the challenges faced by plants due to continuously fluctuating light environments (e.g. high and low light levels) primarily as it relates to photosynthetic electron transport. During this module, I run an active learning exercise in class that meets three goals: i) it serves as a refresher on photosynthetic electron transport for the students; ii) it reinforces the idea that plants are constantly having to acclimate to changing light conditions, iii) it demonstrates how the electron transport chain can become over reduced and lead to electron leakage and the generation of reactive oxygen species. Test questions derived from the content conveyed during the exercise are answered correctly by almost all students indicating that the information presented is effectively retained. Feedback collected from students at the end of the course indicates that they enjoy participating in the exercise and they also request additional active learning opportunities. During my presentation I will model this exercise for the audience as an example of how active learning opportunities can enhance student learning in the plant biology classroom.

3.5. Light, weeds & carbon partitioning - How does a neighbour do it?

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Plants have the ability to detect the presence of neighbouring plants through changes in the red: far-red ratio of light reflected or transmitted off vegetation. This triggers the shade avoidance response before direct shading occurs and has been shown to be important in understanding yield loss especially when it occurs during the critical period for weed control. Previous work in our lab has shown that this response is triggered upon emergence and can have long lasting negative effects on plant yield. While the morphological and hormonal changes associated with this response are well documented, investigations into how carbon and nitrogen assimilation may be impacted are lacking. Through a growth chamber study, we hypothesized that soybean seedlings exposed to weedy conditions and non-limiting resources would have reduced rates of carbon assimilation and in turn direct consequences on the sugar and starch pools, compared to weed-free plants. Through direct measurements of photosynthesis, chlorophyll content and soluble and insoluble sugars, we identified key changes to carbon partitioning in plants exposed to early season neighbouring weeds. While we found significant effects on the carbon cycle, nitrogen assimilation did not seem to be impacted. A better understanding of carbon partitioning during plant-weed interactions, will shed light on the mechanisms of yield loss in soybean. This work can help improve our understanding of yield loss in soybeans and can be applied to management and breeding programs to improve plant health.

3.6. Development of a high-intensity, variable-spectra LED array for optimized plant development in challenging environments

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Plant development can be dramatically influenced by the quality of light present in their environment. Many factors, such as rates of photosynthesis and dry-matter production, synthesis of specific compounds, or time of flowering can be manipulated through the use of specific wavelengths of light. Researchers at the Controlled Environment Systems Research Facility (CESRF) have developed an array of high-intensity, narrow-bandwidth LEDs. The array contains LEDs of nine distinct wavelengths, ranging from short-wavelength UV LEDs to relatively long-wave far-red LEDs. Individually, each wavelength present is sufficient in intensity to drive associated pathways in plants. The intended use for the array is to develop light "recipes" that maintain a plant's optimum light environment for whatever developmental characteristic is desired. The short term goal at the CESRF with this array is to optimize dry matter accumulation in lettuce, tomato, and pepper plants for food production in challenging environments. Design considerations for deploying LEDs, and the influences of spectral quality on lettuce, tomato and pepper photosynthesis are presented.

4.1. Ectopic expression of miR156 improves alfalfa yield and quality

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Alfalfa's position as the world's most valued livestock forage and low-input bioenergy crop are hampered by its relatively low yield and poor biomass quality. Little progress has been made over the last two-three decades in breeding programs to improve these two traits in alfalfa. Here we explore the role of microRNA156 (miR156) in improving yield and quality of alfalfa. Transgenic alfalfa expressing miR156 precursor from *Lotus japonicus* (*LjmiR156*) was generated, and three

SPL genes (*MsSPL6, MsSPL12* and *MsSPL13*) were identified as targets of transcript cleavage by miR156. Ectopic expression of *LjmiR156* induced a number of phenotypes, ranging from a delay in flowering time and enhanced shoot branching to elevated biomass production. Furthermore, the contents of starch and soluble sugars were enhanced in all transgenic plants, whereas the effects on lignin, cellulose, pectin and structural sugars were variable. Our findings highlight the potential of manipulating expression of miR156 and its target *SPL* genes as a strategy to improve alfalfa traits.

4.2. Evidence for lineage-specific selection on gene expression in rice

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Throughout plant domestication and breeding, artificial selection has been used to develop crops, but only a small number of molecular targets of these intense selection processes have been identified. In this work, using genome-wide expression data, we identified gene regulatory changes that very likely have been targets of selection in the breeding of two genetically distinct lines of rice (*Oryza sativa*), Minghui 63 and Zhenshan 97. Selection on regulatory alleles appears to have occurred on several genes with shared molecular functions and shared gene expression patterns. More specifically, we identified evidence of selection for genes with shared gene ontology terms related to nucleic acid binding, and for genes involved in starch breakdown and free radical scavenging. In addition, in an analysis of individual genes, we identified evidence that selection has targeted regulatory loci with strong, controlling effects on gene expression. Predicted functions of genes with signatures of positive selection are consistent with phenotypic differences between Minghui 63 and Zhenshan 97. Our study identifies novel selection targets and suggests that positive selection for regulatory alleles is an important component of plant improvement.

4.3. Combining high density SNP analysis to identify introgression regions associated with CBB resistance in *Phaseolus* species

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OAC-Rex was the first variety of dry bean released in North America that is resistant to Common Bacterial Blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli*. Strong resistance to CBB has not been observed in *P. vulgaris*, but has been described in related species, such as *Phaseolus acutifolius* and *Phaseolus coccineus*. In order to bring CBB resistance to *P. vulgaris*, an interspecific hybridization with the *P. acutifolius* line PI440795 was performed in OAC-Rex's pedigree. As part of the Applied Bean Genomics and Bioproducts Project, the genomic sequences for OAC-Rex and PI440795 are being sequenced using the Illumina HiSeq and PacBio RSII platform. Although the full-length chromosomal sequence is not available at this time, contigs and scaffolds allow us to interrogate both lines using BLAST. Using the SNP markers from the BeanCAP project, we identified over 5000 markers present in OAC-Rex, and 3800 in PI440795. Of these, 321 markers were shared between OAC-Rex and PI440795, but were absent from the control line Compass, which is susceptible to CBB and has no *P. acutifolius* in its pedigree. By comparing the regions identified in this study to 5 known CBB resistance QTLs on chromosomes 1, 2, 6, 8 and 11, we found that the *P. acutifolius*-derived alleles were strongly associated with 4 of the QTLs, with only the QTL on chromosome 1 appearing to be *P. vulgaris*-derived.

4.4. Branching enzymes as agents for modifying glucan structure in industrial processing

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Starch is used as a cheap, renewable, chemically reactive matrix in many industrial processes. During processing, access to chemically reactive groups on starch is essential and largely depends on their exposure, which is a factor of the branching frequency within starch. The ability to manipulate glucan branching in starch and other polyglucans offers many industrial end users (e.g. paints/inks, coatings, adhesives sectors) with superior performance bio-products. Branching enzymes (BE) introduce α-1,6 branch points in starch, consequently increasing the number of non-reducing reactive chemical ends, thus making starch more reactive to synthesis and digestion, and facilitating its solubility. Although existing starch-based crops with desirable structural traits are available (e.g. *waxy* maize), they are more costly than normal starches, and immediate product improvement is currently only possible through post-harvest modification of starch using environmentally hazardous chemical agents. A number of thermotolerant recombinant BEs suited to a range of industrial processing conditions have been developed and their kinetics characterized through various activity assays. The branching patterns introduced by recombinant BEs in a number of test glucans have been analyzed and compared. The BEs developed throughout the course of this project offer an important new tool and platform technology for bio-product development, which will circumvent the use of environmentally hazardous chemical agents. Additionally, the ability to modify the branching of normal starches will potentially open the market for locally grown starch-based crops as a raw material for biopolymer industries.

4.5. Frequency, magnitude, and sources of bias in allele specific transcript abundances' estimates

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Next generation sequencing technologies allow the genome-wide quantification of individual allelic transcripts derived from heterozygous loci, termed allele specific expression (ASE). ASE is quantified by aligning reads to a reference genome and counting the reads that contain distinguishing polymorphisms. Although conceptually straightforward, ASE estimates are frequently incorrect. Here, we use RNA-Seq data from maize, *Zea mays*, as a model system to investigate the causes of ASE misestimates. We also evaluate existing methods for improving ASE estimation, and propose a novel method. We find that sequence divergence between allelic reads and the reference cause ASE misestimates because of alignment software criteria. In addition, at many loci, RNA-Seq reads from one allele align to the correct location within the genome but reads from a second allele map to an incorrect location within the genome. Modifying the reference genome by masking or replacing polymorphic sites to increase the accuracy of ASE globally reduced the frequency of unequal mapping. However, many expression estimates at many sites continue to be misestimated, and the altered references cause misestimates of allelic expression at new sites. We demonstrate that the number of sites with preferential mapping of reads and the magnitude of preferential read mapping is greatly reduced when reads are mapped to transcriptome reference templates specific to each allele.

4.6. A new flexible gateway-compatible vector system for plant functional genomics

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Functional genomic research always needs to clone different DNA fragments into binary vectors, so as to express genes with different tags, from various promoters and with different levels. Gateway technology makes cloning, especially subcloning a gene in multiple expression vectors, much more efficient compare to traditional restriction enzyme based methods. We have developed a large collection of 27 Gateway-compatible binary T-DNA destination vectors called pGTQL vectors for a wide range of different applications in plant functional study including BiFC, subcellular localization, complementation, over-expression, promoter-reporter and purification. All pGTQL plasmids are binary vectors that suitable for *Agrobacterium*-mediated plant transformation. All these vectors are using the same cloning cassette, which eliminated the problem on frame shift caused by cassette change when using destination vectors from different systems. Some of these vectors have been tested in both dicot and monocot plants including *Arabidopsis thaliana*, *Brachypodium distachyon* and *Nicotiana benthamiana*. Our results show that such system is highly efficient and serves as a high-throughput platform for transient or stable transformation in plants. These vectors expand the current plant functional genomics research toolbox, streamline the analysis of gene function and bridge the gap between basic and applied research for the study of valuable agricultural traits.

5.1. *GLUCAN SYNTHASE-LIKE8* Is required for early seedling development in *Arabidopsis*

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Callose, a linear β-1,3-glucan polymer with β-1,6-branches, is synthesized in both sporophytic and gametophytic tissues and plays various roles. Callose is accumulated at the cell plate during cytokinesis, in plasmodesmata, where it regulates cell-to-cell communication, in dormant phloem, where it seals sieve plates after mechanical injury, pathogen attack, and metal toxicity, as well as in male and female gametophytes. *GLUCAN SYNTHASE-LIKE* (*GSL*) genes in *Arabidopsis* have been identified as homologs of fungal glucan synthase, and comprise a family of 12 genes. A new allele of *GSL8*, *essp8*, was identified as having seedling-lethal phenotype through forward genetic screening of an EMS mutant population of *Arabidopsis* showing ectopic expression of seed storage proteins (*essp*) in seedlings. Bulked-segregant analysis and rough-mapping located *essp8* mutation to a 729 kb region on chromosome II. Next-generation mapping was used to detect the gene(s) responsible for the observed mutant phenotype in the mapped region. *essp8* seedlings were sequenced using 300nt paired-end sequencing on the Illumina MiSeq. An EMS-induced point mutation was identified on an intron splicing site which is predicted to introduce a premature STOP-codon. *essp8* seedlings exhibit several growth defects, including disruptions of root tissue patterning and root hair cell morphogenesis, somatic embryo formation, and incomplete cytokinesis. Histochemical callose deposition and cell-to-cell diffusion assays showed defects in callose deposition in the cell wall and significantly increased symplastic macromolecular diffusion between root cells in *essp8* seedlings. Our findings suggest that GSL8 is required for restriction of symplastic movement, maintaining the basic ploidy level, and cell wall integrity.

5.2. Characterization of a novel family of GDP-O-fucosyltransferase in *Arabidopsis thaliana* and *Brassica napus* microspore culture

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Microspore embryogenesis (ME) is a developmental phenomenon in which microspores divert from their

gametophytic pathway towards embryonic development in culture when stressed. It is an effective breeding tool to generate homozygous doubled haploid plants. Although highly efficient, there are only a few crops and genotypes that are very responsive to ME induction and the mechanisms for this developmental switch are not fully understood. Thus, it is important to elucidate the genetic control of the embryogenic response in responsive cultivars and identify the factors limiting regeneration frequencies in recalcitrant lines. We have identified genes that were significantly overexpressed in embryogenic cells. Of special interest is the gene, *At2g44500* whose *Brassica napus* homolog was consistently upregulated in embryogenic cells in microspore cultures, compared to pollen-like or non-responsive cells. *At2g44500* has a conserved GDP-O-Fucosyltransferase (O-FuT) domain in *Arabidopsis* as well as in the *B.napus* homologs that we have isolated. We have also identified conserved amino acid residues that may play a role in the catalytic activity of this putative fucosyltransferase. We are currently characterizing *Arabidopsis* mutants to elucidate the function of this novel family of plant fucosyltransferases. Once characterized, this gene and its paralogs can potentially be used as genetic markers to identify cultivars that are highly responsive in microspore culture. This will also allow us to understand the molecular mechanisms underlying the early stages of embryogenesis in plants, which is the basis of seed development and is fundamental to crop productivity.

5.3. DNA-binding and localization function of the putative nuclear localization signal of *Vitis* CBF1

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C-Repeat Binding (CBF) proteins are a group of AP2 family transcription factors important to ABA-independent cold-acclimation. CBF proteins are transcriptionally regulated by upstream bHLH ICE transcription factors, and once expressed bind to C-Repeat (CRT) elements in the promoters of downstream Cold Regulated (COR) genes. All identified CBFs contain a PKKPAGR signature sequence near the N-terminal, which is predicted to be a Nuclear Localization Signal (NLS). Canella and collegaues demonstrated that the PKKPAGR sequence is not required for nuclear localization of *At*CBF1 but instead is involved in DNA-binding. Conversely, our work in *Vitis* CBF1 has shown the analogous PKKPAGR sequence is required for nuclear localization, as was originally predicted. To separate potential roles in localization and DNA-binding in the PKKPAGR sequence of *Vitis* CBF1 we are using a series of mutant proteins that alter the charge and amino acid composition of the sequence by varying amounts to identify changes that maintain nuclear localization while disrupting DNA binding and vice versa. These functions will be assessed with a transient expression system in tobacco leaves, respectively using either GFP-tagged CBF1 variants alone, or CBF1 variants in concert with a reporter plasmid where reporter expression is regulated by a CRT element. This work was supported by NSERC operating and ORF grants to Annette Nassuth.

5.4. Investigating the role of stigma specific RLCKs during compatible pollen acceptance signalling

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With species in the Brassicaceae family of plants having dry stigmas, pollen must first be recognized by the stigma for successful germination. Upon compatible pollen recognition, water and unknown enzymes are transported through vesicle trafficking from the stigmatic papillae to the pollen, allowing pollen hydration, germination and pollen tube penetration for subsequent fertilization. Despite knowledge of the many of the physiological events following compatible pollen recognition, the signalling events which trigger this response remain unknown. We used a reverse genetics approach to identify possible genes involved in compatible pollen acceptance signalling, whereby stigma-specific genes were identified using publicly available microarray data. We identified a stigma-specific receptor-like cytoplasmic kinase (RLCK) and confirmed its expression profile using RT-PCR. This RLCK is conserved within the Brassicaceae, with orthologues in *Arabidopsis thaliana, Arabidopsis lyrata,* and *Brassica rapa,* indicating its possible involvement in a family-wide compatible pollen recognition pathway. This kinase along with another closely-related gene in *A. thaliana* was knocked-down using RNA interference (RNAi) though the generation of an artificial microRNA construct. Preliminary analysis of these RNAi lines indicates that this kinase is likely involved in compatible pollen acceptance, as plants demonstrated reduced pollen adhesion, pollen tube penetration, and reduced seed set. This project represents an exciting first step towards understanding compatible pollen acceptance signalling.

5.5. Morphological and molecular analysis of stomatal development in grape

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The development of stomatal complexes in *Arabidopsis*, from meristemoid mother cells via meristemoids and guard mother cells (GMC) into guard cells (GC), is regulated in a sequential manner by dimers formed between related basic-helix-loop-helix transcription factors: ICE/SCRM-SPCH, ICE/SCRM-MUTE and ICE/SCRM-FAMA. It is assumed that a similar process takes place in other plants but data to support this have so far been very limited. The aim of this study was to analyze stomatal development in grape. Observations by bench-top SEM were used to determine stomatal density (number of stomatal cells/leaf area) and stomatal index (number of stomatal cells/stomatal cells + pavement cells). Both increased with increasing age of the leaves but decreased again for leaf 10, suggesting that new stomata are no longer initiated in leaf 10 or older. RT-PCR analysis and cloning of the predicted ORFs showed that regular spliced transcripts of the grape *SPCH*, *MUTE*, *FAMA* and *ICE* genes are present in respectively leaves 1and 3, leaves 1, 3 and 5, leaves 3, 5 and 11, and all examined leaves. The detection of alternative transcripts of each gene

in fewer specific leaves suggest that each affects a different step in stomatal development. Transient overexpression of each ORF in tobacco leaves resulted in increased pavement, GMC and/or GC cells. A model which presents a new look at the regulation of stomatal development in plants, including regulation by detected alternative transcripts, will be presented. This work was supported by NSERC operating and ORF grants to AN.

