

Welcome/ Bienvenue

**Proceedings of the of the Canada Society of Plant
Physiologists/Délibérations de la Société Canadienne
de Physiologie Végétal**

Volume 54, Number 1

Eastern Regional Meeting/Congrès Régional de l'Est

Brock University, St. Catharines, Ontario, Canada

December 3rd & 4th, 2010

Local Organizing Committee

Dr. Charles Despres

Dr. Douglas Bruce

Dr. Vincenzo De Luca



Program Overview

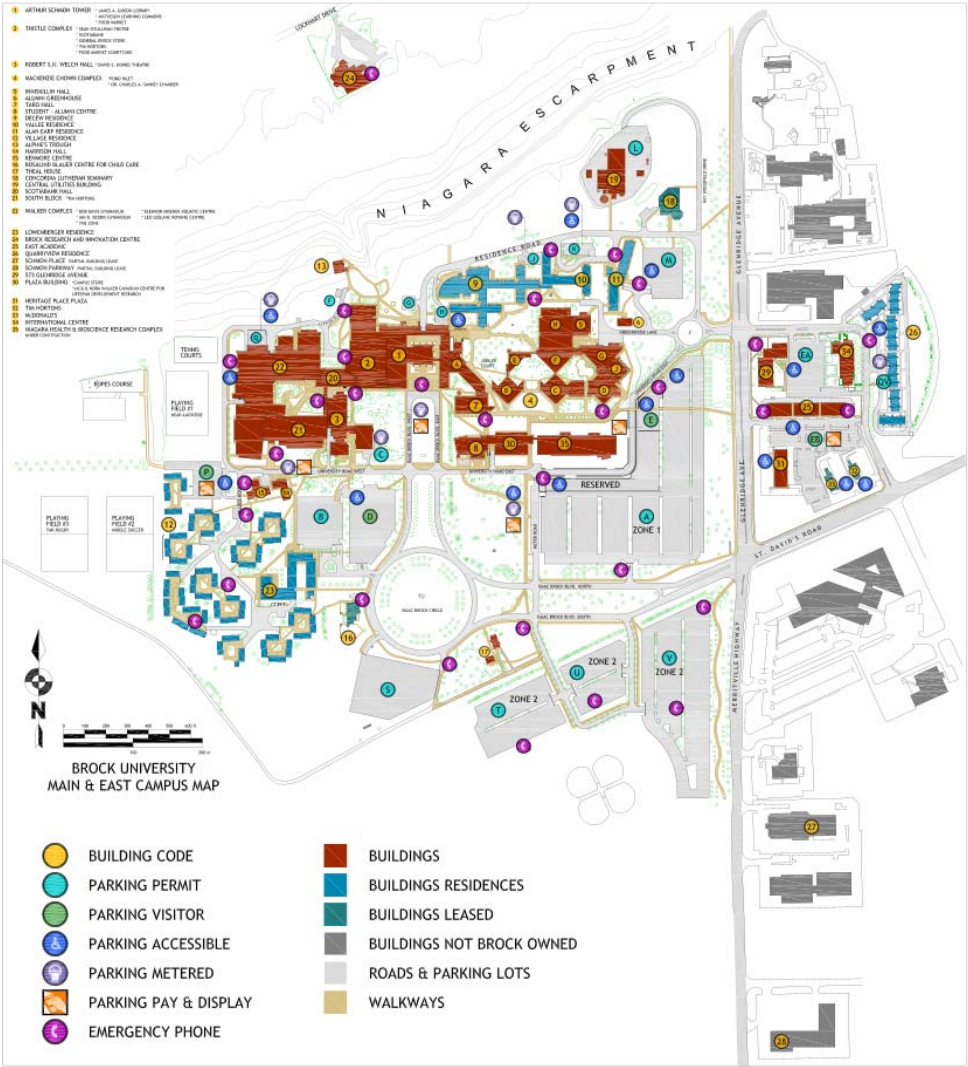


Acknowledgements

We are grateful for financial support to Vineland Research and Innovation Center.

We Thank Brock University Faculty of Mathematics and Science, the Department of Biological Sciences, Facilities Management, Conference and Event Services, Brock Dining Services, Parking Services and Custodial Services for facilitating the organization of this meeting and for helping with meeting expenses.





Registration and the mixer on December 3 will take place In Pond Inlet in the G part of the Mackenzie Chown Building on the right of the map.

The meeting will take place in the Academic South Building (#21), a short 5 minute walk from the Mackenzie-Chown Building.

Parking will likely be in the area near Mackenzie Chown described as E where the reserved parking is found. Note that this parking lot is right in front of the new Cairns Family Health and Bioscience Complex (#35) that is now under construction.

Friday, December 3, 2010 Registration and Mixer

Registration: Pond Inlet 3:00-7:00 pm

Mixer: Pond Inlet 5:00→8:00 pm

Poster Setup Academic South Hallway in front of Rooms AS201 and AS202

Saturday, December 4, 2010 Scientific Program

8:00 AM Registration Academic South Hallway in front of Rooms AS201 and AS202

Opening Ceremonies and Symposium Talks Room AS202

8:45-9:00 am Opening Ceremonies:

9:00-9:45 am **Dr. Peter Moffet, University of Sherbrooke** “Constitutive and induced defenses against plant viruses”

9:45-10:30 am **Dr. Andre Kessler, Cornell University** **Ecological cost of induced plant responses?--The information war in the plant headspace**

10:30-10:50 Coffee break & poster viewing; Academic South Hallway

10:50-12:05 **Oral presentations Session A,B**
Session A (Room AS201)
Session B (Room AS202)

12:05-2:00 pm Lunch & Poster viewing Academic South Hallway

Special Presentation AS202

2:00-2:30 **Dr. Daryl Somers Vineland & Research Innovation Center** “Science and Innovation at Vineland”

2:35 PM – 3:50 PM **Speaker Sessions C & D run concurrently**

Session C (Room AS201)
Session D (Room AS202)

3:50 PM – 4:10 PM: Coffee break & poster viewing.