5.6. Virus induced gene silencing (VIGS) of shikimate kinase like-1 (SKL1) in *Solanum lycopersicum* (tomato) results in an albino phenotype

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SKL1 is a plant shikimate kinase gene duplicate present in all major plant lineages. SKL1 forms a functionally distinct orthologous cluster and biochemical assay revealed that this enzyme does not function as a shikimate kinase. The modeled three-dimensional structure of SKL1 is similar to that of *Arabidopsis* SK and a limited number of active site residues are conserved, more so in the ATP binding pocket. *Arabidopsis skl1* T-DNA mutants are albino and are deficient in thylakoid membrane. However, the biological function of SKL1 remains elusive. We therefore investigated the function of SKL1 in other plants. Using a VIGS system, we have silenced SKL1 in adult tomato plants and observed that some of the leaves of the silenced plant depict a similar albino phenotype. TEM images of these albino leaves depict the absence of the thylakoid membrane and the presence of lipid aggregates within the chloroplasts. Light microscopy also shows an overall reduction of chloroplast abundance per cell. Lipid extraction and analysis by Thin Layer Chromatography (TLC) revealed that there are distinct separation profiles of lipids between the green and the silenced albino tomato leaves. We therefore concluded that the function of SKL1 is conserved between *Arabidopsis* and tomato plants. We hypothesized that SKL1 function is associated with lipid biosynthesis or accumulation in the plastid and this effect is reflected by the inability of the plastid to establish a thylakoid membrane.

6.1. The manipulation of cytosolic nucleoside diphosphate kinase in transgenic *Solanum tuberosum* roots affects growth, respiration and starch metabolism

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Nucleoside diphosphate kinase is found in several subcellular compartments. It catalyzes the transfer of the γ-phosphate from a donor nucleoside triphosphate to an acceptor nucleoside diphosphate and is therefore predicted to play a key function in plant energetic metabolism. However, there is no consensus about its importance in plant metabolism. In this study, we report the effects of the genetic manipulation of cytosolic NDPK on the physiology, respiration and carbon metabolism in potato (*Solanum tuberosum*) roots. Sense and antisense NDPK constructs were introduced in potato using *Agrobacterium rhizogenes* to generate a population of root clones displaying a 40-fold variation in NDPK activity. We used a targeted metabolomic approach as well as measurement of various physiological parameters to understand the impacts of this manipulation on metabolism. Root growth and respiration were positively correlated with NDPK expression levels. No significant variation was observed in the adenylates pools among transgenic roots. However, different NDPK expression levels led to shifts in the pools of important intermediates in primary carbon metabolism and in root starch content. Changing the expression level of cytosolic NDPK also led to (i) alterations in carbon flux through glycolysis; (ii) pleiotropic effects on the activity of other enzymes and (iii) changes in the levels of reactive oxygen species. The implication of cytosolic NDPK in root respiration and metabolism will be discussed in the light of these data.

6.2. An *Arabidopsis thaliana* β-glucosidase is involved in flavonol bisglycoside catabolism

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Flavonol bisglycosides are metabolites with proposed roles as antioxidants, modulators of polar auxin transport and plant-insect communication signals. The flavonol bisglycosides kaempferol and quercetin 3-*O*-β-glucoside-7-*O*-α-rhamnoside (K3G7R and Q3G7R, respectively) accumulate in *Arabidopsis thaliana* under simultaneous abiotic stress (*i.e.* nitrogen deficiency and low temperature, NDLT). Rapid losses of K3G7R and Q3G7R are evident following the transfer of NDLT plants to nitrogen sufficiency and high temperature (NSHT). Here we tested the hypothesis that a flavonol 3-*O*-β-glucoside-7-*O*-α-rhamnoside specific β-glucosidase (BGLU) is involved in the catabolism of K3G7R and Q3G7R in *Arabidopsis* plants. K3G7R and Q3G7R losses coincided with an approximate 1.5-fold increase in flavonol 3-*O*-β-glucoside-7-*O*-α-rhamnoside BGLU activity within 2 days of NDLT recovery relative to plants continually cultured under NSHT (control). QTOF-MS/MS identified the Q3G7R in hydrolysate as quercetin 7-*O*-α-rhamnoside. Plant BGLUs are members of the glycoside hydrolase family 1 (GH1); a phylogenetic analysis of all 47 *Arabidopsis* GH1 proteins revealed *Arabidopsis* BGLU12-17 clustered with Fabaceae hydrolases known to attack isoflavones and isoflavonoids, which are structurally somewhat related to flavonol 3-*O*-β-glucoside-7-*O*-α-rhamnosides. Real time quantitative PCR analysis revealed 300, 148 and 68% respective increases in *BGLU15*, *BGLU12* and *BGLU16* transcripts within 1 day of NDLT recovery relative to control plants. A recombinant thioredoxin-His_6-tagged BGLU15 was expressed in bacteria and purified to homogeneity. Recombinant BGLU15 preferentially hydrolyses the 3-*O*-β-glucosides of flavonols, but does not cleave other sugars. BGLU15 displayed the highest catalytic efficiency for Q3G7R and K3G7R yielding their respective 7-*O*-rhamnosides. These results suggest flavonol 3-*O*-β-glucoside-7-*O*-α-rhamnoside catabolism in *Arabidopsis* involves BGLU15 hydrolysis yielding flavonol 7-*O*-α-rhamnosides.

6.3. Regulation of maize starch synthase II, a key enzyme of starch biosynthesis

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Starch is the most abundant storage carbohydrate in plants, providing 70% of human caloric intake and has many industrial applications. Starch biosynthesis involves the coordination of starch synthases (SSs), starch branching enzymes (SBEs) and debranching enzymes. There is increasing evidence that starch biosynthetic enzymes function through formation of multi-enzyme complexes, and that protein phosphorylation plays a crucial role in enzyme complex formation in cereals. SSIIa is one of the key isozymes of SS whose catalytic regulation remains largely unknown. Recent evidence suggests that SSIIa is post-translationally regulated by protein phosphorylation in amyloplasts of maize endosperm and is found in the plastid stroma and also associated with starch granules. Previous studies demonstrated that maize SSIIa can be phosphorylated by one or more protein kinase(s) in amyloplasts and that granule associated SSIIa is phosphorylated. In addition, protein phosphorylation affects electrophoretic mobility of SSIIa on native gels, suggestive of major conformation changes, and/or association with other proteins. The aim of the current study is to investigate the effect of phosphorylation on the catalytic activity of SSIIa (native and recombinant forms) from maize and to characterize the role of specific phosphorylation sites on SSIIa catalytic activity and protein complex formation. Putative protein kinase(s) and phosphatases involved in regulating the phosphorylation state of SSIIa will be characterized. The proposed study will provide new insights into our understanding of the complex signal transduction system regulating amylopectin biosynthesis in plants. This work is of strategic importance and has the potential to identify novel genes for crop improvement.

6.4. Polyamines are a potential source of 4-aminobutyrate (GABA): Characterization of apple and *Arabidopsis* aminoaldehyde dehydrogenases belonging to ALDH10 family

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4-Aminobutyrate (GABA) accumulates in apple fruit during controlled atmosphere storage. Polyamines (PAs) represent a potential source of GABA. The first step of PA catabolism involves copper-containing amine oxidases (EC 1.4.3.6) and flavoprotein polyamine oxidases (EC 1.5.3.11), resulting in the production of hydrogen peroxide and ω-aminoaldehydes such as 3-aminopropanal and 4-aminobutanal, which are substrates of $NAD(P)^+$-dependent aminoaldehyde dehydrogenases (EC 1.2.1.19). In this study, we chose two members from both *Arabidopsis* (*At*ALDH10A8 and *At*ALDH10A9) and apple (*Md*AMADH1 and *Md*AMADH2) as candidates for encoding 4-aminobutanal dehydrogenase activity, which would result in the production of GABA. The four genes were cloned and soluble forms of the encoded proteins produced using *Escherichia coli*. The pH optimum for recombinant *Md*AMADH1, *Md*AMADH2 and *At*ALDH10A9 was 9.8, whereas the optimum for *At*ALDH10A8 was pH 10.6. Maximal activity and catalytic efficiency were obtained with NAD^+ and 3-aminopropanal, followed by 4-aminobutanal; negligible activity was obtained with betaine aldehyde as substrate. The expression of *Md*AMADH1 was 2-3 times that for *Md*AMADH2 in apple fruit, leaves and flowers. Use of a transient co-expression system with *Arabidopsis* protoplasts and several organelle markers revealed that *Md*AMADH2 was peroxisomal and *Md*AMADH1 cytosolic, although both carry identical peroxisome targeting signal 1 and proximal region. *At*ALDH10A9 was also peroxisomal, but *At*ALDH10A8 was cytosolic, rather than plastidial as previously suggested. Thus, *Arabidopsis* and apple AMADHs may theoretically contribute to GABA production, but their final destination and the influence of stress on *Md*AMADH reconformation requires consideration.

6.5. The Ca^{2+}-dependent protein kinase RcCDPK2 *in vivo* phosphorylates the sucrose synthase isozyme RcSUS1 at serine-11 in developing castor oil seeds

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Sucrose synthase (SUS) catalyzes the UDP-dependent conversion of sucrose into UDP-glucose and fructose, and is becoming an important target for improving seed crops via metabolic engineering. However, the pathways and control of oilseed sugar unloading and metabolism are poorly understood. Recent research established: (i) the SUS isozyme RcSUS1 as the dominant sucrolytic enzyme in the triglyceride-rich endosperm of developing castor oil seeds (COS), (ii) that dynamic and high stoichiometric *in vivo* phosphorylation of RcSUS1 occurs at a conserved Ser-11 residue (this appears to protect RcSUS1from proteolysis), and (iii) the presence of Ca^{2+}-dependent RcSUS1 (Ser-11) kinase activity during COS development (Fedosejevs *et al.* 2014 J Biol Chem). Native RcSUS1 kinase was highly purified via FPLC and identified as RcCDPK2 by Orbitrap Fusion mass spectrometry. *RcCDPK2* cDNA was therefore isolated from COS and heterologously expressed in *E. coli*. Recombinant RcCDPK2 catalyzed rapid Ca^{2+}-dependent phosphorylation of dephospho-RcSUS1 at Ser-11. Ongoing research includes characterizing RcCDPK2's physical and kinetic/regulatory properties, assessing *RcCDPK2* expression in COS, imaging transiently-expressed *35S:RcCDPK2-GFP* in tobacco suspension cells, and comparing RcCDPK2 with RcCDPK1 which *in vivo* phosphorylates COS bacterial-type phosphoenolpyruvate carboxylase at Ser-451 (Hill *et al*. 2014 Biochem J). Although CDPKs are being widely studied owing to their diverse and important roles in plant cell biology and signalling, relatively few *in vivo* CDPK targets have been identified. Our RcCDPK1 and RcCDPK2 research illustrates how integrating classical, native enzyme biochemistry with cutting-edge tools of mass spectrometry, bioinformatics, and genomics can yield important insights into the *in vivo* function of specific CDPK isozymes.

6.6. Role of indoleamines in controlling apple and pear browning by inhibiting polyphenol oxidase activity

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Indoleamines, melatonin (N-acetyl-5-methoxytryptamine, Mel) and its biosynthetic precursor serotonin (5-hydroxytryptamine, Ser) mediate important physiological functions in plants including developmental processes and stress responses. There is limited information regarding the role of these indoleamines in post-harvest processing of fruits and vegetables. Enzymatic fruit browning deteriorates quality of fresh fruits and juices, contributing up to 50% of all losses in fruits and vegetable production. In this study, we investigated the role of Ser and Mel in controlling post-harvest browning in apple and pear. Treatment of cut fruit slices (apple and pear) as well as homogenate (apple) with 10 mM solutions of Ser and Mel was effective in reducing browning; least browning was measured with Ser treatment. Both indoleamines reduced the rate of browning by inhibiting the activity of fruit browning enzyme, polyphenol oxidase (PPO), in browning model solutions containing epicatechin (Epi) and caffeoyl tartrate (CT) as polyphenol substrates. Kinetic parameters suggest Ser as a putative non-competitive inhibitor of PPO activity. Ser and Mel treatment increased total phenolics content and antioxidant capacity of the apple homogenate. Expression of four (*MdPPO4, MdPPO6, MdPPO8 and MdPPO10*) out of the nine *MdPPOs* expressed in apple fruits were down-regulated in apple tissue treated with both the indoleamines, with an additional PPO gene (*MdPPO2*) down-regulated only in the Ser treatment. This is the first report divulging an important role of Ser and Mel acting as anti-browning agents by biochemically and transcriptional regulating PPO enzymes in pome fruits.

7.1. Plant early redox response to caterpillar herbivory

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In response to caterpillar herbivory, plants activate complex signaling networks, of which the phytohormone jasmonic acid (JA) plays a key role, leading to induced defense responses. However, some caterpillar species have evolved strategies to circumvent this induced resistance (IR). Caterpillar labial saliva (LS) activates antagonistic signaling pathway(s) leading to the suppression of the plant's IR. The mechanism underlying this may involve caterpillar LS-dependent activation of the antagonistic salicylic acid and/or ethylene pathways through the action of glucose oxidase, a major enzyme in the caterpillar LS that catalyzes the oxidation of glucose to produce hydrogen peroxide (H_2O_2). At high levels, H_2O_2 is detrimental to cellular components; therefore plants have mechanisms, such as the ascorbate/glutathione cycle, to tightly control levels of these highly reactive molecules. In response to caterpillar herbivory, we measured ascorbate and glutathione levels in two model plants, *Arabidopsis thaliana*, and *Medicago truncatula*. In *A. thaliana*, oxidized glutathione levels decreased in plants fed upon by normal caterpillars at 35 minutes. As well, total glutathione level decreased in response to herbivory by caterpillars with impaired labial salivary secretions. Thus, the caterpillar LS helped to maintain a reductive cellular environment. In contrast, in the legume M. truncatula, the ratio of oxidized-to-reduced ascorbate as well as oxidized glutathione and, therefore, the ratio of oxidized-to-reduced glutathione increased in plants fed upon by caterpillar with intact labial salivary secretions. Thus, in these plants, caterpillar LS leads to an induction of oxidative stress.

7.2. *Arabidopsis* triphosphate tunnel metalloenzyme2 (*AtTTM2*) is a negative regulator of the salicylic acid-mediated feedback amplification loop for defense responses

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The triphosphate tunnel metalloenzyme (TTM) superfamily represents a group of enzymes that are characterized by their ability to hydrolyze a range of tripolyphosphate substrates. *Arabidopsis* encodes three TTM genes, *AtTTM1*, *2* and *3*. Despite its annotation as adenylate cyclase, we found that AtTTM2 did not produce cyclic AMP, but rather exhibited pyrophosphatase activity. *AtTTM2* knockout (KO) mutant plants exhibit an enhanced hypersensitive response, elevated pathogen resistance against both virulent and avirulent pathogens, and elevated accumulation of salicylic acid (SA) upon infection. In addition, stronger systemic acquired resistance was also observed. These enhanced defense responses are dependent on SA, *PAD4*, and *NPR1*. Despite their enhanced pathogen resistance, *ttm2* plants did not display constitutively active defense responses, suggesting that *AtTTM2* is not a conventional negative regulator, but a negative regulator of the amplification of defense responses. The transcriptional suppression of *AtTTM2* by pathogen infection or treatment with SA and the SAR activator, BTH, further supports this notion. Such transcriptional regulation is conserved among *TTM2* orthologues in the crop plants, soybean and canola, suggesting that TTM2 is involved in immunity in a wide variety of plant species. This indicates the possible usage of *TTM2* KO mutants for agricultural application to generate pathogen resistant crop plants.

7.3. The arbuscular mycorrhizal symbiosis in pea: insights from a hypermycorrhizal mutant

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E151 (*Pssym15*) is an ethyl methanesulfonate-generated mutant of *Pisum sativum* L. cv. Sparkle. During investigations into the root symbioses of this mutant, it was discovered to be hypermycorrhizal; this is in stark contrast to its low-nodulating phenotype, especially when considering

that both mycorrhizal and rhizobial symbioses are known to share common developmental and genetic pathways. In this work we have further examined E151 hypermycorrhizal phenotype using histochemical staining techniques in order to determine if any metabolic perturbations arise from this atypical association. We were able to assess the functionality of the symbiosis by staining roots of 35 day-old Sparkle and E151 plants inoculated with *Rhizophagus irregulare* for the activity of alkaline phosphatase (ALP) and for the accumulation of polyphosphate compounds (PolyP). ALP for both lines was located in arbuscules, intraradicular hyphae and vesicles while PolyP accumulated only in vesicles. In a majority of Sparkle root segments, ALP activity was high and consistent throughout the intraradicular fungal hyphae and PolyP accumulation was minimal. In E151, however, ALP activity was often patchy and localized, with PolyP accumulation ranging from not present to more than in the colonized Sparkle root segments. Knowing that the ALP enzyme is involved in fungal carbohydrate metabolism and PolyP accumulation occurs when phosphorous transfer from the fungus to the plant is reduced, we conclude that the mycorrhizal functionality is altered in E151. A model to explain the observed alterations will be proposed, and implications for the differing symbiotic phenotypes addressed.