4:10 PM - 5:25 PM Speaker Session E (Room AS202)

5:25 PM **Student Award Presentations, Concluding Remarks** (room AS202)

5:35 PM Conclusion of Meeting

Detailed Scientific Program on Saturday, December 4

8:00 AM **Registration, Breakfast and Poster Organization:** Academic South Hallway in front of Rooms AS201 and AS202.

Opening Ceremonies and Symposium Talks Room AS202

8:45 - 9:00 AM **Opening ceremonies**

Chairperson: Charles Despres, Brock University

9:00 - 9:45 AM **Dr. Peter Moffet Invited Speaker**

Enjoying the silence: Argonaute proteins in anti-viral responses.

Peter Moffett

Département de Biologie, Université de Sherbrooke, Sherbrooke, Québec

To successfully infect a plant, a virus must usurp the cell host machinery and overcome plant defense mechanisms. A major mechanism of constitutive antiviral immunity is ensured by RNA silencing which relies on the recognition and degradation of viral double-stranded RNA into virus-derived small RNAs (vsRNAs) by DICER-like enzymes. These vsRNAs are then incorporated into complexes containing members of the Argonaute (AGO) family of endonucleases, whereupon act as guides to target viral RNA. However, most viruses are able to overcome this constitutive defense through the use of virus-encoded suppressors of RNA silencing (VSRs). In turn, plants possess a type of induced resistance to viruses based on recognition of viral proteins mediated by the products of plant disease resistance (*R*) genes, which encode NB-LRR proteins. We have investigated the role of different AGO family members in both constitutive and induced anti-viral defenses. We find that, whereas constitutive anti-viral responses are based on vsRNA-mediated RNA degradation, the mechanisms induced by NB-LRR proteins permit viral RNAs to accumulate but prevent them from associating with ribosomes. These results suggest that NB-LRR proteins induce AGO-dependant anti-viral mechanisms that specifically inhibit the translation of viral transcripts, rather than inducing their degradation. Furthermore, we find that induced anti-viral responses employ a different set AGO proteins than those required for constitutive RNA silencing-mediated defenses. Our results indicate a specialization in AGO function during different types of anti-viral defenses and provide new insights into the mechanisms of induced defense responses.

9:45-10:30 am Dr. Andre Kessler Invited Speaker

Ecological cost of induced plant responses?--The information war in the plant headspace.

Andre Kessler

Ecology & Evolutionary Biology, Cornell University, Ithaca, NY 14853

Plants respond to insect herbivore damage with an array of changes in primary and secondary metabolism. Some of these metabolic changes can cause increased resistance of the plant to current and subsequent herbivore attack and may function as direct and indirect defences. Such induced resistance effects have been extensively studied but very little is known about their role in structuring arthropod communities, influencing insect herbivore population dynamics, and conversely the distribution of herbivory within plant populations.

I will discuss ecological consequences of plant defences and evaluate the resulting costs and benefits of induced plant responses. In particular I will focus on volatile organic compound (VOC) -mediated effects of herbivory on insect community dynamics and interactions with plant mutualists, such as pollinators and natural enemies of herbivores. Results of recent studies suggest that herbivore-induced plant responses cause that plant-insect interactions are played out in an arena that is much larger than the plant itself and has significant plant and arthropod community consequences. Thereby VOCs play a crucial role in transmitting information between the different organisms. The community-wide effects that plant phenotypic plasticity has are analogue to those of animal behaviour and can be functionally analysed in similar ways.

10:30-10:50 AM Coffee break
Poster viewing; Academic South Hallway

10:50-12:05 PM Speaker Sessions A and B run concurrently

Speaker Session A (Room AS201)

Chairperson: **Jacquie Bede**, McGill University

10:50 AM A1

Effect of stress on chloroplast arrangement in single cell C4 photosynthetic species. Sarah M. Schoor* and Simon D.X. Chuong; *University of Waterloo, 200 University Ave W, Waterloo, Ontario N2L 3G1.*

11:05 AM A2

Distribution of photosynthetic enzymes and transcripts in chlorenchyma cells of *Bieneria sinuspersici* during leaf development. Makoto Yanagisawa* and Simon D. X. Chuong; *Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1.*

11:20 AM A3

Refixation of photorespiratory CO₂ as a method to minimize carbon loss in C₃ grasses. Florian Busch¹, Tammy L. Sage¹, Asaph B. Cousins², Rowan F. Sage¹; ¹*Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON;* ²*School of Biological Sciences, Washington State University, Pullman, WA.*

11:35 AM A4

The effects of genotype and clonal history on shaping the drought response in *Populus*. Katharina Bräutigam^{1,2}, Sherosha Raj^{1,2}, Olivia Wilkins^{1,2}, Malcolm M. Campbell^{1,2}; ¹*Department of Cell & Systems Biology, and* ²*Centre for the Analysis of Genome Evolution & Function, University of Toronto, 25 Willcocks St., Toronto, ON M5S 3B2, Canada.*

11:50 AM A5

Genotype by environment interaction for fibre and starch profiles of potato (*Solanum tuberosum* L.). Stephanie Bach*¹, J. Alan Sullivan¹, Benoit Bizimungu², Agnes Murphy² and Rickey Yada³; ¹*Department of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1;* ²*Agriculture and Agri-Food Canada, Fredericton, NB E3B 4Z7;* ³*Department of Food Science, University of Guelph, Guelph, ON N1G 2W1.*