7.4. Natural plant volatiles: A sustainable pest control strategy

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Plant volatiles are important attractants or deterrents for insects in the selection of a plant for feeding and oviposition. Apocarotenoids are carotenoid-derived volatiles, such as β-ionone, that have demonstrated a repellent effect with some insects. Other apocarotenoids are also produced by the enzyme activity of carotenoid cleavage di-oxygenase on carotene, but their role in plant-insect interactions is not as well understood. In oviposition choice experiments, we evaluated the response of cabbage looper moth (*Trichoplusia ni*) to volatiles of transgenic *Arabidopsis* and Tomato (*Lycopersicon esculentum)* plants overexpressing *carotenoid cleavage di-oxygenase* (*CCD* and *NCED*) genes. Transgenic plants overexpressing one of *NCED3, CCD4* and *CCD1*genes exuded volatiles at relatively higher intensity when compared to the wild type control. The *NCED3* and *CCD4* transgenic *Arabidopsis* plants demonstrated significant attraction and increased oviposition of the cabbage looper moth compared to the wild type plants. In contrast, *CCD1* overexpression in *Arabidopsis* led to a decreased rate of oviposition while *CCD1* overexpression in Tomato favoured increased egg deposition. Analysis of the plant volatile profiles by GC-MS showed differences in the volatile spectra of the four types of transgenic plants including changes in the apocarotenoid profile and concentration. Our findings suggest that manipulating the carotenoid-based volatile profile of plants could provide a novel strategy contributing to existing integrated pest management to further reduce the reliance on undesirable chemical pesticides.

7.5. PLASMODESMATA-LOCATED PROTEIN over-expression negatively impacts the manifestation of systemic acquired resistance and the long-distance movement of DEFECTIVE IN INDUCED RESISTANCE1 in *Arabidopsis*

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Systemic acquired resistance (SAR) is a plant defense response that provides broad-spectrum immunity to distant uninfected leaves after an initial localized infection. The lipid transfer protein (LTP) DEFECTIVE IN INDUCED RESISTANCE1 (DIR1) is an essential component of SAR that moves from induced to distant leaves following a SAR-inducing local infection. To understand how DIR1 is transported to distant leaves during SAR, we analyzed DIR1 movement in transgenic *Arabidopsis* lines with reduced cell-to-cell movement caused by the overexpression of PLASMODESMATA-LOCATED PROTEIN1 (PDLP1) and PDLP5. These PDLP-overexpressing lines were defective in the manifestation of the SAR response, and DIR1 antibody signals were not observed in phloem sap enriched petiole exudates collected from distant leaves. Our data support the idea that cell-to-cell movement of DIR1 through plasmodesmata is important during long-distance SAR signalling in *Arabidopsis*.

7.6. Revealing the microbial diversity between the rhizospheres of six wetland plants

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To investigate the degree of species-specificity of plant - rhizosphere microflora associations, six wetland plant species (*Chamaedaphne calyculata, Eriophorum vaginatum, Eriophorum virginicum, Carex utriculata, Carex oligosperma* and *Glyceria canadensis*) were examined using pyrosequencing. Plants from three wetland sites near Sudbury, Ontario were transplanted into a constructed wetland, inoculated from soils from all the sites of collection, and grown for two growing seasons. Rhizosphere samples were then collected prior to senescence from three replicates of the plant species. Root rhizosphere samples and root fragments were used for total microbial community analysis by using 454-pyrosequencing and extracellular enzyme activity analysis. Any findings that suggest the microbial community displays specificity to plant species will expand our understanding of plant-microbe interactions. Gaining insight into wetland plant species specificity of microbes will aid in determining their importance and functional role in wetland plant ecological strategies. Preliminary data indicates specificity of some microbial taxa to certain plant species.

8.1. Knockdown of mitochondrial alternative oxidase induces the "stress state" of signalling molecule pools in *Nicotiana tabacum,* with implications for stomatal function

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The mitochondrial electron transport chain (ETC) includes an alternative oxidase (AOX) that may control the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS act as signalling intermediates in numerous plant processes, including stomatal movement. The role of AOX in controlling ROS and RNS concentrations under both steady-state and different stress conditions was evaluated using *Nicotiana tabacum* plants lacking AOX due to RNA interference. A potential functional implication of changes in ROS and RNS homeostasis was also evaluated by an examination of stomatal function. The leaves of non-stressed AOX knockdowns maintained concentrations of H_2O_2 and nitric oxide (NO) normally seen in wild-type plants only under stress conditions. Further, NO amounts in guard cells were much higher in knockdowns. These guard cells were altered in size and were less responsive to NO as a signal for stomatal closure. The results reveal a possible role for AOX in stomatal movement. We present a working model in which AOX respiration in the guard cells maintains NO homeostasis by preventing over-reduction of the ETC. This becomes particularly important during periods of stress when high concentrations of NO acting as a signal for stomatal closure may also be inhibiting cyt oxidase respiration.

8.2. Regulatory elements in the untranslated regions (UTRs) of ribosome-bound mRNAs of *Arabidopsis thaliana* involved in translational control of gene regulation under stress

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Gene regulation at the transcriptional and translational level leads to diversity in phenotypes and functional organisms. Regulatory DNA or RNA sequence motifs adjacent to the gene coding sequence act as binding sites for proteins that in turn enable or disable expression of the gene. Whereas the known DNA and RNA binding proteins range in the thousands, only a few motifs are known and have been experimentally verified. In this study, we used a novel bioinformatics strategy for identifying putative conserved regulatory sequence motifs in different groups of sequences. Our test sets are groups of untranslated regions (UTR) sequences from genes regulated at the translational level in *Arabidopsis thaliana* under normal and stressed conditions. The test group of sequences is divided into random sub-groups and subjected to three *de novo* motif finding algorithms (Seeder (Fauteux *et al*., 2008), Weeder (Pavesi *et al*., 2004) and MEME (Bailey *et al*., 2006)). Significant motifs detected by those algorithms will be experimentally tested to elucidate its role during translational process. In addition to identifying sequence motifs, using an *in silico* tool we have predicted microRNA target sites in the 3'UTRs of the regulated genes, as well as identified upstream open reading frames (uORFs) located in the 5'UTRs. Our bioinformatics strategy and the knowledge generated will contribute to understanding gene regulation during stress, and can be applied to disease and stress resistant plant development.

8.3. A possible role for the Arabidopsis HOTHEAD protein in plant stress response pathways

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This study focuses on HTH protein localization using a native promoter-driven HTH fluorescent fusion protein (HTH-FP). Based on a survey of plants at various developmental stages, we have determined that HTH expression appears to be developmentally regulated. For young seedlings, HTH-FP was expressed in the epidermis and in the seedling vasculature. No expression was detected in rosette leaves. In flowers, expression was observed in all floral organs. In individual ovules, expression was localized to the chalazal end of the embryo sac, and appeared to be restricted to a subset of cells in the female gametophyte. Most interestingly, at the subcellular level, HTH-FP protein was found to reside in endoplasmic reticulum (ER)-derived structures that resemble stress-associated ER bodies. HTH-FP localization in these specialized organelles suggests that one function of the HTH protein may be in responding to various types of plant stress. As genome instability is induced by stress, it may be that mutation at the *HTH* locus can lead to mis-regulation of stress responses and subsequently result in elevated genetic variability, as reported previously. Future work is aimed at verifying HTH ER-body localization using ER-targeted markers, as well as investigating the effect of various types of stresses on relative HTH-FP expression and protein localization.

8.4. *Ascophyllum nodosum* (L.) Le Jolis. extract enhances drought stress tolerance in tomato (*Solanum lycopersicum* L.)

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Plant growth and development is affected by a range of biotic and abiotic stresses such as; drought, salinity, frost and pathogens. Drought is one of the major causes for low productivity in agriculture. Seaweeds and their extracts have been used in agriculture to mitigate biotic and abiotic stresses. However, the effect of seaweed extract on plant under drought stress has not been investigated. In this study, we investigated the effect of the brown alga *Ascophyllum nodosum* (L.) Le Jolis. extract (ANE) to mitigate drought

stress in tomato. The application of ANE affected wilting, stomatal conductance, water potential and plant recovery after drought stress. A larger percentage (85%) of plant that received the extract treatment recovered from a severe drought stress as compared to control plants (30-40%). It was also evident ANE treatment resulted in higher stomatal conductance, high plant water potential and subsequently reduced wilting. Moreover, the transcript abundance of stress response genes were less in treated plants. Taken together, the results suggest that ANE had significant positive effect in protecting tomato plant against drought stress.

8.5. A novel calmodulin-binding site regulates the induction of programmed cell death by Arabidopsis cyclic nucleotide-gated ion channel 12.

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Cyclic nucleotide-gated channels (CNGCs) are non-selective cation channels found across eukaryotes and comprise a 20 member family in Arabidopsis. CNGC isoforms have been implicated in various plant signaling pathways, including immunity, however, little is known regarding CNGC structure-function or regulation, particularly in contrast to the well-studied mammalian CNGC family. It has been hypothesized that plant CNGCs, like mammalian isoforms, are regulated by the conserved Ca^{2+} sensor calmodulin (CaM), and most CNGCs are predicted to share a CaM-binding domain (CaMBD) in their cytosolic C-terminus, which overlaps with the cyclic nucleotide-binding domain (CNBD). However, CNGC12, a positive regulator of plant defense, shares low sequence conservation at this region. Our results show that CNGC12 possesses a novel domain organization featuring CaMBDs in both the cytosolic N- and C-termini of the channel. These CaMBDs do not overlap with the CNBD, and bind CaM with high but differing affinities, while one site may bind CaM independently of Ca^{2+}. To assess the physiological function of these CaMBD(s), heterologous expression of mutant *CNGC12* isoforms was used to examine the role of CaM-binding in the formation of hypersensitive response (HR)-like programmed cell death. We have found that one CaMBD is required to provide allosteric inhibition of CNGC12 function, while the *in planta* role(s) of the additional CaMBDs remain unclear. We will discuss the emerging evidence that, like mammalian CNGCs, plant CNGC isoforms are more divergent in their regulatory domains than initially concluded, and that CaM may regulate CNGC function in multiple ways.

8.6. Using ion-selective optrode sensors to characterize water chemistry in hydroponic systems and in extreme environments in the context of space exploration

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Part I – Advanced Life Support Systems
Human space exploration requires life support systems to provide food for astronauts and treat their wastes. Hydroponic production offers efficiently grown plants and participates in waste water treatment; however, sensors must be used to monitor and manage the flow of nutrients through the system. A prototype ion-selective optical sensor (optrode) system was developed to characterize the ionic composition of water samples. The Ion-Selective Optrode System (ISOS) was tested in closed-environment hydroponic chambers to monitor uptake of nutrients by plants [NO_3^-, Ca^{2+}, K^+, Na^+]. Results demonstrate that the ISOS is capable of selectively measuring concentration changes of individual ions with minimal interferences. This information can be used as feedback control input for nutrient dosing, which is required to achieve complete solution recycling – an absolute requirement for plant production systems in space craft.
Part II – Astrobiology
The ISOS was also field tested in an extreme, high-altitude location (Laguna Negra, Chile) as part of the Planetary Lake Lander project of the SETI Institute – a mission to develop adaptive science technologies to detect biological signatures on distant moons and planets. Successful operation of ISOS was demonstrated as calcium ions were detected in a turbid river outflow - this data was confirmed by in-lab HPLC analysis of samples returned from the site. Sodium ions from NaOH, added to adjust pH, were also detected. The ISOS field performance compared well with laboratory performance indicating that ion-selective optrode sensors are suitable for remote use in extreme environments.

9.1. Effects of elevated temperature on the spring phenology of Eastern white pine (*Pinus strobus* L.)

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In boreal forests, phenological events in evergreen conifers are sensitive to environmental cues such as temperature and photoperiod. With the predicted increase in global temperature, the timing of temperature cues will shift while the timing of photoperiod cues will remain unchanged. The aim of this study was to assess the effects of elevated temperature on the spring recovery of photosynthesis and timing of budburst of Eastern white pine (*Pinus strobus* L.) that have adapted to different temperature and photoperiod regimes. Seedlings from a northern, a local, and a southern provenance were planted together at Koffler Scientific Reserve, ON and heated (+1.5/3ºC; day/night) using infrared

heaters to simulate 2050 temperature predictions for southern Ontario. A positive relationship between temperature, bud development, and photosynthetic efficiency was observed, as well as an increase in chlorophyll that corresponded with a decrease in photoprotective carotenoid pigments, during the spring recovery period. In early spring, the southern provenance initiated bud development before the other provenances, but did not recover maximum quantum yield of photosystem II (F_v/F_m) and effective quantum yield (Φ_{II}) as quickly. In contrast, the northern provenance began bud development later than the other provenances, but recovered F_v/F_m and Φ_{II} more rapidly. In general, elevated temperature had minor effects on the spring recovery of all three provenances. Therefore, we conclude that slight warming will have no major effect on the spring recovery of Eastern white pine, but highlights the adaption of different genotypes to local environmental conditions.

9.2. Sugar insensitivity in a model *Arabidopsis thaliana* cell suspension culture

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Typically, when sugars accumulate to high levels in vegetative tissue, photosynthetic gene expression is repressed. When wild type *Arabidopsis* seedlings are grown in media containing 6% sucrose or higher, photosynthesis is almost completely repressed. Since the cells have an abundant supply of sugar available, they do not need to expend energy on metabolically expensive photosynthetic machinery to fix CO_2. However, we have identified a cell suspension culture of *Arabidopsis thaliana* that is insensitive to sugar. When grown in media containing 0-15% sucrose this cell culture has a dark green phenotype and expresses all the major photosynthetic proteins. Why are the cells expressing these photosynthetic proteins when they already have an ample supply of sugar available? A problem in sugar sensing or signaling pathways could explain this strange phenotype. These pathways were examined using RNA-Seq. The cell culture was grown in media containing 0%, 3%, 6%, and 9% sucrose. RNA-Seq was used to get global gene expression profiles. A few candidate genes were identified which could explain the sugar insensitive phenotype of the cell culture, *ABI4* the most promising among them.

9.3. Influence of senescence-associated pigment changes on leaf spectral properties in *Acer saccharum*

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Remote sensing techniques are increasingly used to monitor the effect of climate change on plant phenology and to improve estimates of ecosystem productivity. Numerous vegetation indices have been developed but often fail to adequately reflect the physiological changes occurring in autumn. This study aims to assess the ability of several vegetation indices to reflect leaf autumn physiology and the associated changes in pigment composition in sugar maple (*Acer saccharum*). We hypothesized that changes in pigment composition during autumn affect leaf optical properties and allow assessing fall phenology and photosynthetic efficiency remotely. For this purpose we studied chlorophyll fluorescence, pigment content and leaf spectral reflectance of green, yellow, orange and red leaves sampled in autumn in comparison with non-senescent summer leaves. A fast decrease of chlorophylls was followed by a sharp increase of anthocyanins, while carotenoids demonstrated a slower degradation rate, causing leaf color to change from green over yellow to red, along with a decline in the photosynthetic efficiency. The commonly used spectral reflectance indices Normalised Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI) only reflected the presence of chlorophylls, while the Photochemical Reflectance Index (PRI) did not distinguish between yellow and red leaves. We propose that indices combining red and green color detection improve the remote-sensing of gradual changes in autumn physiology, e.g. by using the Green-Red vegetation index (GRVI) or simultaneously assessing the Anthocyanin Reflectance Index (ARI). Similarly, indices for digital repeat photography represent autumn changes best if they include red and green color information, like Hue and GRVI.

9.4. Mitochondrial alternative oxidase maintains respiration and preserves photosynthetic capacity during moderate drought in *Nicotiana tabacum*

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The mitochondrial electron transport chain includes an alternative oxidase (AOX) that is hypothesized to aid photosynthetic metabolism, perhaps by acting as an additional electron sink for photo-generated reductant or by dampening the generation of reactive oxygen species. Gas exchange, chlorophyll fluorescence, photosystem I absorbance, and biochemical and protein analyses were used to compare respiration and photosynthesis of *Nicotiana tabacum* L. cv Petit Havana SR1 wild-type (WT) plants with that of transgenic AOX knockdown (RNA interference) lines, under both well-watered and moderate drought-stressed conditions. During drought, AOX knockdown lines displayed a lower rate of respiration in the light than WT. Further, CO_2 and light response curves indicated a non-stomatal limitation of photosynthesis in the knockdowns during drought, relative to WT. Compared to WT, the knockdowns under drought maintained photosystems I and II in a more reduced state, showed greater regulated non-photochemical energy quenching, and displayed a higher rate of cyclic electron transport. The origin of these differences may lie in chloroplast ATP synthase amount, which declined dramatically in the knockdowns during drought. We conclude that AOX is necessary to maintain mitochondrial respiration during moderate drought. In its absence, respiration rate slows and the lack of this electron sink feeds back on the

photosynthetic apparatus, resulting in a loss of chloroplast ATP synthase that then limits photosynthetic capacity. This interpretation is supported by on-going studies showing that the overexpression of AOX can be used as a means to stimulate respiration and improve the photosynthetic performance of tobacco plants subjected to severe drought.

9.5. *In vitro* propagation and genetic improvement of sugar maple (*Acer saccharum* Marsh.)

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Sugar maple (*Acer saccharum* Marshall) is an important tree of North America and the basis for $350 million maple syrup industry. The energy to concentrate sap accounts for about 40% of syrup production costs and has a significant carbon footprint. Ecological impacts and costs could be reduced while increasing yields by developing elite germplasm with higher sap sugar content (SSC). While SSC is under genetic control, selection and propagation of “sweet” trees has not been feasible due to difficulties in vegetative propagation and lack of information on genetic markers. Attempts of clonal propagation through tissue culture have been largely unsuccessful and the basis for this longstanding problem has remained unknown. However, in recent experiments we have identified that the critical factor limiting sugar maple response is light intensity. We are currently evaluating light parameters (intensity and wavelengths) to facilitate efficient *in vitro* multiplication. Further, Sugar maples are heterozygous and heterogeneous, hence seed propagation results in phenotypic variation. Selection of trees with desirable traits at the seedling stage could be feasible using high density genetic markers associated with SSC. We are currently generating SNPs through genotyping by sequencing to associate them with SSC using a large (>200), evenly spaced, seedling based population of trees of uniform age (~22 yrs) phenotyped over several years. The phenotyping indicates considerable variation in SSC (1.1-7.6%) and tree morphology. Development of an efficient propagation system and information on genetic markers, will fulfill the industry need and provide tools to enhance the genetic potential of this species.

9.6. Photoacclimation in *Chlorella vulgaris* UTEX 265 is both redox- and photoperiod-dependent

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Chlorella vulgaris was grown under either low (28/150 [°C / µmol photons m^{-2} sec^{-1}]) or high (28/2000) excitation pressure (EP) with 24 h, 18 h or 12 h photoperiods in a 24 h cycle. High EP acclimated *C. vulgaris* developed a yellow-green phenotype compared to the dark green phenotype of low EP cells characterized by low chlorophyll content, high chlorophyll a/b ratio with concomitantly reduced light-harvesting polypeptide abundance as well as low photosynthetic capacity and efficiency. We hypothesized that if EP was the sole regulator of phenotype as previously suggested, *C. vulgaris* should photoacclimate in response to the steady-state EP during the light period regardless of photoperiod. At 28/150, the green phenotype combined with expected photosynthetic characteristics were typical of acclimation to low EP regardless of photoperiod. Although cells grown under an 18 h photoperiod at 28/2000 developed a typical yellow-green phenotype comparable to growth under continuous high light, cells grown at 28/2000 but a 12 h photoperiod exhibited a dark green phenotype. *C. vulgaris* grown at 28/2000 under either a 12 h or 18 h photoperiod were structurally and photosynthetically comparable to cells acclimated to continuous low or high EP, respectively. The "uncoupling" of EP and phenotype at 28/2000 indicates that EP cannot be the sole regulator of photoacclimation in *C. vulgaris*. We conclude photoacclimation in *C. vulgaris* is dependent on the extent of EP during the light period and the duration of the photoperiod. A model that integrated photosynthetic redox regulation, photoperiod and phenotype will be discussed.

Poster Abstracts

P1. Effect of mercury chloride and other toxic substances on root water transport of maize seedlings

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The transient stimulatory effect of $HgCl_2$ on volume flow ascending from the excised seedling root of Zea mays has been investigated using a microflow sensor. When both stele and cortex were connected to the sensor the maximum flow induced by different poisons (250 µM $HgCl_2$, 0.1% acetic acid, 5 mM dinitrophenole) was 5 to 10 times higher than the original exudation rate. The transient flow phenomenon was not observed, when the flow sensor was connected to the stellar cylinder only. At the period of the transient flow stimulation, the cortical cells died off and the cortex became infiltrated. Necrosis proceeded from the outer to the inner cortex. At the end of the transient flow phenomenon cellular glucose had been nearly completely released. The observed axial flow through cortical intercellular flow channels might be explained by a transient gradient of osmotic pressure across the cell walls of the denatured epidermis resulting from solute release to the apoplast. This would require a sufficiently small cell wall pore size. Creation of osmotic flow through the epidermal and hypodermal walls proved with denatured roots that had been washed free from cellular solutes. When these roots were connected to the flow sensor and the osmotic potential of the medium was decreased by low-molecular-weight solutes, significant volumes fluxes through the root were induced. Functional aspects of the extremely small cell wall pore size in the outermost cortical cell layers of the maize root need further studies.

P2. Characterization of tomato (*Solanum lycopersicum*) phosphatidylinositol 3-kinase C2 domain

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Phosphatidylinositol 3-kinase (PI3K) is a ubiquitous enzyme that phosphorylates phosphatidylinositol (PI) which is a key step in signal transduction pathway, by enabling membrane translocation of the enzyme by the activity of a calcium-sensitive C2 domain. Genomic analyses have confirmed that only one copy of gene encoding PI3K (Class III) is present in plant genome as compared with mammalian systems which have all three classes. Complementary DNA encoding *Solanum lycopersicum* PI3K (*Sl*-PI3K) C2 domain was cloned into PET28b (+) vector and expressed in *E. coli* (Rosetta^{TM}2) as a chimeric protein along with a carboxy terminal His-Tag. Expressed protein was localized in inclusion bodies, and was purified by solubilization/reconstitution using Nickel-affinity column. PI3K C2 domain had a higher binding affinity toward all tested phosphoinositides, with the strongest binding toward phosphatidylinositol 3-phosphate and phosphatidylinositol 3,5-biphosphate. A much lower affinity towards PI was observed. Binding was stronger towards monophosphates of PI as compared bisphosphates. By contrast, PI3K C2 domain did not bind to phosphatidic acid (PA), phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Transient expression revealed that *Sl*-PI3K was localized on plasma membrane. It is suggested that PI3K C2 domain enables the binding of cytosolic PI3K enzyme to the membrane. The ability of PI3K C2 domain to bind to all phosphoinositides including PI suggests that PI3K may participate in ethylene signal transduction as an early event during cell senescence, upstream of phospholipase D action. A possible model for the action of plant phosphoinositides in plant senescence will be discussed.

P3. The efficacy of ozone and the advance oxidation process in reclaiming horticultural wastewater

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Pathogenic microbes and residual pesticides in greenhouse irrigation water can hinder crop production and have a large environmental impact when released back into the environment. This wastewater must therefore be treated sufficiently before being released; well treated water can even be reused by growers to further reduce water consumption for both environmental and economical reasons. Pesticides intended to be degraded by UV light applied to foliar regions of plants, which leach off into irrigation water or aerial drift from herbicides sprayed on weeds on the greenhouse floor can have detrimental effects even at low concentrations. It is not common for greenhouse growers to reuse their irrigation water and typical greenhouse sanitation systems include UV and chlorine based systems which are primarily intended to remove pathogenic microbes from reused irrigation water. These systems may not be as effective at removing Non-Organic Pollutants (NOCs) as ozone based technologies. A bioassay using the model organism *Lemna minor* was conducted by sampling water containing one of two common pesticides of different classes, the fungicide and plant growth regulator paclobutrazol and the herbicide glyphosate at intervals before and after treatment with UV, Ozone and the Advance Oxidation process (O3 + UV) and culturing *Lemna* in the sampled water. Effective protocols for removal of pollutants from irrigation water will allow crop production in locations otherwise unsuitable for cultivation due to water restrictions such as areas of low fresh water reserves (deserts and islands) and places with a finite amount of water such as in space.

P4. Evaluation of potato varieties for chipping and nutritional qualities

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In North America, potato chips constitute one of the two major processed potato products. Numerous factors affect chip processing, nutritional qualities and safety qualities such as color specific gravity, sugars, polyphenol levels and antioxidant potential as well as asparagine, acrylamide content, which are influenced by the genotype, environment during growth and storage. The present study evaluated thirteen elite lines including colored lines along with five promising varieties grown under various environments in several locations in Ontario and after storage at a commercial storage facility over two years. Beacon Chipper, W8603-1, Snowden, Waneta, W6822-3, W5955-1, Tundra and W8587-4 were identified as the best varieties for light chip color after various storage periods. Colored varieties such as F06058 and F06053 were identified as the healthiest varieties based on higher total phenolic contents and antioxidant activities in both potatoes and chips followed by the non-colored varieties Beacon Chipper, Waneta, Nicolette, W2438-3Y, and W8615-5. Only W8641-4 had the consistently lowest asparagine content during both growing seasons. No significant difference was noted between varieties in acrylamide content of chips.

P5. Effects of soil temperature on growth of species with different root turnover strategies

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Among perennial herbaceous wetland plants in Northern Ontario there are two distinct types of strategies with respect to root turnover: roots survive the winter, or entire root systems senesce in the fall. Species with fall-senescing roots have leaf and root traits generally associated with fast growth and short organ life span. Such a strategy is often associated with disturbed sites, but for the wetland plants in Northern Ontario such an association is not obvious, as many species with annually renewed roots form stable communities. We hypothesize that one environmental factor related to distribution of these species is soil temperature. We predict that plants with a necessity to produce new root systems each spring are more sensitive to cold soil temperatures in spring than species with long-lived root systems, and due to allocation of resources to new root production they show a slower shoot growth in spring. We investigated the sensitivity of these species to cool temperatures by comparing field soil temperatures at sites dominated by species with different root turnover strategies, and by experimentally testing growth response of these species to a cooling of the rooting zone. We investigated spring shoot growth of these species in the field and in a garden experiment. The results show that species with annually produced new roots avoid the coldest wetland sites and are more negatively influenced by cool soil temperatures than species with long-lived roots. Such species also are smaller in early growing season compared to their final size later in the summer.

P6. Irrigation management strategies for nursery trees based on plant water status measured with automated stem psychrometers

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Tree nurseries are among the heaviest users of irrigation water in the ornamental horticulture industry sector. Environmental concerns and the rising cost of water have prompted this industry to moderate its use of water but effective and efficient irrigation management strategies remain elusive. This study will attempt to correlate key environmental variables, such as vapour pressure deficit, with directly monitored plant water stress. Automated stem psychrometers were used to measure total plant water potential at thirty minute intervals over extended periods between irrigation or rainfall events. Concurrent measurements of ambient humidity (VPD) were correlated with plant water status (Ψ) measurements on eleven tree species in a southern Ontario nursery using either sprinklers or drip irrigation. It is proposed to use these plant-environment interactions to contribute to a model for irrigation management under a range of environmental conditions. Preliminary results indicated that the relationship between Ψ and VPD was predictable under irrigated and non-irrigated conditions. However, additional work is required to clarify the role of other factors such as stomatal function.

P7. Molecular and biochemical analysis of *chalcone reductase* genes in soybean

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Soybean (*Glycine max*) is one of the most important legume crops in Canada, generating $1 billion in exports. Though this number may be promising, soybean farmers lose about $50 million worth of yields annually due to root rot disease caused by *Phytophtora sojae*. Many strategies have been

developed to reduce the infection however, these methods are expensive and mostly ineffective. An alternative approach to this problem is to select a trait naturally found in soybean that can increase resistance. One such trait is the increase in the production of root isoflavonoids, more specifically glyceollins. These compounds act as antimicrobial agents and, they are synthesized by the isoflavonoid branch of the phenylpropanoid pathway. One of the key enzymes exclusively involved in glyceollin synthesis is chalcone reductase (CHR). The objective of this research is to identify the *GmCHR* gene family members, investigate their biochemical properties, and functionally characterize the root-specific *GmCHR*s. Using *in silico* analysis, 17 putative *GmCHR*s were identified in the soybean genome. Among these, 9 *GmCHRs* were selected for the study as they contain all active site residues, and are transcribed. All candidate GmCHRs localize in both the nucleus and cytoplasm and display tissue specific expression pattern. They are heterologously expressed for protein purification and enzymatic assay. RNAi constructs were generated for silencing four root-specific *GmCHR*s. The degree of silencing and the effects on isoflavonoid contents of the roots is being evaluated. Identification of root-specific *GmCHR*(s) will allow us to manipulate glyceollin content and eventually improve resistance against *P. sojae*.

P8. Role of melatonin and serotonin in alleviating salt stress in *Arabidopsis thaliana*

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Salinity is a major environmental stress in agriculture with significant detrimental effects on plant productivity. The development of strategies to enhance salinity stress tolerance in plants is essential to ensure crop production in saline environments. Melatonin (Mel) and serotonin (Ser) accumulate in response to environmental stresses and are presumed to play protective roles, and to improve growth of tissues during recovery. In this study, the effects of Mel and Ser were investigated in *Arabidopsis thaliana* under salt stress. Mel and Ser treated plants had significantly higher fresh weight, primary root length, and shoot height in the MS medium containing salt (25 and 50mM) compared to the plants grown in their absence. The effects of Mel and Ser treatments were further investigated on the expression of ABA responsive and reactive oxygen species (ROS)-related antioxidant genes. Mel (30 µM) pre-treatment for 24 hrs followed by 50 mM salt treatment for 24 hrs was effective in up-regulating three ABA responsive genes, *ABI3, ABI5* and *RD29B* compared to the non-Mel pre-treated control plants. Both Mel and Ser pre-treatment significantly up-regulated the *ZAT10* and *ZAT12* genes involved in antioxidant defense and improvement of Arabidopsis salt tolerance. These results indicate that Mel facilitates salt tolerance by up-regulating some of the ABA responsive genes as well as controlling the salt-induced ROS overproduction, whereas Ser is involved in controlling the salt-induced ROS overproduction and contributes to the increased growth of plants.

P9. *Lotus japonicus STYLISH* gene family and its role during nitrogen fixing symbiosis

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Lotus japonicus is a model leguminous plant which, like most legumes, forms root nodules that host nitrogen-fixing rhizobial bacteria. Nodule development is initiated with a molecular cross-talk between a specific strain of *Rhizobium* and a compatible legume partner to establish an effective symbiotic relationship without evoking plant defenses. We have hypothesised that activation of at least some of the *STYLISH* (*STY*) genes is required for nodule formation, presumably to regulate auxin biosynthesis. In order to test this hypothesis, I have initiated a series of experiments aimed at the impairment of STY protein-dependent signalling *in planta*, which should allow for the evaluation of their role during nodule formation. I have characterized the primary structure of one of the *STY* genes, namely *STY3*, and determined its full length mRNA sequence. As *STY* gene family members are known to act redundantly, a dominant repressor construct was developed by making a C-terminal translational fusion between the entire coding region of the *LjSTY3* cDNA and a DNA fragment encoding a 12 amino acid long ERF-associated amphiphilic repression domain, called the SRDX domain. Both the *L. japonicus* cognate *LjSTY3* promoter and the ubiquitin promoter are being used to guide *in planta* expression of this chimeric repressor. This is predicted to act as a dominant suppressor of STY protein targets, hence causing impairment in downstream signalling events. Whether these genes are indeed required for nodule formation should be determined by this approach.

P10. Measurement of neutral detergent fibre, acid detergent fibre and acid detergent lignin in corn stalk fibres by Fourier-transform infrared spectroscopy

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This study was initiated to investigate the potential of using partial least squares (PLS) and Fourier-transform infrared (FTIR) spectroscopy to predict neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) parameters in corn stalk fibres as alternative procedure to wet chemistry. Forty recombinant inbred lines (RILs) from a CG62 x CO387 cross were grown in replicated plots (3) in four environments in Ontario. The dried corn stalks were ground in Wiley mill using 2 mm sieve. NDF, ADF and ADL were measured in 48 corn stalk fibre samples by ANKOM sequential analysis to develop the PLS predictive model. Infrared spectra were collected from 252 dried ground samples (in duplicate) by attenuated total reflectance (ATR) from 400 to 4000 cm^{-1} using a FTIR spectrometer. All spectra were averaged over 64 scans at a resolution of 4 cm^{-1}, derivatized to the first Savitsky-Golay derivative (15 units of filter width and polynomial order of 2), normalized

and mean centre scaled. A PLS statistical procedure was used to predict dependent variables from a set of independent variables. The predicted NDF, ADF and ADL values were positively and highly significantly correlated with the measured values (R^2 of NDF, ADF and ADL 0.92, 0.92 and 0.87, respectively). The corn stalk RILs showed significant variations for NDF, ADF and ADL contents in their fibres. This study demonstrated that FTIR spectroscopy as a cost effective and high through-put alternative to wet chemistry for compositional analysis in a large set of agricultural feed stocks.

P11. Physiological disorders and oxidative stress metabolism in stored pear fruit

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1-Methylcyclopropene (1-MCP, ethylene antagonist) application and controlled atmosphere (CA) storage are used to delay ripening-related senescence in fleshy fruit. CA treatment of pears typically consists of low temperature in combination with low O_2 and elevated CO_2 partial pressure. A drawback of CA, specifically prolonged exposure to low O_2, is the tendency to promote storage-related disorders. Preliminary evidence indicates that fruit from the newly developed cultivar 'Harovin Sundown' are susceptible internal breakdown and senescent scald, and that optimization of the CA regime is required. Furthermore, limited information is available on early biochemical mechanisms underlying disorder development in pears, but the involvement of oxidative stress metabolites seems likely. In this study, we investigated the effect of 1-MCP, CA and low O_2 on disorder development in 'Harovin Sundown' pear fruit (stored for up to 180 d) and the change in ascorbate/dehydroascorbate and glutathione/glutathione disulphide balance, as well as γ-amino-butyrate, which were quantified by enzyme-coupled spectrophotometric and HPLC assays, respectively. Senescent scald and internal breakdown were apparent in fruit chilled under ambient air; the latter disorder was also apparent with CA in non 1-MCP fruit. Disorders were effectively minimized by the combination of 1-MCP and 2.5 kPa O_2. Canonical powered partial least squares analysis revealed that senescent scald and internal breakdown were positively correlated with GABA accumulation and negatively with ascorbate and glutathione redox balance. This project may provide diagnostic information on storage disorders of pears.