Speaker Session B (Room AS202)

Chairperson: **Alan Bown**, Brock University

10:50 AM B1

Chemical screen uncovers link between sugar signalling and one-carbon metabolism. Michael E Stokes*¹, Abhishek Chattopadhyay¹, Olivia Wilkins¹, Malcolm M Campbell^{1,2}; ¹*Department of Cell & Systems Biology,* ²*Centre for Analysis of Genome Evolution & Function, University of Toronto, Toronto, Ontario, Canada M5S 3B2*

11:05 AM B2

Characterization of GDP-O-FucosylTransferase in *B.napus* Microspore Embryogenesis. Jerlene Nessia*¹, K. Peter Pauls; *Department of Plant Agriculture, University of Guelph, Guelph, ON*

11:20 AM B3

Vinca drug components accumulate exclusively in leaf exudates of Madagascar periwinkle. Vonny Salim*^a, Jonathon Roepke^a, Maggie Wu^a, Antje M. K. Thamm^a, Jun Murata^a, Kerstin Ploss^b, Wilhelm Boland^b, and Vincenzo De Luca^a; ^a*Department of Biological Sciences, Brock University, St Catharines, ON* and ^b*Max-Planck-Institut für Chemische Ökologie, 07745 Jena, Germany.*

11:35 B4

Identification and characterization of members of the Chalcone Isomerase gene family involved in isoflavonoid biosynthesis of *Glycine max*. Mehran Dastmalchi^{1,*} and Sangeeta Dhaubhadel^{1,2}; ¹*Department of Biology, University of Western Ontario, London, ON;* ²*Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, ON.*

11:50 B5

Plants have two COG0354 proteins with folate-dependent functions in the metabolism of iron-sulfur clusters. Jeffrey C. Waller^A, Gaozhong Shen^B, Sophie Alvarez^C, Karen Loizeau^D, Stéphane Ravanel^D, John H. Golbeck^{B,E}, and Andrew D. Hanson^A; (^A*Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, USA,* ^B*Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA,* ^C*Donald Danforth Plant Science Center, St. Louis, MI 63132, USA,* ^D*Laboratoire de Physiologie Cellulaire Végétale, CNRS/CEA/INRA/Université Joseph Fourier, CEA-Grenoble, F-38054 Grenoble cedex 9, France,* and ^E*Department of Chemistry, The Pennsylvania State University, University Park, PA 16802).*

12:05-2:00 pm Lunch and Poster viewing Academic South Hallway

Chairperson: **Douglas Bruce**, Brock University

Special Presentation AS202

2:00-2:30 PM **Dr. Daryl Somers Vineland and Research Innovation Center "Science and Innovation at Vineland"**

2:30 PM – 3:45 PM **Speaker Sessions C and D run concurrently**

Speaker Session C (Room AS201)

Chairperson: **Simon Chuong**, University of Waterloo

2:30 PM C1

Siderophore-Independent Iron Uptake in the Cyanobacterium *Anabaena flos-aquae*. Nikki L Wirtz¹, Ron G Treble¹, Harold G Weger²; ¹*Dept. of Chemistry & Biochemistry, Univ. of Regina;* ²*Dept. of Biology, Univ. of Regina.*

2:45 PM C2

Examining the role of the TOC complex in selective protein import into dimorphic chloroplasts in the single-cell C₄ species *Bienertia sinuspersici*. Terry S.C. Lung*, Simon D.X. Chuong; *Department of Biology, University of Waterloo, 200 University Ave W, Waterloo, ON, Canada N2L 3G1.*

3:00 PM C3

Expression, purification, and biophysical analysis of Tic20, the putative pre-protein channel of the chloroplast inner envelope membrane. Spence Macdonald*, Matthew D. Smith. *Department of Biology, Wilfrid Laurier University, Waterloo, Ontario.*

3:15 PM C4

Preprotein specificity of the Toc159 family of chloroplast protein import receptors. Siddhartha Dutta & Matthew D Smith; *Department of Biology, Wilfrid Laurier University, Waterloo, ON.*

3:30 PM C5

Investigating the genetic basis for naturally occurring primary root growth variation in *Zea mays*. Meyer, Ann*, Lukens, Lewis; *Department of Plant Agriculture, University of Guelph, Guelph, ON, N1G 2W1.*

Speaker Session D (Room AS202)

Chairperson: **Shelley Hepworth**, University of Ottawa

2:30 PM D1

Genetic variation for *Arabidopsis thaliana* heterotic responses is in large part explained by the epistatic interaction of flowering time genes FRIGIDA and Flowering Locus C. Siobhan Moore* and Lewis Lukens; *Department of Plant Agriculture, University of Guelph, Guelph, ON, N1G 2W1*

2:45 PM D2

Patterns of *cis* natural antisense transcripts across stress growth conditions in *Arabidopsis thaliana* suggest RNA interference activity. Shuhua Zhan* and Lewis Lukens; *Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada N1G 2W1.*

3:00 PM D3

Ultrastructural analysis of compatible and self-incompatible pollinations in *Arabidopsis* spp. Darya Safavian* and Daphne Goring; *Department of Cell and Systems Biology, University of Toronto, Toronto, ON*

3:15 PM D4

Arabidopsis Basic Leucine-Zipper Transcription Factors TGA9 and TGA10 Interact with Floral Glutaredoxins ROXY1 and ROXY2 and Are Redundantly Required for Anther Development. Jhadeswar Murmu¹, Michael J. Bush¹, Catherine DeLong², Shutian Li³, Mingli Xu¹, Madiha Khan¹, Caroline Malcolmson¹, Pierre R. Fobert², Sabine Zachgo³, and Shelley R. Hepworth¹; ¹ *Department of Biology, Carleton University, Ottawa, Ontario, Canada K1S 5B6*; ² *National Research Council Canada, Plant Biotechnology Institute, Saskatoon, Saskatchewan, Canada S7N 0W9*; ³ *Department of Botany, University of Osnabrück, 49076 Osnabruck, Germany*

3:30 PM D5

Utilizing synteny between common bean and soybean for molecular characterization of key genes for folate synthesis pathway in common bean. Weilong Xie^{1,2}, Youn-Seb Shim¹, Alireza Navabi^{1,2} and K. Peter Pauls¹; ¹ *Department of Plant Agriculture, University of Guelph, and* ² *Agriculture and Agri-Food Canada, Guelph, Ontario, Canada, N1G 2W1.*

3:50 PM – 4:10 PM. Coffee break and poster viewing.

Chairperson: **Sheila Macfie**, University of Western Ontario

4:10 PM - 5:25 PM Speaker Session E & Award Ceremony (RoomAS202)

4:10 PM E1

Newly developed *in vivo* vector system to analyze promoter activities. Michelle Moody¹, Mahbuba Siddiqua¹, Zamir Jetha and Annette Nassuth; ¹These authors contributed equally to this work; *Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON*

4:25 PM E2

The Plant U-box/ARM E3 ligases as potential signalling proteins for S-Domain Receptor Kinases. Emily Indriolo, Pirashaanthy Tharmapalan, and Daphne R. Goring; *Department of Cell & Systems Biology, University of Toronto, Toronto, Ontario*

4:40 PM E3

1. Alternative Oxidases of Non-Angiosperm Plants. Karina Neimanis and Allison E. McDonald; *Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, N2L 3C5, Canada.*

4:55 PM E4

2. Arabidopsis RING E3 Ligase XBAT32 Regulates Lateral Root Production through Its Role in Ethylene Biosynthesis. Madhulika E. Prasad, Andrew Schofield, Wendy Lyzenga^{*}, Hongxia Liu and Sophia L. Stone; *Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4R2.*

5:10 PM Student Awards and Concluding Remarks (room AS202)

Poster Titles: Please Use Poster Board with your Number on it.

- 1. The Use of Various Microscopy Techniques to Image Bacteria-Root Associations.** Melanie Columbus^{1*}, Sheila M. Macfie¹, Gordon Southam²; ¹*Department of Biology, University of Western Ontario, London, ON*; ²*Department of Earth Sciences, University of Western Ontario, London, ON*
- 2. Histochemical distribution of cadmium in lettuce (*Lactuca sativa*) and barley (*Hordeum vulgare*) roots.** M.F. Akhter^{*} and S. M. Macfie; *Department of Biology, University of Western Ontario, London, Ontario N6A 5B7.*
- 3. Isolation of antibacterial compound from rhizobacteria antagonistic to *Clavibacter michiganensis* subsp. *Michiganensis*.** Fazli Mabood^{*}, Alfred Souleimanov, Donald L. Smith; *Department of Plant Science, McGill University/Macdonald Campus, Ste Anne de Bellevue, Qc.*
- 4. Ancient leaf beetles reflexively bleed azoxyglucosides sequestered from their host cycad plants.** Alberto Prado¹, Jacqueline C. Bede¹, Donald Windsor²; ¹*Department of Plant Science, McGill University, Ste-Anne-de-Bellevue, QC*; ²*Smithsonian Tropical Research Institute, Balboa-Ancon, Panama*
- 5. Sauvignon Blanc expresses a gene highly similar to AMAT, responsible for the “foxy” aroma of *Vitis labrusca* berries.** Brent Wiens^{*}, Vincenzo De Luca. *Department of Biological Sciences, Brock University, St. Catharines, ON*
- 6. DAT gene expression in *Catharanthus*; Reactivation and specific expression in homologous and heterologous systems.** Abdullah Makhzoum^{*}, Geneviève Petit-Paly, Benoit St. Pierre and Mark A. Bernards; *Environmental Stress Biology Group, Department of Biology, The University of Western Ontario, London, ON, Canada N6A 5B7*
- 7. Screening for high and altered monoterpenoid indole alkaloid lines in EMS-treated *Catharanthus roseus* plants.** Matthew Czerwinski^{*}, Kyung Hee Kim, Antje Thamm, Vincenzo De Luca, *Department of Biological Sciences, Brock University, St. Catharines, ON, L2S 3A1.*
- 8. Comparative metabolic and genomic analysis of monoterpenoid indole alkaloid (MIA) medicinal plants.** Sayaka Masada-Atsumi, Kyung Hee Kim, Dylan Levac, Vincenzo De Luca; *Department of Biological Sciences, Brock University, St. Catharines, ON*
- 9. Cloning and Characterization of Amyrin Synthase, CrAS Involved in Triterpenes Biosynthesis in Leaf Epidermis of *Catharanthus roseus*.** Fang Yu, Antje Thamm, and Vincenzo De Luca; *Department of Biological Sciences, Brock University, St Catharines, Ontario, L2S 3A1, Canada*