P12. Identification of the PP2C HAI1 as an interactor of ABA2, an ABA biosynthetic enzyme

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Abscisic acid (ABA) is a plant hormone that plays a regulatory role in the water stress response, as well as the maintenance of seed dormancy and the mitigation of other abiotic stresses. ABA2 is an ABA biosynthetic enzyme, which converts xanthoxin to abscisic aldehyde. Its expression is not coordinated with the expression of other ABA biosynthesis genes, as well as with stress. Perhaps this points to a unique function of ABA2, one that lies beyond the realm of ABA biosynthesis and requires its expression to remain constant. In order to elucidate potentially novel roles of ABA2, we analyzed interaction partners of this enzyme by conducting yeast-two hybrid (Y2H) assays using a full-length cDNA collection for ABA inducible genes. Using this assay, we found that ABA2 interacts with itself, which is consistent with the finding that ABA2 functions as a multimeric complex. HAI1, a Protein Phosphatase Type 2 C (PP2C), was also found to interact with ABA2, in addition to other members of this family, including ABA-hypersensitive germination 3 (AHG3) and hypersensitive to ABA1 (HAB1). These proteins are members of the core ABA signaling complex and act as negative regulators of ABA-induced gene expression. We are currently examining if ABA2 and PP2Cs interact *in planta* by BiFC. We will discuss the possible interaction between ABA biosynthesis and signaling at a protein level.

P13. Fungal endophytes that promote plant growth on saline and dry soil

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Drought and soil salinization are critical abiotic stressors for plant growth that affect crop yield through catastrophic weather events and prolonged irrigation practices. Some fungi grow symbiotically inside plants as endophytes, and specifically. Class 2 fungal endophytes include strains that may promote plant growth and survival under abiotic stress like salt or dry soil. Strains of Class 2 endophytes isolated from highly saline sites in Saskatchewan were used to colonize tomato seeds. Seedlings were grown in double-decker Magenta boxes, so that water quantity and salinity could be controlled precisely to assess endophyte-related plant performance under salt and drought stress. Endophyte-colonized plants had higher root (20-60 %) and shoot biomass (10-20 %) in no-salt control or salt stress (300 mM or 500 mM NaCl) treatments than non-colonized plants, indicating a fitness benefit due to endophyte colonization. In another experiment, plants were assessed for drought tolerance by exposure to 10 days without water.

Endophyte-colonized plants had 30-40 % increased root and 10-20 % increased shoot biomass in comparison to control plants, consistent with 10-25 % better water use efficiency due to colonization. Class 2 endophytes isolated from plants naturally growing on saline soils have high potential to improve agriculture on dry or saline soil.

P14. Environmental parameters influencing clubroot incidence and severity on canola

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Canola (*Brassica napus* L.) is a major crop in Canada, with an economic value of over $19 billion per year. *Plasmodiophora brassicae* Woronin, the causative agent of clubroot, can cause substantial decreases in yield of susceptible crucifer species. The objective of this study was to assess the effects of temperature and soil moisture on the incidence and severity of clubroot by seeding canola at multiple dates throughout the growing season. Canola was seeded in soils naturally infested with pathotype 6 of *P. brassicae* approximately every 2 weeks in 2013 and 2014, and 50 plants per experimental unit were assessed at 2-week intervals starting 4 weeks after seeding. Clubroot symptoms at 6 weeks after seeding were correlated with certain weather events and environmental conditions. Soil moisture at 5–15 days after seeding (r = 0.89) and rainfall at 3 weeks before harvest (r = 0.74) were correlated with clubroot severity. Air temperature during the first 2 weeks after seeding was correlated with clubroot incidence (r = 0.72). These data will be used to model clubroot development on canola based on temperature, rainfall and soil moisture.

P15. The SUGAR-DEPENDENT1 lipase associates with the peroxisomal surface and interacts with PEROXISOMAL ABC-TRANSPORTER1

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Triacylglycerol lipases (TGLs) are interfacial enzymes that associate with the surface of cytosolic lipid droplets (LDs) in order to access their substrates. One of the major TGLs responsible for initializing the breakdown of storage oils in germinated *Arabidopsis* seeds is SUGAR-DEPENDENT1 (SDP1). Previous sub-cellular fractionation experiments using the *sdp1* mutant suggested that SDP1 activity is associated with LDs. Here, however, we show that stable expression of a C-terminal green fluorescent protein (GFP)-tagged SDP1 fusion protein driven by the *SDP1* promoter is overwhelmingly associated with peroxisomes and not LDs in *Arabidopsis* seedlings, and can also functionally complement the *sdp1* mutant. Peroxisomal localization of both N- and C-terminal GFP-tagged SDP1 is also observed in transient expression experiments using tobacco suspension cells and leaves, even when induced to accumulate LDs by supplying exogenous fatty acids and upregulating lipid biosynthesis by co-expressing DIACYLGLYCEROL ACYLTRANSFERASE1, respectively. Furthermore, isosurface imaging and differential detergent-permeabilization assays indicate that GFP-SDP1 localizes to the outer surface of the peroxisomal boundary membrane. We also show, using yeast two-hybrid assays, that SDP1 interacts with the cytosolic-facing C-terminal portion of PEROXISOMAL ABC-TRANSPORTER1, a peroxisomal membrane protein responsible for transporting a variety of substrates into peroxisomes for β-oxidation including fatty acids derived from the lipolysis of stored oils in LDs. Given that LDs in germinating seeds are frequently in close physical proximity with peroxisomes, the unique localization of SDP1 and its interaction with PXA1 appears to reflect a novel means by which SDP1, compared to other characterised TGLs, participates in TAG breakdown in plants.

P16. *Lonicera japonica* and *Catharanthus roseus* produce secologanin via a common pathway

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Secologanin, an iridoid glucoside, is a terpene-derived precursor to the monoterpenoid indole alkaloids (MIAs) produced in *Catharanthus roseus*. Iridoids are produced in many species of plants, including those that do not produce MIAs, such as *Lonicera japonica*. Feeding experiments and biochemical studies have raised questions about the order of reactions involved in secologanin biosynthesis in different plant species. Substrate specificity studies with recombinant *C. roseus* loganic acid *O*-methyltransferase (CrLAMT) suggest that hydroxylation precedes methylation and is the penultimate step in secologanin biosynthesis, while feeding experiments with cell suspension cultures of *L. japonica* suggest that hydroxylation could take place after methylation. This study shows that recombinant *L. japonica* LAMT (LjLAMT) has extremely similar properties to those of CrLAMT. The pH optimum was determined to be between pH 7.0 and 9.0, while the K_M and V_{max} values with loganic acid were calculated to be 0.89 ± 0.14 mM and 0.0397 ± 0.003 pkat/mg protein, respectively. In addition, the recombinant LjLAMT did not methylate 7-deoxyloganic acid, thus exhibiting the same narrow substrate specificity seen with recombinant CrLAMT and indicating that these two unrelated plant species produce secologanin using the same biosynthetic pathway.

P17. Inhibiting phenylpropanoid biosynthesis facilitates protoplast isolation and plant regeneration in American elm (*Ulmus americana)*

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The use of protoplast fusion to develop Dutch elm disease resistant elm hybrids (ie.*Ulmus americana* x *U. parvifolia*) has been proposed as early as 1980. However, despite several attempts by various research groups developing a protoplast fusion system has been unsuccessful. Previous attempts have failed primarily due to difficulties in cell wall digestion and isolating *U. americana* protoplasts, presumably due to cell wall bound phenylpropanoids interfering with enzyme activity. Culturing American elm tissue on 100 µM 2-aminoindan-2-phosphonic acid, an inhibitor of the phenylpropanoid pathway reduced phenylpropanoid accumulation and facilitated efficient protoplast isolation with a yield of approximately 2×10^6 protoplasts/ml packed cell volume. While the isolated protoplasts failed to survive in liquid or alginate bead culture systems, they initiated cell division and continued to divide when embedded in a two phase low melting point agarose bead system at a cell density. Protoplast-derived callus proliferated and differentiated into shoot buds in response to 10 or 20 µM thidiazuron. Differentiated buds elongated and continued to proliferate on elm shoot medium supplemented with 3.0 µM GA_3. The protoplast-derived shoots rooted and acclimatized to greenhouse conditions and continued to grow. This system provides the first protoplast-to-plant regeneration system for American elm and provides a framework for the development of protoplast fusion or genome editing technologies.

P18. Repression of lateral organ boundary genes by PENNYWISE and POUND-FOOLISH is essential for meristem maintenance and flowering in *Arabidopsis thaliana*

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The switch to flowering is a major developmental decision in plants. The timing of this transition is coordinated by a network of signals acting on the shoot apical meristem (SAM). Chemical and structural changes in the meristem, termed floral evocation, results in new patterns of aerial development: leaf development is repressed, internodes are elongated, and axillary meristems proliferate to form flowers. This response depends on the activities of two paralogous BELL-like homeodomain proteins: PENNYWISE (PNY) and POUNDFOOLISH (PNF) expressed in the meristem. Loss-of-function *pny pnf* mutants have defects in meristem maintenance that block flowering but allow the continued production of leaves. We show here that *pny pnf* defects are caused by misexpression of lateral organ boundary genes *BLADE-ON-PETIOLE1/2* (*BOP1/2*) and *KNOTTED*-like from *ARABIDOPSIS THALIANA6* (*KNAT6*) together with *ARABIDOPSIS THALIANA HOMEOBOX GENE1* (*ATH1*) whose products function in a linear pathway. Inactivation of genes in this module fully rescues *pny pnf* defects including meristem maintenance, internode elongation, and flowering. This is associated with the restoration *FD*, *LEAFY*, and *APETALA1* transcript accumulation whose products promote flowering. We provide evidence that *BOP1/2* acting through *KNAT6/ATH1* promote accumulation of jasmonic acid which counteracts the growth and floral-promoting activities of gibberellin. These data reveal a mechanism by which repression of lateral organ boundary genes by *PNY-PNF* is essential for flowering.

P19. NLP8 is required for nitrate to promote seed germination in *Arabidopsis thaliana*

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Nitrate is the primary nitrogen source for most plants, yet it can also act as a signal required for numerous aspects of plant developmental processes, like seed germination. Here, we show that Arabidopsis NIN-LIKE PROTEIN 8 (NLP8) is required for nitrate to promote seed germination. Loss-of-function mutants of NLP8 display significantly lower germination rates when compared with Col-0 in the presence of nitrate. RNA-sequencing carried out on *nlp8-2* mutants in the presence of nitrate, demonstrated a dramatic alteration in expression for most of the nitrate-regulated genes. This suggests that NLP8 acts as a central regulator of nitrate signaling during seed germination. Furthermore, NLP8 directly binds to the promoter of the major ABA catabolic gene, *CYP707A2*, and upregulates its expression to degrade ABA in seed germination. In *nlp8-2* mutants, hormone quantification showed an impairment of the nitrate-induced decrease of ABA in germination. Genetic evidence places the ABA biosynthetic enzyme ABA2, epistatic to NLP8. Finally, we have found that NLP8 is regulated by nitrate post-transcriptionally through its N-terminal region.

P20. A jasmonate signaling suppressor from peach mediates the transition from outcrossing to self-pollination

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Variations in floral display represent one of the biggest features associated with the transition from allogamy to autogamy in angiosperms. The promotion of autogamy under stress conditions suggests the involvement of indigenous pathways with a dual role in flower development and stress response. Jasmonic acid (JA) pathway was thought to be a good candidate to play such role because of its involvement in many aspects of plant responses to different environmental and developmental cues. In the present study we used peach (*Prunus persica* L.) varieties with showy and non-showy flowers in order to investigate the role of JA in floral display. Our results show that PpJAZ1, a member of jasmonic acid (JA) signaling pathway in peach, is involved in the regulation of petal expansion during anthesis and promotion of self-pollination. *PpJAZ1* transcript levels were higher in petals of the non-showy flowers than that of showy flowers at anthesis. The overexpression of *PpJAZ1* in tobacco (*Nicotiana tabacum* L.) converted the showy, chasmogamous tobacco flowers into non-showy, cleistogamous flowers. Stability of PpJAZ1 was further confirmed *in vivo* using PpJAZ1-GFP chimeric protein. Despite its inhibitory effects on JA-dependent functions in leaves and roots, PpJAZ1 had less negative effect on the fertility of transgenic plants, indicating that PpJAZ1 regulates the spatial localization of JA signaling in different plant organs. Our results together reveal that under stress conditions, e.g. herbivore attacks, such stable forms of JAZ proteins might alter JA signaling in different organs to promote autogamy, as a reproductive assurance mechanism.

P21. The role of ethylene action on *CYP707A2* in seed germination

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The phytohormone abscisic acid (ABA) acts as a negative regulator of germination, and must be degraded upon imbibition of the seed. *CYP707A2* is a member of the cytochrome P450 *CYP707A* family of ABA 8'-hydroxylases, and is rapidly upregulated in imbibed seeds acting as the primary ABA degradation enzyme during seed germination. To examine the role of ethylene in the regulation of *CYP707A2*, we used qPCR to analyze its expression in the seed for ethylene biosynthetic and signaling mutants *acs124567911*, *Etr1-1*, and *ein3-1*. We found that *CYP707A2* is downregulated in 3h imbibed *acs124567911* seeds, and may be affected in *Etr1-1* seeds. In accordance with this, ABA hormone measurements of *ein2-44* showed that the ABA content was higher when compared to Col-0 at 6h post-imbibition. A time-course analysis of *CYP707A2* expression in Col-0 in the presence of 10μM 1-Aminocyclopropane-1-carboxylic acid (ACC), demonstrated no appreciable effect on *CYP707A2* expression. This pattern of expression was consistent in ethylene signaling mutants, but showed a stimulatory effect on *CYP707A2* expression in the ethylene biosynthetic mutant *acs124567911*. To visualize the CYP707A2 expression pattern and to quantify its expression in response to ACC*, CYP707A2pro::GUS* and *CYP707A2pro::CYP707A2-GUS* lines were constructed. GUS quantification and staining showed that the application of ACC did not significantly alter GUS expression. An understanding of how *CYP707A2* is upregulated will contribute to our knowledge of how the imbibed seed initiates germination programs that stimulate or inhibit germination progression.

P22. Differential effects of mycorrhizal inoculation on seedling growth and survival of two *Cuscuta* species with contrasting ecology

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Seedling longevity in the non-parasitic phase of *Cuscuta* life-cycle is a crucial characteristic to ensure a successful plant population establishment. To survive, *Cuscuta* seedling must attach to the host plant and parasitize it; if not, the seedling can only stay alive for a short period of time. Because the seedlings have a transient root-like organ, considered not a 'true' functional root by most studies, and because previous reports of seedling survival times among *Cuscuta* species varied widely, the effects of potential factors, including mycorrhiza-formation, on the development and survival of *Cuscuta* seedlings were studied. Two species with a contrasting ecology, *Cuscuta gronovii* and *C. campestris*, were used. Mycorrhizal fungal colonization and its potential effects on the growth and survival of the seedlings were assessed using Promix® soil inoculated with *Rhizophagus intraradices* and chicory Root Organ Culture (ROC) inoculated with *R. irregulare*. Furthermore, the morphology, structure, and absorptive ability of the root-like organ were examined. We found that the root of *Cuscuta* seedling functions as a typical root despite its transitory nature. The roots of both species are capable of absorption and interact with mycorrhizal fungi but differently. Higher fungal colonization caused a better growth performance and a higher survival in *C. gronovii* than in *C. campestris*. This may correlate with the different ecology of the two species and suggest that fungal specificity plays an important role in the evolution and biogeography of dodders.

P23. Measuring cell wall mechanical properties on single plant cells using microfluidics

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Biomechanical and mathematical modeling of plant developmental processes requires quantitative information about the structural and mechanical properties of living cells, tissues and cellular components. A crucial mechanical property of plant cells is the mechanical stiffness or Young's modulus of its cell wall. Measuring this property in situ at single cell wall level is technically challenging, in particular when the cell is alive and only possesses a primary wall. We used microfluidics to quantify this stiffness of the primary cell wall for a cylindrically growing, single cell - the pollen tube. Pollen grains are trapped and positioned within a microfluidic chip, the growing pollen tube guided into a microchannel and then exposed to a bending force created through directed fluid loading. The flexural rigidity of the pollen tube and the Young's modulus of the cell wall are estimated through finite element modeling of the observed fluid-structure interaction. For one of our model species, the *Camellia* pollen tube, we determined an average value for the Young's modulus of 350 MPa - similar to teflon. This value is in agreement with the result of an independent method based on cellular shrinkage after plasmolysis and with the mechanical properties of in vitro reconstituted cellulose-callose material.

P24. Evaluating the role of mitochondrial alternative oxidase during nitrogen-limited growth and growth on ammonium

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Effective carbon and energy metabolism requires fine-tuned turnover of the adenylate and pyridine nucleotide pools. Abiotic stresses can disrupt this metabolism resulting in the increased expression of a mitochondrial electron transport chain component termed alternative oxidase (AOX). AOX provides a means to uncouple electron transfer from ATP production in the mitochondrion and consume excess reducing power. In this way, AOX may play an important role in promoting energy and redox balance while preventing oxidative/nitrosative damage, but its significance in these regards is not well understood. We are investigating the role of AOX during two "nitrogen stress" scenarios. Both N-limited growth and growth on ammonium may disrupt metabolism and are reported to induce AOX abundance in different species. *Nicotiana tabacum* are being grown hydroponically at three different N concentrations (100 µm, 1 mM, 5 mM) being supplied as either $Ca(NO_3^-)_2$ or $(NH_4^+)_2SO_4^-$. An array of physiological, biochemical and molecular analyses are being used to evaluate growth, metabolism (respiration, photosynthesis), gene expression, and the function of several key mitochondrial matrix proteins (eg. manganese superoxide dismutase, glycine decarboxylase, aconitase) potentially prone to oxidative/nitrosative damage. To date we have shown leaf AOX gene expression increases with increasing nitrogen limitation and is higher during growth on ammonium than nitrate. Comparison of wild-type plants with transgenic AOX knockdown and overexpression lines is in progress. These comparisons will critically evaluate the role of AOX during N-limited *versus* N-sufficient growth conditions, as well as during growth on nitrate *versus* ammonium, two N sources with strongly contrasting reductant requirements.

P25. An ancient African crop hosts a novel bacterial endophyte that suppresses modern crop diseases

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Ancient crops may serve as novel sources of microbes to benefit modern agriculture. Finger millet is an ancient African crop, domesticated 7000 years ago in Ethiopia and described by local farmers as being resistant to many pathogens. We hypothesized that finger millet may host beneficial microbes (endophytes) that may contribute to its ability to resistant pathogens. Here we report the first ever discovery of endophytes from finger millet. We describe a novel *Enterobacter* species (strain M6) based on total genome sequencing. *In vitro* experiments demonstrate that strain M6 could suppress the toxigenic fungal pathogen, *Fusarium graminearum*. Replicated greenhouse experiments demonstrated that M6 could potentially suppress *Fusarium* disease in modern corn and wheat. GFP-tagged M6 was visualized by confocal microscopy inside the plant tissues which confirmed that M6 is a true endophyte. I*n vitro* microscopic interaction demonstrated that M6 causes cleavage of fungal hypha at septa. To discover the microbial genes responsible for the anti-fungal trait, Tn5 mutagenesis was conducted. Screening of 4800 Tn5 insertion events led to the discovery of 10 candidate genes, including genes encoding a surface lipoprotein, Phenazine biosynthesis enzyme, a phenolic oxidase, Fusaric acid resistance protein, a transcriptional regulator, and a translational regulator. The importance of the Tn5 knockouts were confirmed in replicated greenhouse trials. This endophyte showed a potential for practical application to suppress GER in corn, FHB in wheat. Since *Fusarium* pathogen and millet plant appear to be ancient in Africa, this may represent an interesting example of 3-way co-evolution between endophyte, pathogen and host.

P26. Do plant growth-promoting rhizobacteria reduce production of ethylene in *Arabidopsis* under cadmium stress?

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Stressed plants produce ethylene, which leads to stunted growth and senescence. *Pseudomonas putida*, a plant

growth-promoting rhizobacterium (PGPR) promotes plant growth under stress conditions. The proposed mechanism for this involves metabolizing the precursor to ethylene, 1-amino-cyclopropane-1carboxylate (ACC), using the bacterial enzyme ACC deaminase (AcdS). *Pseudomonas putida* UW4/*AcdS*$^{+}$ (UW4) and a mutant, *P. putida* UW4/*AcdS*$^{-}$ (ACC^{-}) that lacks *AcdS*, were used to test the hypothesis that, under cadmium stress, *Arabidopsis. thaliana* (Col-0) inoculated with UW4 will have reduced ethylene and larger size than both uninoculated control plants and those inoculated with ACC^{-}. Confocal microscopy combined with BacLight ™ Live/Dead® staining confirmed that both bacterial strains grew on the plant roots. Furthermore, the expression of *AsdS* in UW4 and lack of *AcdS* expression in ACC^{-} was confirmed by PCR. Moreover, PCR analysis revealed that *A. thaliana* contains an *AcdS*-like gene. Plants inoculated with UW4 were larger and produced less ethylene than did plants inoculated with ACC, when grown in the presence of cadmium. However, uninoculated plants had comparable growth and ethylene levels to plants inoculated with UW4 when grown on a cadmium-containing medium. This investigation supports the hypothesis that UW4 reduces plant ethylene and increases plant growth under cadmium-stress conditions, but it is unclear why uninoculated plants did not produce cadmium-induced ethylene and maintained high growth. Therefore, further work is needed to determine the mechanisms responsible for reduced ethylene and increased plant growth seen in uninoculated plants. This will include sequencing the *AcdS*-like PCR product found in *A. thaliana*.

P27. In vivo interactions between starch branching enzymes require phosphorylation at specific serine residues

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Protein phosphorylation is a key method of regulating enzyme activity. In the starch biosynthetic pathway of maize (*Zea mays*), protein phosphorylation is necessary for activation of the maize starch branching enzyme IIb (SBEIIb). SBEIIb also forms homodimers and multimeric complexes with starch synthases (SS). Phosphorylation was recently shown to occur at Serine residues Ser649, Ser286, and Ser 297. The present study analyzed SBEIIb mutagenized at Ser649 and transformed into *Nicotiana benthamiana* leaves. Tobacco leaves were also transformed with Zm SBEI and *Arabidopsis thaliana* SSII. Bimolecular fluorescence complementation showed in vivo that phosphorylation at the Ser649 residue is critical for association of SBEIIb into multimeric complexes with SBEI. SBEIIb homodimer formation was observed even in mutagenized SBEIIb, suggesting that phosphorylation at Ser649 is not essential to SBEIIb homodimer formation. ZmSBEIIb and ZmSBEI could interact with AtSSII, indicating a degree of structural conservation with AtSBE2.2, particularly in phosphorylation and protein-protein interaction sites. The data reinforces the hypothesis that phosphorylation of SBEIIb at Ser649 is critical for its activity in the maize starch biosynthetic pathway.

P28. Investigation into the role of the Arabidopsis calmodulin-like protein, CML39, in hormonal regulation of early seedling development and fruit formation.

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Calcium (Ca^{2+}) is an important and versatile second messenger in organisms. Cytosolic Ca^{2+}-oscillations are evoked by environmental stimuli such as biotic or abiotic stresses and are subsequently detected by Ca^{2+}-binding proteins, termed Ca^{2+} sensors, which help coordinate cellular responses. Calmodulin (CaM) is an evolutionarily conserved eukaryotic Ca^{2+} sensor involved in many signal transduction pathways. Interestingly, plants possess expanded families of unique Ca^{2+}sensors related to CaM known as calmodulin-like (CML) proteins. Several CMLs have been implicated in developmental and stress-response signalling but the roles of most CMLs remain unknown. We recently reported the importance of Arabidopsis CML39 in early seedling establishment (Bender et al 2013, Plant J: 76:634). *CML39* knock-out mutants display developmental arrest in the absence of exogenous sucrose. Our ongoing phenotypic analysis of *cml39* knock-out plants has identified several additional developmental abnormalities in these mutants. In comparison to wild-type plants, *cml39* mutants display perturbations in response to exogenous hormones, altered fruit morphologies, and unusual germination properties. Qualitative and quantitative studies describing the phenotypic characteristics of *cml39* vs. wild-type plants are presented.

P29. *De novo* regulatory motif discovery identifies significant motifs in promoters of five classes of plant dehydrin genes

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Plants accumulate dehydrins in response to osmotic stresses. Dehydrins are divided into five different classes, which are thought to be regulated in different manners. To understand the transcriptional regulation of the five dehydrin classes, *de novo* motif discovery was performed on 350 dehydrin promoters from a total of 51 plant genomes. Overrepresented motifs were identified in the promoters of five dehydrin classes. K_n dehydrin promoters contain motifs linked with cold/dehydration. KS dehydrin promoters contain motifs with a GATA core linked to light-regulated expression. SK_n and Y_nSK_n dehydrin promoters contain motifs that match elements connected with cold/dehydration, abscisic acid and light response. Y_nK_n dehydrin promoters contain motifs that match abscisic acid and light response elements, but not cold/dehydration response elements. Conserved promoter motifs are present in the dehydrin classes and across different plant lineages, indicating that dehydrin gene regulation may also be conserved.

P30. Post-translational regulation of starch synthase II in *Arabidopsis thaliana* and *Zea mays*

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Starch is the major form of carbon storage in plants and the most important caloric energy source for humans. It is produced in amyloplasts of plant storage tissues, such as tubers and seed endosperm, and chloroplasts of leaves to temporarily store photosynthates, which are degraded at night to support growth. Mutations affecting starch biosynthesis can alter granule functional properties, resulting in more desirable products in terms of nutrition or industrial applications. The maize *sugary-2* (*su2*) mutant lacks activity of starch synthase IIa (SSIIa), leading to profound alterations in starch structure. One *su2* allele is caused by a single amino acid substitution (Gly^{522}>Arg) in a glucan-binding domain of SSIIa. SSIIa forms the core of a heteromeric protein complex, involved in amylopectin cluster formation. The G522R mutation in its glucan-binding domain affects SSII catalytic activity and prevents the trafficking of all associated enzymes into the granule. SSIIa is regulated by protein phosphorylation, but the site(s) of phosphorylation are unknown. Despite SSIIa being well characterized in cereals, the universality of its role in protein complex assembly and post-translational regulation of starch synthesis amongst all plant species is unknown. This study aims to identify and characterize post-translational modifications of SSII in *Arabidopsis thaliana, in vitro*, using recombinant proteins, and *in vivo*, by complementing *SSII*-null mutants with modified SSII sequences using *Agrobacterium*-mediated transformation.

P31. The role of the exocyst complex in the early stages of compatible pollen-pistil interactions in *Arabidopsis thaliana*

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The initial events of pollen-pistil interactions are fundamentally important in flowering plants as they will influence successful fertilization. We are particularly interested in the exocyst complex, known to be necessary for tethering exocytic vesicles to specific sites at the plasma membrane in yeast and animals. Exo70A1, a member of the exocyst complex, have been previously identified as an essential factor in the stigma for compatible pollen-pistil interactions in *Arabidopsis thaliana* and *Brassica napus*. We hypothesized that Exo70A1 functions as part of the exocyst complex to tether secretory vesicles to the plasma membrane under the pollen attachment site to deliver essential stigmatic resources for the compatible pollen grain to undergo hydration and pollen tube entry. In transmission electron microscope images taken at different time points following pollination, we have observed targeted exocytosis to the stigmatic papillar plasma membrane under the compatible pollen grain. To provide further support for the role of the exocyst complex in compatible pollen acceptance, the roles of the remaining seven subunits of the exocyst complex, Sec3, Sec5, Sec6, Sec8, Sec10, Sec15 and Exo84 were investigated in *Arabidopsis*. Stigma-specific knock-down constructs were used to suppress the expression of each exocyst subunits individually. The early post-pollination stages of pollen hydration, pollen grain adhesion, pollen tube penetration, seed set and overall fertility were analyzed to determine the requirement of each subunit. Interestingly, our collective data demonstrated that all eight exocyst subunits are required for the early stages of compatible pollen-pistil interactions. This novel finding is the first demonstration for the requirement of all eight subunits in a specific biological process in plants.

P32. A novel C-terminal motif serves as the targeting signal for a subset of plastid outer envelope tail-anchored proteins

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Tail-anchored (TA) proteins are a unique class of functionally diverse, integral membrane proteins that are defined by their single C-terminal transmembrane domain and their ability to insert post-translationally into specific organelles in an N_{out}-C_{in} orientation. While in recent years considerable progress has been made toward understanding the biogenesis of TA proteins in yeast and mammals, relatively little is known about how these proteins are properly partitioned within plant cells. One main reason for this paucity of knowledge is that so few plant TA proteins have been identified, let alone characterized in terms of their organelle-specific targeting signals, particularly for plastid TA proteins. Here we show the results of an *in silico* analysis aimed at cataloguing all of the known and putative plastid outer envelope TA proteins (OEP) in the Arabidopsis deduced proteome. Notably, at least three of the TA OEPs identified, namely OEP6, OEP9, and TOC34 are similar, in that they possess relatively long (≥15 residues) C termini that contain a novel motif enriched in R/K and S/T residues, suggesting they utilize the same molecular targeting information. Indeed, the C termini of OEP6, OEP9 and TOC34 are both necessary and sufficient for targeting to plastids, and are interchangeable. We show also that several other previously unknown OEP TA proteins possess the same C-terminal physicochemical characteristics as OEP6, OEP9 and TOC34 and are indeed localized to plastids. Collectively, these results provide important insight into the molecular mechanisms that underlie the biogenesis of plastid TA proteins.

P33. Subcellular localization of *Zea mays* calcium-dependent protein kinase 11

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Calcium-dependent protein kinases (CDPKs) constitute a superfamily of calcium-dependent Serine/Threonine protein kinases found in plants. In maize (*Zea mays*) there are 40

different CDPK isoforms, many with uncharacterized functions. CDPKs are structurally similar in that all have a kinase domain at or near the N-terminus, a pseudosubstrate domain (for autoinhibition) and calcium binding regulatory domains (EF hands). CDPKs undergo a conformational change when the EF hands bind Ca^{2+}, exposing active sites for phosphorylation. The variable N-terminus of CDPKs acts as a targeting sequence and determines their subcellular localization. In this experiment we investigated the subcellular localization of ZmCDPK11, a CDPK identified and isolated from maize, through transient expression in the dicot species *Nicotiana Benthamiana*. Using ChloroP software, ZmCDPK11 scored 0.505 predictive of a chloroplast transit peptide. Removing 22 amino acids from the N-terminus of ZmCDPK11, software targetP resulted in a score of 0.578 predicting a mitochondrial transit peptide. Two cDNAs for ZmCDPK11, with and without the predicted 22 amino acid transit peptide, were ligated into a pCAMBIA vector and fused at the c-terminus with green fluorescent protein (GFP). The ZmCDPK11 constructs were transformed into *Agrobacterium tumefacians* LBA4404 and syringe infiltrated into the leaves of 4-6 week old *N. Benthamiana* leaves. Transient expression of the GFP fused ZmCDPK11 constructs was observed in epidermal pavement and guard cells under a Leica SP2 confocal microscope 2 to 4 days post infiltration. Observations of both ZmCDPK11 constructs are suggestive of cytoplasmic and plastid localization.

P34. Expression analysis of the water stress Inducible promoter *Wsi18*, in the model monocot *Brachypodium distachyon*

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Gene expression is mostly controlled at the transcriptional level, which is mainly regulated by the promoter region. Promoters are generally found upstream of the coding region of a gene, and are responsible for binding the RNA polymerase II enzyme complex, which then goes onto transcribe the gene. Within the promoter sequence are *cis*-acting elements, which bind transcription factors. Transcription factors further mediate gene expression by affecting the binding of the RNA polymerase II complex. *Wsi18* is a promoter native to rice, which is induced by drought stress, salt stress and ABA. In an effort to discover the *cis*-elements primarily involved in the drought inducible nature of *Wsi18*, promoter constructs were made in which regions predicted to have drought inducible *cis*-elements were removed. These promoter constructs driving the GUS gene are being introduced into *Brachypodium distachyon* where their level of drought induced expression will be evaluated. In addition, the closest native homologue to *Wsi18* in *Brachypodium distachyon* has been identified as *Bradi2G47700*. Constructs of *Bradi2G47700* have also been made and experiments are being conducted to evaluate the expression of *Bradi2G47700* in *Brachypodium distachyon* under drought conditions. Identifying the regions within these promoters that are essential for drought inducibility, will lead to a greater understanding of what makes *Wsi18* and other monocot promoters drought inducible.

P35. Investigating the role of a new class of lipid droplet-associated proteins (LDAPs) in lipid droplet biogenesis

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We recently identified a new class of plant-specific lipid droplet-associated proteins (LDAPs) that share extensive sequence similarity with small rubber particle proteins found in rubber-producing plants. The majority of higher plants, however, including those that do not produce large amounts of lipids in non-seed tissues, contain three LDAP-like genes, suggesting these genes are involved in conserved mechanisms of lipid droplet (LD) biogenesis. In *Arabidopsis*, the three LDAP genes display unique expression in various tissues and during certain stages of growth and development indicating they have specific, non-overlapping roles in LD formation and regulation. Here we show that all three isoforms of *Arabidopsis* LDAP (LDAP1/2/3) do indeed target to the surface of LDs in plant cells and when mis-expressed via ectopic overexpression or T-DNA knockout/RNAi-mediated knockdown cause distinct alterations in the number of LDs in leaves. To gain a better understanding of how LDAPs target specifically to LDs, we also performed a series of experiments on LDAP3. Through a combination of mutagenesis experiments, protein-protein interaction analyses, and *in vitro* liposome-binding assays, we show that the targeting of LDAP3 is determined in part by *cis*-acting information within the protein, weak but significant protein-lipid interactions, and potential oligomerization on the LD surface. Screening of an Arabidopsis cDNA library using LDAP3 as 'bait' revealed additional heteromeric protein-binding partners that may be involved in LDAP targeting and/or LD biogenesis. The identity of these proteins, including results from our preliminary investigations, are presented.