10. **¹⁴C-fixation and export of sugars and monoterpenes in leaves of two genera of the Plantaginaceae.** I. Szucs*, M. Escobar, R. Cloutier, C. Beninger, D. Leonardos, and B. Grodzinski; *Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada N1G 2W1.*
11. **Quantitative Trait Loci (QTL) for Fiber Characteristics in Soybean Residue for Composite Materials.** Yarmilla Reinprecht¹, Mohammad Arif¹, Gary R. Ablett², Vaino W. Poysa³, Istvan Rajcan¹, Leonardo Simon⁴ and K. Peter Pauls¹; ¹*University of Guelph, Department of Plant Agriculture, Guelph, ON N1G 2W1*; ²*University of Guelph, Ridgetown Campus, Ridgetown, ON N0P 2C0*; ³*Agriculture and Agri-Food Canada, Greenhouse and Processing Crops Research Centre, Harrow, ON N0R 1G0*; ⁴*University of Waterloo, Department of Chemical Engineering, Waterloo, ON N2L 3G1*
12. **Fatty Acid ω -Hydroxylases in Soybean.** Jessica Koteles* and Mark A. Bernards; *Department of Biology, University of Western Ontario, London, ON, Canada.*
13. **Arabidopsis LONG-CHAIN ACYL-COA SYNTHETASE 1 (LACS1), LACS2, and LACS3 facilitate fatty acid uptake in yeast.** Ian Pulsifer*, Sabine Kluge, and Owen Rowland; *Department of Biology and Institute of Biochemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6.*
14. **Three Arabidopsis Fatty Acyl-CoA Reductases, FAR1, FAR4, and FAR5, Generate Primary Fatty Alcohols Associated with Suberin Deposition.** Sollapura Vishwanath^{*,1}, Frédéric Domergue², Jérôme Joubès², Jasmine Ono¹, Jennifer Lee¹, Matthieu Bourdon², Reem Alhattab¹, Christine Lowe¹, Stéphanie Pascal², René Lessire², and Owen Rowland¹; ¹*Department of Biology and Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada, K1S 5B6*; ²*Laboratoire de Biogenèse Membranaire, Université Victor Ségalen Bordeaux 2, CNRS, UMR 5200, 146 rue Léo Saignat, Case 92, 33076 Bordeaux Cedex, France*
15. **The role of extracellular glycosidases in the *Pythium irregulare* - ginseng pathosystem.** Dimitre A. Ivanov*, Mark A. Bernards; *Department of Biology, University of Western Ontario, London, ON.*
16. **Adenosine kinase activity contributes to intracellular cytokinin homeostasis.** B. Moffatt¹, S. Schoor¹, S. Lee¹, S. Farrow², H. Turčinov³, K. von Schwartzberg³, N. Emery²; ¹*University of Waterloo, Waterloo, Canada*; ²*Trent University, Peterborough, Canada*, ³*Universität Hamburg, Hamburg, Germany*
17. **Accumulation of therapeutic protein IL-10 in model plant *Arabidopsis thaliana*.** Ling Chen, Lazlo Gyenis, Jim Brandle, Sangeeta Dhaubhadel; *Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, ON*

18. **Characterisation of the GTP binding domains of Toc159 and Toc33 using Circular Dichroism and Fluorescence Spectroscopy – A new approach to investigate structural and mechanistic details of heterodimerization.** Geetika Patel^{1,2}, Matthew D. Smith¹ and Arthur G. Szabo². *Department of Biology¹ & Department of Chemistry², Wilfrid Laurier University, Waterloo, ON.*
19. **Cold acclimated winter cereals exhibit an enhanced CO₂ assimilation under long-term growth at elevated CO₂** Keshav Dahal^A, Khalil Kane^B, Pooja Sharma^A, Fathey Sarhan^B, Bernard Grodzinski^C and Norman PA Hüner^A; ^A*Department of Biology and The Biotron Environmental Climate Change Research Centre, University of Western Ontario, London, Ontario Canada N6A 5B7.* ^B*Department of Biological Sciences, Université du Québec à Montréal, Montreal, Quebec H3C 3P8.* ^C*Department of Plant Agriculture, University of Guelph, Bovey Building, Guelph, Ontario, Canada N1G 2W1.*
20. **Shade-Intolerant *Embotrium coccineum* (Proteaceae) exhibits plasticity with respect to photoacclimation.** Zuñiga-Feest, A., Krol, M, Dahal, K., Ivanov, A. and Hüner, N.P.A. *Universidad Austral de Chile, Valdivia, Chile; Dept. of Biology and The Biotron Experimental Climate Change Research Centre, University of Western Ontario, London, Canada.*
21. **Non-Photochemical Quenching of Chlorophyll Fluorescence Among Marine Diatoms.** Allen Derks*, Doug Bruce; *Department of Biological Sciences, Brock University, St. Catharines, Ont., L2S 3A1*
22. **Growth of Green *Arabidopsis thaliana* Cell Cultures Occurs Independently of Photosynthesis.** Michelle Chung*, Marianna Krol, Alexander G. Ivanov, Aimen Naeem, Norman P.A. Hüner *Department of Biology and The Biotron Experimental Climate Change Research Centre, University of Western Ontario*
23. **Effect of light, ABA and cytoskeletal inhibitors on the clumping of chloroplasts in leaves of *Kalanchoë blossfeldiana*, a succulent CAM plant.** Ayumu Kondo^{*1,2}, Shingo Suminoe¹, Kato Hideyuki¹, Msahiro Tawata¹, Toru Funaguma¹; *Simon Chuong*². ¹*Faculty of Agriculture, Meijo University, Nagoya, Japan.* ²*Department of Biology, University of Waterloo, Waterloo, ON.*
24. **The impact of artificial night lighting in an urban environment on plant photosynthesis and gene expression;** J. Skaf^{†1*}, E. T. Hamanishi^{†2}, O. Wilkins¹, S. Raj¹, M. M. Campbell^{1,3}; ¹*Department of Cell & Systems Biology, University of Toronto, Toronto, ON M5S 3B2,* ²*Faculty of Forestry, University of Toronto.*
25. **In vitro regenerated wetland sedge *Eriophorum vaginatum* L. via callus culture is genetically stable;** Monika Rewersa,^b Jennifer Drouina,^b Elwira Sliwinskab and Ewa Cholewaa; ^a *Department of Biology, Nipissing University, North Bay, ON, P1B 8L7, Canada;* ^b *Laboratory of Molecular Biology and Cytometry, Department of Plant Genetics and Biotechnology, University of Technology and Life Sciences, Bydgoszcz, Poland*