P36. Epidermal and meristematic cell type-specific gene regulation in soybean, *Glycine max* (L.) Merr.

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Most soybean cultivars are covered in trichomes and sport three leaflets per compound leaf. Trichomes develop and differentiate from the epidermis, and the fate of leaves –

whether they are compound or simple – is decided in the meristem. Though both characters can have agronomical impact, neither trichome initiation nor compound leaf development is well investigated in soybean. In this study, the transcriptomes of the shoot apical meristem (SAM) and the adjacent epidermis of two mutant soybean isolines of Clark standard cultivar, one trichome-less and one with five leaflets per compound leaf, were compared with a wild type cultivar to detect gene expression differences that could help explain the mutant phenotypes. Cell-specific transcriptomes were obtained using laser capture microdissection and RNA-Sequencing. Numerous differentially expressed genes were identified in the two tissues (meristem and epidermis) of each of the two mutants and Clark cultivar. *De novo* motif analysis of the promoter sequences identified overrepresented motifs in the promoter sequences that can help elucidate transcriptional regulation of up- and down-regulated genes in two tissues of the two mutant isolines.

P37. Exploring genomic contributions in a common bacterial blight resistant navy bean population

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The Ontario navy bean breeding program has released several varieties resistant to common bacterial blight (CBB), caused by Xanthomonas axonopodis pv. phaseoli. This resistance is derived from two interspecific crosses with Phaseolous acutifolius (tepary bean) and is conditioned by several loci on different chromosomes. While the contribution of P. acutifolius has been thoroughly studied in the context of CBB resistance, little is known about its possible contribution to other agronomic traits. A greater understanding of genomic introgression could identify valuable, detrimental or, neutral genes and alleles for important traits conferred by tepary bean in Ontario bean breeding germplasm. In this study, a population developed from a cross between two resistant parents, Rexeter and Apex, three-way crossed to a susceptible parent with no tepary introgression, Compass, (Compass2*//Rexeter/Apex) was used. In a preliminary study of the genomes in this population, twenty F1 genotypes, their parents and, the two tepary plant introductions (PI319443 and PI440795) were screened with a panel of 5830 SNPs. Graphical genotyping was used to compare potential sources of introgression where genomic regions were identical by state to their tepary ancestors and the genetic distances of this population from tepary bean and its parents were compared to CBB susceptibility values obtained by image analysis of X. axonopodis pv. phaseoli infected leaves. F1s which segregated into groups with greater similarity to Rexeter and Apex, their resistant parents, were also more resistant to CBB than those that segregated into groups with Compass, their susceptible parent**.**

P38. Transcriptional responses to exogenous asparagine in Arabidopsis roots

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Asparagine is a major form of nitrogen storage and transport in higher plants. This amide amino acid can sustain Arabidopsis growth as a single nitrogen source. However, at the seedling stage, it inhibits root elongation and root hair formation in a dose-dependent manner, relative to control conditions without nitrogen. These responses are related to the amount of internal asparagine, as demonstrated in studies performed with asparaginase deficient mutants. To investigate transcriptional responses to asparagine, Arabidopsis seedlings were grown for ten days and transferred to media with or without 20 mM asparagine for two hours. Under these conditions, internal asparagine concentration in roots was raised by five-fold. RNA was extracted from quadruplicate samples and transcripts profiled by Illumina Gaiix sequencing. Paired-end, 100 base pair reads were mapped to Arabidopsis gene models. A total of 95 genes had transcript levels elevated by more than two-fold, with 12 were decreased by more than two-fold, at a false discovery rate less than 0.001. These include several genes related to nitrogen transport, metabolism and storage. The results provide information on transcriptional responses elicited by asparagine in root, and identify several marker genes which could be used to further investigate the signal transduction pathway leading to these responses.

P39. Identification of GmMYB176-specific protein kinase in soybean

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GmMYB176 is an R1 MYB transcription factor that regulates isoflavonoid biosynthesis in soybean. The previous studies showed that 14-3-3 proteins, which are phospho-protein binding proteins, interacts with GmMYB176 and regulate its intracellular localization. Deletion of phosphorylation sites in GmMYB176 affected its interaction with 14-3-3 and thus its intracellular localization. Therefore, phosphorylation may play a crucial role in the regulation of nucleo-cytoplasmic shuttling of GmMYB176. In an effort to identify GmMYB176 interactors, 7 putative protein kinases were identified by pull down assay. The current project aims to identify GmMYB176-specific protein kinase in soybean. The interaction of all 7 candidate kinases with GmMYB176 will be validated *in planta*. The validated candidates will be used in kinase assay to study their ability to phosphorylate GmMYB176 *in vitro*. Two protein kinases, Gm08PK and Gm17PK, showed interaction with GmMYB176 *in planta*. The results revealed that GmMYB176 interacts with

Gm08PK in the nucleus whereas Gm17PK in the nucleus and the cytoplasm. The subcellular localization studies showed that Gm08PK and Gm17PK were localized in nucleus and plasma membrane, respectively. The validated protein kinases will be expressed in bacteria for protein expression and purification for kinase assay. This research will provide insight into the regulation of GmMYB176 and isoflavonoid biosynthesis and thus will help in metabolic engineering of isoflavonoid biosynthesis in plants.

P40. A bacterial biosensor for *in situ* visualization of glutamine dynamics in maize tissue

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The essential macronutrient nitrogen (N) is assimilated into glutamine (Gln) after uptake through maize roots for shuttling to leaf tissue. Knowledge of exact spatial and temporal dynamics of Gln *in situ* is important for advancing research in plant biotechnology. A method of visualizing N assimilation and uptake ability in maize may not only help researchers better understand this key physiological process but also lead to the development of crop varieties with increased yields. Biosensing is an emerging field in which biological processes are altered to detect metabolites. Our lab has engineered a strain of *Escherichia coli* named the GlnLux biosensor to serve as a plant analytical tool by sensing Gln within plant tissue. GlnLux is auxotrophic for Gln and luminesces constitutively due to an introduced *lux* operon. The biosensor releases light enabling spatial and temporal mapping of nitrogen assimilation within maize tissue. Output is shown to correlate with amount of N supplied to maize plants, and movement of Gln around the plant can be mapped across time. Additionally, *in situ* pictures of Gln localization in maize leaves have been generated at resolutions which conventional methods of Gln analysis like high-performance liquid chromatography (HPLC) cannot achieve. Future implementation of the biosensor might lead to better understanding of N utilization and the development of highly efficient crop plants. This could reduce N fertilizer inputs, watershed N contamination, and greenhouse gases emitted in the manufacture of synthetic N fertilizer.

P41. Purification of the *L*-methionine:2-oxoglutarate aminotransferase from *Ulva intestinalis* and identification of gene candidates

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Dimethylsulfoniopropionate (DMSP) is an important algal metabolite enzymatically synthesized from methionine by a 4-step pathway. Algae synthesize DMSP in massive quantities and appear to use DMSP for osmoprotection and scavenging reactive oxygen species to cope with environmental stresses. After release into the ocean, bacteria use DMSP as a carbon and sulfur source, yielding dimethylsulfide (DMS) as a byproduct. Once volatilized, DMS constitutes ~50% of the global atmospheric sulfur budget and may play a role in global climate regulation. As the source of DMS, it is surprising that little is known about DMSP biosynthesis and no biosynthetic enzymes or the genes encoding them are known. Given the importance of DMSP, identifying DMSP metabolic enzymes and the genes encoding them should be a priority, which we are now pursuing. In 1997, Andrew Hanson's group used isotope labelling and radiometric enzyme assays to elucidate the DMSP pathway in *Ulva intestinalis* macroalgae. These radiometric assays for DMSP enzymes are time-consuming, expensive, messy, and slow - seriously hindering protein purification efforts. To overcome this challenge, we developed a high-throughput, inexpensive, rapid, ultrasensitive enzyme activity assay for *L*-methionine:2-oxoglutarate aminotransferase (Met AT, E.C. 2.6.1.88), the first enzyme in the pathway. With this new assay, we explored traditional protein purification approaches including ammonium sulfate and polyethylene glycol precipitation plus different anion exchange, gel filtration, dye-ligand affinity, and amino acid-affinity chromatographic separations. Results of these experiments and insights into this step of DMSP biosynthesis will be presented. The identification of gene candidates will also be presented.

P42. Chloroplast chaperone HSP90C assists in the targeting of thylakoid lumen protein OE33 in *Arabidopsis thaliana*

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The chloroplasts in higher plants are the central hubs for oxygenic photosynthesis, cell signalling, and synthesizing amino acids, lipids and phytohormones. Although having its own genome, most chloroplast proteins are encoded by nuclear genes as higher molecular weight precursors containing transit peptides that target the protein to their final destination within chloroplasts. While the mechanism of protein import across the chloroplast membranes has been extensively studied, much less is known about the underlying mechanisms for intra-chloroplast protein targeting and translocation. The chloroplast stroma localized HSP90C is a member of the highly conserved HSP90 family molecular chaperones and has been reported to facilitate protein translocation across the outer and inner membranes into the organelle. In this study, we identified OE33, an intrinsically disordered protein and a subunit of the PSII complex localized in the thylakoid lumen, as a new client protein of HSP90C. By fusing GFP to the C-terminus of OE33, we were able to delay thylakoid targeting of the fusion protein and observed three distinct processing forms, the pre-protein, the intermediate and mature forms. Using OE33GFP as a reporter protein, we showed that by overexpressing HSP90C in the form of HSP90CFLAG, we were able to enhance the thylakoid targeting of OE33GFP as revealed by the increase of mature OE33GFP compared to intermediate OE33GFP. We also observed similar results when using an OE33T200A mutant which interacts more strongly with HSP90C. Taken together, we propose that HSP90C binds OE33 protein within the stroma and specifically aids in its thylakoid targeting.

P43. Root suberin load and composition change with age and different soil moisture levels in *Arabidopsis thaliana*

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Suberin is a lipid and phenolic hetero-polymer found in the cell walls of specific tissues, such as root endodermis and peridermis. Suberin is known to limit the apoplastic movement of water and ions in roots. Besides its constitutive synthesis in certain tissues, suberin can also be changed by environmental conditions. It has thus been proposed that suberin plays a role in protecting plants against unfavourable environmental stresses. In this study, we developed a drought assay using a sub-lethal level of water deficit (chronic drought) to investigate changes in suberin load and composition in response to drought stress in *Arabidopsis thaliana.* We also evaluated changes in suberin loads after re-watering. In addition, we monitored root suberin levels through the course of plant development. We found that individual monomers as well as total suberin loads in root tissues increased with age and reached a plateau after five weeks of age, coinciding with the transition to the reproductive stage. Plants grown under water deficit conditions (5% V/V moisture level) for three weeks had a significant increase (22%) in total root suberin loads relative to non-stressed plants grown at 20% moisture level. The main aliphatic suberin monomers induced were 22:0 and 24:0 fatty acids, 18:1 dicarboxylic acid and 18:1 hydroxy fatty acid. Suberin ferulate levels were not induced by this water deficiency. Suberin load and composition were unchanged when plants were subjected to one week of re-watering after the two week drought stress. Future work using this drought assay will assess the effects of mutants affected in suberin composition in order to better understand the role of suberin in drought adaptation.

P44. Early Arabidopsis responses to *Tetranychus urticae* feeding: Local vs. systemic responses

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The two-spotted mite *Tetranychus urticae* (TSSM) is a major agricultural pest able to feed on a wide range of plant species by sucking the content of individual mesophyll cells. Plants respond to TSSM attack by inducing the expression of defense genes, mostly regulated by jasmonic acid (JA). Although plant-herbivory interactions have been widely studied, the perception of herbivory at the site of feeding and its systemic transmission to the rest of the plant is unknown. We dissected early Arabidopsis defense responses against TSSM by comparing LOCAL (leaves where mites are feeding) and SYSTEMIC (tissues from infested plant but where no mites are feeding) transcriptomes in a time-course experiment. LOCALLY, we show an induced expression of defense genes within 30 min of mite feeding, a response that overlaps with wounding. For SYSTEMIC responses, we find a general down-regulation of transcription programs in a response that differs from systemic responses to wounding. Comparison of systemic responses after wounding and TSSM attack showed that both depend on JA-signalling, but that different JA-regulated programs are activated. Systemic TSSM-induced responses are regulated by the ERF-JA branch that integrates ethylene and JA signals, while systemic wound-induced responses are regulated by the antagonistic JA-branch, mediated by MYC2. These findings suggest that plants mount different systemic responses to TSSM and wounding. It is unclear if these differences result from mites' ability to manipulate the plant defenses or if the plant distinguishes between mechanical wounding and TSSM herbivory. Experiments aimed in resolving these mechanisms will be presented.

P45. A two-component enzyme complex is required for dolichol biosynthesis in plants

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Dolichol is a long-chain polyisoprenoid that plays an indispensable role in the post-translational modification of eukaryotic proteins. As proteins enter the secretory pathway they are typically decorated by a glycan that is first assembled on an ER membrane anchored dolichol molecule. The glycan is then transferred *en bloc* from dolichol to the appropriate asparagine acceptor site on the nascent glycoprotein. Although the enzymes that synthesize the glycan are well established, dolichol synthesis has only been demonstrated with crude microsomal preparations, suggesting that more than one protein may be involved. Using tomato (*Solanum lycopersicum*) as our model, we show that dolichol synthesis requires the participation of two proteins: a cis-prenyltransferase (CPT) and a distantly related member of the CPT family. These two proteins interact, *in vivo*, and co-localize to the endoplasmic reticulum. Both proteins are required to functionally complement a yeast mutant that is deficient in dolichol and by combining both proteins in *E. coli*, dolichol synthase enzyme activity could be recovered. Finally, RNAi-mediated knockdown of the CPT results in a significant reduction in dolichol and a pleiotropic phenotype, which is consistent with the essential role of this polyisoprenoid.

P46. Using small molecules to prime and explore anthocyanin accumulation response to low temperature exposure

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As sessile organisms, plants must contend with frequent fluctuations in environmental conditions, especially during germination and early development. Some of these environmental fluctuations can generate stress responses, thus limiting plant growth. Priming is a mechanism in which early exposure to one stressful environmental condition results in more rapid or vigorous acclimation when the plant is exposed to subsequent challenges. This preparation therefore enhances plant tolerance to fluctuations in environmental conditions and can minimize losses in productivity. We have undertaken a high-throughput approach to ask if small molecules can act as "chemical environments" to alter *Arabidopsis thaliana* phenotype subsequent to early exposure and removal, consistent with the concept of a priming effect. A screening protocol was developed, and from thousands of small molecules eight compounds were identified with the ability to reduce the intensity of a visible phenotype, anthocyanin accumulation induced by chilling stress. Dose-response curves have been established, with most compounds demonstrating a statistically-significant correlation between increased compound dose and decreased pigment accumulation. The timing of early chemical exposure necessary to effect changes in anthocyanin accumulation before and during chilling has also been examined, with significant differences observed between treatment regimes at different early developmental stages. The data at this point are insufficient to determine if the observed phenotypic response represents a primed relief from chilling treatment stress, or instead simply a perturbation in the anthocyanin biosynthetic pathway. Further investigation will focus on transcriptomic and/or epigenomic changes responsible for the observed chemical treatment effect and phenotypic response.

P47. Investigating the role of plant mitochondria in NO synthesis and signalling

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Plant mitochondria are proposed to act as signaling organelles that orchestrate acclimation responses to abiotic and biotic stress. While the primary signals being generated by mitochondria are largely unknown, reactive oxygen and nitrogen species are considered strong candidates. In this study, laser-scanning fluorescence confocal microscopy was used to detect leaf mesophyll and guard cell nitric oxide (NO) amounts in wild-type and transgenic tobacco plants with varying amounts of mitochondrial alternative oxidase (AOX). AOX knockdowns showed much higher amounts of NO than wild-type when treated with inhibitors of Complex III or ATP synthase, but not following treatment with an inhibitor of the TCA cycle enzyme aconitase, suggesting an influence of AOX on NO production under specific mitochondrial dysfunction scenarios. Interestingly, treatment with antimycin A, an inhibitor of the Qi-site of Complex III, generated much higher NO amounts than treatment with myxothiazol, an inhibitor of the Qo-site, even though both inhibitors were equally effective at inhibiting oxygen consumption. When both inhibitors were used concurrently, NO amount was similar to that with myxothiazol alone. Further, myxothiazol was able to reduce the high levels of NO seen in AOX knockdown guard cells. These results suggest that Complex III is a site of normoxic generation of NO in plant mitochondria and that this generation is regulated by AOX amount. Further studies are examining the nitrite-dependence of this NO generation, as well as the influence of this NO signal on expression of previously described mitochondrial dysfunction genes.