26. **Regeneration of pepper (*C. annuum* L.) haploid plants through *in vitro* androgenesis.** Anna Kisiała¹, Paweł Nowaczyk²; ¹*Nipissing University, North Bay, ON*; ²*University of Technology and Life Sciences, Bydgoszcz, Poland*
27. **Comparative rooting response of chrysanthemum cuttings to short and long methods of exogenous auxin application.** Hussain Ahmad* and Theo Blom; *Department of Plant Agriculture, University of Guelph, Guelph, ON, N1G 2W1*
28. **Effect of light quality on total nonstructural carbohydrate (TNC) content and rooting of cuttings of chrysanthemum cultivars.** Hussain Ahmad*, David Kerec and Theo Blom; *Department of Plant Agriculture, University of Guelph, Guelph, ON, N1G 2W1. Toronto, ON M5S 3B2*, ³*Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto, ON M5S 3B2*.
29. **Low-temperature photosynthetic performance of two winter annual grass invaders.** Olga Bykova* & Rowan F. Sage; *Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON*
30. **Genotype by environment interaction for fibre and starch profiles of potato (*Solanum tuberosum* L.).** Stephanie Bach*¹, J. Alan Sullivan¹, Benoit Bizimungu², Agnes Murphy² and Rickey Yada³; ¹*Department of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1*; ²*Agriculture and Agri-Food Canada, Fredericton, NB E3B 4Z7*; ³*Department of Food Science, University of Guelph, Guelph, ON N1G 2W1*.
31. **Photoprotective isoprenoids increase drought stress tolerance of Douglas fir.** Laura Junker*^{1,2}, Anita Rott³, Jürgen Kreuzwieser³, Ingo Ensminger^{1,2}; ¹*Forest Research Institute Baden-Wuerttemberg, Department of Forest Ecology, Freiburg, Germany*; ²*University of Toronto at Mississauga, Department of Biology, Mississauga, Canada*; ³*University of Freiburg, Chair of Tree Physiology, Freiburg, Germany*
32. **Stomatal development and drought in *P. balsamifera*.** Hamanishi, E.T.^{1*} and M.M. Campbell^{2,3}; ¹*Faculty of Forestry*, ²*Department of Cell and Systems Biology*, ³*Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto, ON M5S 3B2*.
33. **Inorganic carbon uptake at acid pH by the alga *Chlorella kessleri*.** Omar El-Ansari and Brian Colman; *Department of Biology, York University, Toronto*
34. **Inorganic carbon acquisition in the acid-tolerant alga *Stichococcus bacillaris*.** Christopher Powe and Brian Colman; *Department of Biology, York University, Toronto, ON*
35. **Mapping and characterization of a developmental mutant in *Zea mays*.** Luis M. Avila*, Gregory Downs, Lewis Lukens; *Department of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1*

36. **Genetic and physiological characterization of a water stress sensitive *Zea mays* mutant.** Robert Bruce*, Lewis Lukens; *Department of Plant Agriculture, University of Guelph, Guelph, ON.*
37. **Re-annotation of microarray platforms using recent genomic sequence data.** Gregory Downs* and Lewis Lukens; *Department of Plant Agriculture, University of Guelph, Guelph, ON.*
38. **14-3-3 proteins regulate the nuclear-cytoplasmic distribution of GmMYB176 involved in isoflavonoid synthesis in soybean.** Xuyan Li¹, Dasom Kim², Sangeeta Dhaubhadel^{1,2}; ¹*Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, ON;* ²*Department of Biology, University of Western Ontario, London, ON*
39. **Characterisation of a transcriptional circuit involving the transcription factor, *AtMYB61*;** Michael Prouse*^{1,2}, Julia Romano^{1,2}, Christian Dubos¹, and Malcolm M. Campbell^{1,2}; ¹*Department of Cell & Systems Biology,* ²*Centre for the Analysis of Genome Evolution & Function, University of Toronto, ON M5S 3B2, CANADA*
40. **Antagonistic interaction between *BLADE-ON-PETIOLE1/2* and *BEL1*-like homeobox genes *PENNYWISE* and *POUND-FOOLISH* co-ordinates flowering and *Arabidopsis* inflorescence architecture.** Paul Tabb*, Madiha Khan, Michael Bush, Jinhyung Cheong, and Shelley R. Hepworth. *Department of Biology, Carleton University, Ottawa, Ontario, Canada, K1S 5B6.*
41. **Antagonistic interaction of *BLADE-ON-PETIOLE1* and 2 with *BREVIPEDICELLUS* and *PENNYWISE* regulates *Arabidopsis* inflorescence architecture.** Madiha Khan*, Mingli Xu*, Tieqiang Hu, Paul Tabb, Jhadeswar Murmu, Sarah M. McKim, Lama Musa, Kathryn Storey, Jethro Mercado, and Shelley R. Hepworth; *Department of Biology, Carleton University, Ottawa, ON, K1S 5B6.*
42. **The role of the exocyst subunit, *Sec15*, in the *Arabidopsis thaliana* compatible pollen response.** Yara Zayed*, Laura A. Chapman, and Daphne R. Goring; *Department of Cell and Systems Biology, University of Toronto, ON M5S 3B2, Canada*
43. **RNA Expression Analyses of Plant U-box/ARM family members in *Arabidopsis thaliana*.** Pirashaanthi Tharmapalan*, Emily Indriolo, and Daphne R. Goring; *Department of Cell & Systems Biology, University of Toronto, Toronto, Ontario.*
44. **Validation of *de novo* bioinformatic predictions of *Arabidopsis thaliana* cis-regulatory elements using *in planta* GFP/GUS expression assays.** Shuxian Hiu¹, Ryan Austin¹ and Nicholas Provar^{1,2}; ¹*Department of Cell & Systems Biology,* ²*Genome Biology & Bioinformatics, University of Toronto, Toronto, ON M5S 3B2.*

CONFERENCE ABSTRACTS

Oral Presentations

A1. Effect of stress on chloroplast arrangement in single cell C4 photosynthetic species

Sarah M. Schoor* and Simon D.X. Chuong

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In the family Chenopodiaceae, *Bienertia sinuspersici* is able to perform single cell C4 photosynthesis by partitioning chloroplasts into two distinct cytoplasmic compartments: one with chloroplasts containing decarboxylation enzymes (peripheral chloroplast; PCC), the other RuBisCO (central chloroplast; CCC). Since the discovery of single cell C4 photosynthesis in terrestrial plants, much interest has been focused on how this process is able to occur without Kranz anatomy. To assess the importance of this unique chloroplast arrangement, various stresses such as light, temperature and hormones were applied to *Bienertia* cells. PCC arrangement was not disrupted as a result of stress treatment, but CCC was. To determine whether C4 photosynthesis is still capable of being performed in plants with dissociated CCC, immunoblot analysis was performed; preliminary results show C4 photosynthetic enzymes are still present. Further immunolabelling studies show a loss of microtubule organization as a result of stress treatment, implicating the cytoskeleton in the disorganization of the CCC. Future study will focus on the recovery of the cytoskeleton chloroplast arrangement and assessing photosynthetic activity.

A2. Distribution of photosynthetic enzymes and transcripts in chlorenchyma cells of *Bienertia sinuspersici* during leaf development

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Bienertia sinuspersici is a C₄ species that can perform C₄ photosynthesis within a single cell by partitioning chloroplasts and enzymes in two cytoplasmic compartments, the central cytoplasmic compartment (CCC) and peripheral cytoplasmic compartment (PCC). TEM immunolocalization studies were used to examine the relative abundance of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) large subunit and orthophosphate dikinase (PPDK) polypeptides in the dimorphic chloroplasts at different stages of leaf development. High levels of Rubisco proteins were present in chloroplasts of both compartments at early stages and became preferentially localized in chloroplasts of the CCC of mature leaves. In contrast, low levels of PPDK proteins were detected in both chloroplasts at early stages and increased substantially in the PCC chloroplasts as the leaves matured. Real time RT-PCR and in situ hybridization studies were used to examine the expression of Rubisco large subunit (*rbcL*) transcript. The expression of *rbcL* transcripts appeared to correlate the protein accumulation. *rbcL* transcripts accumulated in both types of chloroplasts at early stages and was detectable only in the CCC chloroplasts in mature cells. These results indicate that the regulation of transcript abundance and selective accumulation of proteins in different chloroplasts are essential biochemical processes in the development of single-cell C₄ photosynthesis.

A3. Refixation of photorespiratory CO₂ as a method to minimize carbon loss in C₃ grasses

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Plants lacking a C₄ cycle do not have the means to efficiently suppress photorespiration. Some C₃ grasses may be able to limit their carbon loss due to their chloroplast structure, enhancing refixation of respired and photorespired CO₂. We developed a new method involving the measurement of carbon isotopes to estimate the rates of refixation in attached leaves of *Triticum aestivum* and *Oryza sativa*. The method distinguishes between CO₂ fluxes into and out of the leaf, enabling us to determine the rates of net and gross CO₂ assimilation as well as CO₂ evolution. Using different concentrations of ¹³CO₂, the method allowed us to quantify the fraction of respired CO₂ that was refixed from the intercellular space, as well as intracellular refixation of respired CO₂. Since the method can distinguish between photo- and dark-type respiration, it can be used as an independent measurement of mitochondrial respiration in the light. In *Triticum aestivum*, almost 50% of the CO₂ evolved was refixed by the plant, about half of which via intracellular and the other half via intercellular refixation. In *Oryza sativa*, the total fraction of CO₂ being refixed was slightly higher as a result of higher intracellular refixation. Our observations correspond to the chloroplast structure found in the two studied species.

A4. The effects of genotype and clonal history on shaping the drought response in Populus

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Water availability is a key determinant of tree growth and survival. Exposure to episodic drought can impinge significantly on forest health and the establishment of productive tree plantations. There is therefore great interest in understanding the mechanisms underpinning drought responses in ecologically and economically important tree genera, like *Populus*. To test hypotheses related to the transcriptional regulation of *Populus* drought responses, genome-wide changes in transcript abundance in response to water limitation were examined in economically important *Populus* clones. To study the interaction between clone history and drought, individuals of the same genotype were obtained from geographically distinct locations and their response to water limitation was studied at the physiological and molecular level under controlled laboratory conditions. Intriguingly, transcriptome analyses using the Affymetrix GeneChip technology uncovered differences in transcript abundance patterns based on differences in geographic origin of clones with identical genotypes. Similarly, differences in DNA methylation based on clone history were observed. The data provide insights into the interplay between genotype and environment, and hint at the potential role played by epigenetic phenomena in this interaction. Moreover, the data provide mechanistic insights into applied questions related to the nursery source of poplar clones and how that impacts on future clone performance in plantations, and into remarkable plasticity in poplar drought responses.

A5. Genotype by environment interaction for fibre and starch profiles of potato (*Solanum tuberosum* L.)