P48. Characterization of the molecular targeting information for the peroxisomal membrane transport protein PXA1

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The Arabidopsis PEROXISOMAL ABC TRANSPORTER 1 (PXA1) plays a number of important roles in plant lipid metabolism, including the transport into the peroxisome of fatty acids and lipophilic precursors of the jasmonate and auxin biosynthetic pathways. Unlike most of its counterparts in yeasts and animals, which are 'half-size' ABC proteins that dimerize to form a functional transporter, PXA1 is a 'full-sized' transporter consisting of two homologous but distinct halves, each composed of a transmembrane domain region and a nucleotide- and substrate-binding region. Notably, the two halves of PXA1 can be artificially expressed as separate proteins that assemble to form the functional transporter, but also have distinct differences in their substrate specificity. Here we show that the two halves of PXA1 also differ with respect to their peroxisomal targeting information. Through a combination of *in vivo* mutagenesis experiments and structural modeling, we show that the N-terminal half of PXA1 (N-PXA1), but not the C-terminal half of the protein (C-PXA1), targets to peroxisomes in plant cells. We show also that this difference appears to be due to at least three amino acid sequences in N-PXA1 that are predicted to form part of the structural interface responsible for mediating peroxisomal targeting, but are conspicuously divergent in C-PXA1. Overall, these and other results provide important insights into the dichotomy of PXA1 in terms of the function and targeting of its two halves and the evolution of peroxisomal ABC transporter proteins in general, as well as the mechanisms underlying peroxisomal membrane protein targeting in plants.

P49. An *in silico* analysis of cell wall related gene families in soybean stem

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The use of plant fibers in composite production is an attractive new market for agricultural residue but is limited by their variability and poor performance when incorporated into composite materials. Our initial study demonstrated that the chemical composition of the fibre affects the physical traits of the composites. We also mapped Quantitative Trait Loci (QTL) for major cell wall components (cellulose, hemicellulose and lignin content) on 19 soybean chromosomes and identified their co-localization with several genes coding for enzymes involved in cell wall biosynthesis/modification. The focus of the current work was to map additional cell wall-related QTL [NDF (neutral detergent fiber), ADF (acid detergent fiber) and indices for level of lignification calculated from them] and analyze genome regions underlying those QTL. RNAseq data (Phytozome v.10) and soybean genome annotation and feature coordinate files (SoyBase) were used to analyze cell wall-related QTL regions (*in silico*). Self-alignment of the soybean genome identified syntenic and gene duplication regions. For example, two cinnamyl alcohol dehydrogenase (*CAD*)genes (ADH, class V family), involved in lignin biosynthesis, were found on duplicated regions on chromosomes Gm10 (Glyma.10g262400) and Gm20 (Glyma.20g128600). Over 60 cellulose synthase-like (Csl) genes (hemicellulose biosynthesis) were identified on all 20 soybean chromosomes while 19 members of cellulose synthase (*CesA*) gene family (cellulose biosynthesis) were identified on 10 chromosomes. Some gene clustering was identified. The results could be used to develop molecular markers to select soybean lines with stem fibres that are superior for composite manufacturing.

P50. Autophagy's link to self-incompatibility in the Brassicaceae

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Monecious flowering plants have both male (pollen) and female (pistil) reproductive organs within their flowers. Some species reject their own pollen to prevent inbreeding depression and produce offspring with greater genetic diversity. In the Brassicaceae, this self-incompatibility response is controlled by the pistil through the failure to transfer factors required for pollen hydration and germination. Signalling events responsible for this are initiated by the allelic binding of the pollen *S* Cysteine Rich (SCR) ligand to the pistil *S* Receptor Kinase (SRK). Downstream signalling events are believed to include the ARC1 E3 ubiquitin ligase which, following phosphorylation by SRK, ubiquitinates and inhibits Exo70A1. With compatible pollen, Exo70A1, as part of the exocyst complex, is normally proposed to be responsible for directing cargo-filled secretory vesicles to the stigmatic papillar plasma membrane. With the inhibition of Exo70A1, resources are not delivered to self-incompatible pollen grains, resulting in self-pollen rejection. Recent transmission electron microscopy work from our research group preliminarily implicates autophagy in the arrest of resources from self-incompatible pollen. To further investigate autophagy's role in self-incompatibility we are now reconstituting the self-incompatibility pathway in autophagy mutant backgrounds of naturally self-compatible *Arabidopsis thaliana* with the addition of *A. lyrata* SCR, SRK, and ARC1. We hypothesize that a breakdown in autophagy will lead to a breakdown in self-incompatibility. Fluorescent protein tagged autophagy and vesicle markers will be used to visualize changes in self-incompatibility in autophagy mutant backgrounds. To rescue autophagy in these mutant backgrounds, lines will be backcrossed to wild-type *A. thaliana,* which we hypothesize will also rescue self-incompatibility.

P51. Determining physiological processes underlying HopZ1a recognition in *Arabidopsis thaliana*

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Pseudomonas syringae is a Gram-negative bacterium that infects multiple plant species by manipulating cellular processes via injection of type three secreted effectors (T3SEs) into host cells. Nucleotide-binding leucine-rich repeat (NLR) resistance proteins recognize specific T3SEs and trigger an immune response, called effector-triggered immunity (ETI). ETI is a rapid immune response capable of preventing pathogen spread, and manifests as localized programmed cell death, known as the hypersensitive response (HR). Ion leakage assays can be used to quantify leakage of intracellular ions that occurs during the development of an HR. The T3SE HopZ1a elicits a strong ETI response mediated by a NLR protein called HopZ-ACTIVATED RESISTANCE1 (ZAR1), and the unique ETI-associated pseudokinase called HopZ-ETI-DEFICIENT1 (ZED1). Interestingly, ion leakage assay data suggests that ZAR1/ZED1-mediated recognition of HopZ1a is impaired by high relative humidity. Therefore, we sought to characterize the role of humidity in modulating ETI responses. In further investigating the effect of humidity on the development of HopZ1a-induced ETI, we will be able to learn more about the physiological processes underlying ETI.

P52. The use of chemical compounds to help *Arabidopsis* acclimate to a nitrogen limiting environment

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Plants are unable to escape adverse conditions; consequently, they have evolved mechanisms to contend with fluctuating environmental conditions. For example, plants often experience varying soil nitrogen levels during

their life cycle, and adjust growth and development to accommodate these changes. A typical plant response to limiting nitrogen involves an increase in root growth relative to shoot growth, as well as the breakdown of chlorophyll in order to remobilize nitrogen to younger tissues. As such alterations in plant development and physiology have a profound impact on plant growth and survival, as well as productivity in agricultural and forestry settings, there is interest in better understanding the molecular determinants that underpin these responses. Besides conventional genetic methods, a chemical genetics approach can be employed to discover molecular components involved in generating plant responses to altered soil nitrogen. Using a high-throughput screening method, a chemical library consisting of thousands of chemicals was screened to identify compounds capable of generating an altered phenotype in *Arabidopsis thaliana* seedlings under limiting nitrogen conditions. It is centred on the premise that prior chemical exposure may simulate a molecular response that subsequently impacts how a seedling contends with low nitrogen growth conditions. Following multiple chemical screens, four chemical compounds were identified that appear to pre-condition plant responsiveness to reduced nitrogen, as characterised by differences in root morphology and chlorophyll retention. These compounds will be further characterized to identify their role in producing an altered phenotype in *Arabidopsis* seedlings under nitrogen deprivation.

P53. Characterization of effector genes in common blight bacteria isolates showing differential pathogenicity on common bean

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Common bacterial blight, caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is a damaging disease of common bean (*Phaseolus vulgaris* L.) throughout the world. Four *Xap* isolates, collected locally, showed differential pathogenicity on a set of bean genotypes. Non-pathogenic Xanthomonads were also detected and isolated in pathogenic *Xap* populations. Six single *Xap* colonies, purified from those four *Xap* isolates showing differential pathogenicity, and one non-pathogenic Xanthomonas colony were sequenced using a combination of Illumina and Pacbio sequencing approaches. Genome sizes of the pure line isolates range from 5.2-5.4 Mbp with 64.7% G+C content. Sequences are highly conserved but there are sequence inversions in the middle of chromosomes between fuscans and non-fuscans isolates. In total, 27-29 Type III effector genes including one or two Transcription Activator-Like Effectors (TALEs) were identified in the genomes of the pure lines. Preliminary results showed that there was a sequence polymorphism between a pathogenic line and a non-pathogenic line in a TALE sequence. Characterization of effector genes would help understand the mechanism of the differential pathogenicity, provide tools for monitoring emergence of new aggressive *Xap* strains, and facilitate breeding durable resistant bean varieties.

P54. RNA-Seq genotyping and its application in constructing a high-density genetic linkage map of *Zea mays*

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Genetic linkage maps are generated by genotyping individuals within a segregating population at many polymorphic nucleotide sites. RNA-Seq data is a promising way both to identify and to assay these nucleotide polymorphisms, but low transcriptome coverage, sequencing errors, and genome alignment problems can cause an individual's alleles to be misidentified. Here, we utilize RNASeq data both to identify and to assay *Zea mays* molecular polymorphisms. First, we identified 25,822 high-confidence single nucleotide polymorphisms (SNPs) between two inbred maize lines by filtering aligned RNASeq reads. Second, using RNASeq data from over 100 maize recombinant inbred lines, we identified a subset of 2,588 sites with high minor allele frequencies (>0.3) and low missing data (<5.0%). Analysis of these markers revealed high levels of heterozygosity (>9.1%) in eight inbred lines, indicating that these lines are genetic contaminants. We also used the markers to construct a high density linkage map. At a number of loci, the order of markers within the genetic map differs from their order in the B73 reference genome, identifying possible errors in the maize reference genome assembly. In summary, RNASeq based genotyping methods provide powerful tools for genome analyses.

P55. Dissecting phenylpropanoid pathway in common bean (*Phaseolus vulgaris*) by RNA sequencing

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Common bean (*Phaseolus vulgaris*) is an important grain legume that is rich in nutrient content such as complex carbohydrates, vegetable protein, dietary fiber, vitamins and minerals. It also contains a substantial amount of antioxidants present in polyphenolic compounds which are synthesized via the phenylpropanoid pathway (PP). Literally no study is done yet about PP genes in common bean and their overall expression in potential germplasms that would facilitate in developing antioxidant rich common bean cultivars. Therefore, an RNA-Seq analysis was performed with 19 different common bean classes with different seed colour and germplasm sources using Illumina deep sequencing. Our analysis show that a total of 27726 genes, 29665 CDS, 37287 TSS, 73235 isoforms, and 4512861 differentially expressed genes was identified among all these 19 classes. Among 260 PP related genes, significant changes in gene expression were observed in 2 paralogs of dihydroflavonol 4-reductase, 8 chalcone synthases, 5 cinnamoyl CoA-reductases, anthocyanidin 4-reductase, 4-

coumarate:CoA ligase, leucoanthocyanidin reductase, cinnamate 4-hydroxylase, flavonoid 3 hydroxylase, and flavonoid 3′ hydroxylase in coloured seeds compared to the white ones. Increased expression was also observed in putative transcription factors (TFs), for example 6 MYBs, 5 MYCs including 3 EGL3 type MYCs, one TT8 and one AN11 type WD40 TF in coloured seeds. No difference was observed when RNA-seq gene expression of three DFR genes was validated by real time PCR. This finding will facilitate understanding the transcriptional regulation of phenylpropanoid pathway to identify candidate genes and classes that would ultimately benefit applied common bean breeding programs.

P56. Wild and ancestral members of the corn family may host endophytes that can suppress a fungal disease in their modern turfgrass relatives

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Wild and ancient relatives of modern crops may possess endophytes that can benefit modern agriculture. The grass family compromises the world's most important cereals (rice, maize, wheat) in addition to turfgrass used for recreational, aesthetic and environmental purposes. Creeping Bentgrass is the most widely used turfgrass on golf greens in the US and Canada. Dollar spot disease is one of the most important diseases affecting Creeping Bentgrass. Our lab bioprospected 14 maize genotypes for bacterial endophytes. We hypothesized that some maize endophytes may suppress dollar spot. In this study, 5 out of 200 tested endophytes showed inhibition of *S. homoeocarpa in vitro*. Greenhouse trials demonstrated that 3 strains partially suppressed dollar spot disease. One strain, A12, was selected further to understand the mechanism of activity. GFP-tagging and confocal microscopy revealed the location of A12 in turfgrass plants. A mutant screen was conducted on 3000 Tn5-mutagenized colonies; 12 mutants showed loss of antifungal activity *in vitro* and *in planta*. Several genes were found to be involved in the antifungal activity. These results suggest that wild and ancestral members of the grass family may host microbes that can benefit grasses of importance to modern agriculture.

P57. An assessment of cell-penetrating peptide transfection in soybean somatic embryos

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The genetic modification of soybean has had limited success using the methods currently available. Cell-penetrating peptide (CPP) transfection presents a novel approach to the alteration of the soybean genome. The CPP system involves harnessing the properties of highly invasive peptide sequences and using them to assist with the delivery of macromolecular cargo into cells. The Trans-activator of transcription (Tat) protein from HIV-1 contains a peptide sequence known as Tat that possesses such properties. The aim of my research is to investigate the application of Tat_2 CPP transfection in soybean somatic embryos. The somatic embryogenesis system has been established and the work has now moved on to the assessment of a variety of parameters associated with transfection success. The plasmid pBI221, containing a β-glucuronidase (GUS) reporter gene, is the cargo molecule of interest. The transient expression of GUS will be measured to determine the efficacy of various treatments, including complex incubation times, a range of CPP-DNA concentrations, and the use of permeabilization treatments. Early results indicate that there is potential for the use of CPP transfection in soybean somatic embryos; however there remains a large amount of information to be uncovered concerning the ideal conditions for transfection. This study represents one of the early assessments of CPP use in soybean somatic embryos.

2014 CSPB/SCBV ERM Attendees

The following are the names of attendees for the 2014 CSPB/SCBV ERM as of Nov 18, 2014.

Al-Daoud, Fadi	University of Guelph	
Akhtar, Tariq	University of Guelph	Plenary Lecture II, P45
Allan, Wendy	Carl Zeiss Canada	
Arif, Muhammad	University of Guelph	P10
Alber, Nicole	University of Toronto Scarborough	8.1, P47
Anderson, Erin	University of Guelph	P48
Aung, Banyar	University of Western Ontario	4.1
Azad, Kumkum	University of Saskatchewan	P13
Bajwa, Vikramjit Singh	University of Guelph	6.6, P8
Barton, Kiah	University of Guelph	
Bede, Jacqueline	McGill University	7.1
Behdarvandi, Behrang	Wilfrid Laurier University	P22
Bertrand, Brenden	University of Guelph	P3
Blakney, Andrew	McGill University	
Bozzo, Gale	University of Guelph	2.2, 2.3, 6.2, P11
Bräutigam, Katharina	University of Toronto Scarborough	1.3
Brasher, Megan	University of Guelph	P45
Brikis, Carolyne	University of Guelph	2.3
Cameron, Robin	McMaster University	7.5
Campbell, Malcolm	University of Toronto Scarborough	1.3, P46, P52
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Carlow, Chevonne	University of Guelph	1.4
Challa, Sneha	University of Western Ontario	7.4
Champigny, Marc	University of Toronto Scarborough	
Chang, Pearl Pei-Chun	University of Toronto at Mississauga	8.3
Charbonneau-Bérubé, Eva	McGill University	
Chen, Ling	Agriculture and Agri-Food Canada	
Cholewa, Ewa	Nipissing University	
Coskun, Devrim	University of Toronto	1.5
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Dahal, Keshav	University of Toronto Scarborough	8.1, 9.4
Dastmalchi, Mehran	University of Western Ontario	2.6
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Duermeyer, Lisza	University of Toronto	P21
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Efatpour, Mohammad	University of Guelph	
Faultless, Trent	University of Guelph	5.3
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Ferouz, Atiyyah	Agriculture and Agri-Food Canada	P57
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Frank, Joshua	University of Western Ontario	P26
Fréchette, Emmanuelle	University of Toronto at Mississauga	3.3. 9.1
Freixas Coutin, José Antonio	University of Guelph	2.2
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Geitmann, Anja	Université de Montréal	P23

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Grimberg, Nicholas	Wilfrid Laurier University	
Guinel, Frédérique	Wilfrid Laurier University	7.3, P22
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Ludovice, Dominic	University of Toronto	
Lum, Geoffrey	University of Guelph	P11
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Mano, Noel Anthony	University of Guelph	P27
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McCarthy, Avery	University of Western Ontario	9.2
McDonald, Allison	Wilfrid Laurier University	3.4
McKenzie-Gopsill, Andrew	University of Guelph	3.5
Mehrpooyan, Fatemeh	University of Guelph	6.3
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Naik, Eshan	University of Toronto	P52
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Neil, Kevin	University of Guelph	
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Nowak, Julia	Agriculture and Agri-Food Canada	1.6
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Patterson, Jenelle	University of Guelph	P30
Paudel, Jamuna Risal	McGill University	7.1

Pauls, K. Peter	University of Guelph	2.2, 4.3, 5.2, P10, P37, P49, P53, P55
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Perry, Gregory	University of Guelph	4.3, P53
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Pinhero, Reena	University of Guelph	2.5, P4
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Ranathunge, Kosala	University of Guelph	
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Stinziano, Joseph	University of Western Ontario	3.1
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Thompson, Cody	University of Guelph	8.6
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