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Potatoes have been a staple for hundreds of years but can be relatively high in glycemic index (GI). Due to this, the potato has lost favor in many diets and the positive benefits are being outweighed by potential health problems. Potatoes contain two different types of starch: amylose and amylopectin. Structural differences between amylose and amylopectin contribute to variations in breakdown after ingestion, affecting GI. These differences contribute to three categories of starch: rapidly digestible (RDS), slowly digestible (SDS) and resistant (RS). RS is commonly associated with dietary fibre and health benefits. There are two components to fibre: soluble and insoluble, which have different functional properties in the colon. Our research examines the genotype by environment influence on fibre and starch profiles. Field trials were conducted in 2009 and 2010 with 12 potato genotypes in three locations across Southern Ontario. Analysis of fibre and starch profiles will help elucidate the genotypic and phenotypic framework through which human health is affected by what we ingest. Potatoes were analysed using protocols adapted from Sigma, Ankom, Megazyme and Englyst *et al.* (1992). Analysis from one field season indicates genotypic, environmental and genotype by environment influences on fibre and starch.

B1. Chemical screen uncovers link between sugar signalling and one-carbon metabolism

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Sugars play an important role throughout plant development, acting as structural components, metabolic intermediates, and sources of energy. Because of this, plants have evolved numerous mechanisms through which cellular carbohydrate abundance can be detected. Forward-genetic screens have proven fruitful in uncovering multiple sugar-perception pathways, but can be limited by functional redundancy and seedling lethality. As a means of circumventing these issues, a chemical genetic approach was employed to uncover novel aspects of plant sugar perception. Over 2100 compounds were screened for the ability to perturb seedling responses to exogenous sucrose. This screen revealed a group of chemicals belonging to the sulfonamide family of compounds, known to inhibit one-carbon (C1) metabolism in plants, to restrict hypocotyl elongation in a sucrose-dependent fashion. Mutant and pharmacogenetic analyses suggest this is a HEXOKINASE1-independent phenomenon related to the synthesis of folates. Microarray-based transcriptome analysis identified a small set of transcripts that show altered responses to the compound when administered in the presence of sucrose, including genes implicated in auxin signal transduction. Complementary reverse- and forward-genetic screens are currently being pursued to unveil the genetic basis for crosstalk between C1 metabolism and sugar signalling in Arabidopsis.

B2. Characterization of GDP-O-Fucosyltransferase in *B.napus* Microspore Embryogenesis

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Microspore embryogenesis (ME) is a developmental phenomenon in which microspores divert from their gametophytic pathway towards embryonic development when stressed or cultured. It is an effective breeding tool to generate doubled haploids for efficient canola (*Brassica napus*) cultivar development. Although highly efficient, there are only a few genotypes that are highly 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.

Our previous microarray analysis enabled us to identify genes that were overexpressed in embryogenic cells. Of special interest is the Arabidopsis gene, AT2G44500 in which a Brassica transcript hybridized to and was consistently upregulated in embryogenic cells compared to pollen-like cells. AT2G44500 is highly expressed in the rib meristem and has a conserved GDP-O-Fucosyltransferase (O-FuT) domain in Arabidopsis. We have cloned and sequenced two complete genomic *B.napus* homologs, *BnOFuT1* and *BnOFuT2* and show that the intron-exon organization and the O-FuT domain are conserved in these homologs. We are currently endeavouring to characterize the function of this novel GDP O-Fucosyltransferase in *B.napus* and its involvement in microspore embryogenesis.

B3. Vinca drug components accumulate exclusively in leaf exudates of Madagascar periwinkle

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The vinca alkaloids of Madagascar periwinkle (*Catharanthus roseus*) are well known to be the most important source of drugs used in various cancer chemotherapies. These monoterpene indole alkaloids (MIAs) are derived from the coupling of catharanthine and vindoline to produce dimers that prevent cell division. However, the precise mechanisms for their assembly within plants are poorly understood. In this work, we report that the highly regulated MIAs pathway is matched by secretory mechanisms that keep catharanthine and vindoline separated from each other in living plants. Analysis of the leaf surface extract of *Catharanthus* leaves confirmed that catharanthine accumulates in the leaf wax exudates of leaves whereas vindoline is found within leaf cells. The ability of catharanthine to inhibit the growth of fungal zoospores and its insect toxicity provide an additional ecological role for its secretion. The spatial separation of MIAs provides a biological explanation for the low levels of dimeric anticancer alkaloids found in periwinkle plants that result in their high cost of commercial production.

B4. Identification and characterization of members of the *Chalcone Isomerase* gene family involved in isoflavonoid biosynthesis of *Glycine max*

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Isoflavonoids are plant secondary metabolites produced via a legume-specific branch of the phenylpropanoid pathway. They are essential to the physiological functions of the legume, as signaling molecules for nodulation of nitrogen-fixing bacteria, and as precursors to phytoalexins that inhibit plant pathogen infections. Isoflavonoids are noted for their human health benefits including, roles in reducing the risk of hypercholesterolemia, cardiovascular disease and hormone-dependent cancers. A better understanding of the molecular and genetic basis of isoflavonoid biosynthesis will allow manipulation of isoflavonoid composition in legumes, including soybean and its introduction into non-legumes for human health, as well as agricultural benefits.

The second step in the isoflavonoid biosynthetic pathway is the conversion of a chalcone to a flavanone catalyzed by Chalcone Isomerase (CHI). We have identified eight CHI genes in the soybean genome, including the novel CHI3B using a bioinformatic approach. We have established that CHI2 expression corresponds with higher isoflavonoid levels in soybean roots, through quantitative gene expression analysis of CHI genes in cultivars with different isoflavonoid content. The function of CHI2 in planta will be determined through RNAi silencing using soybean hairy roots, and subsequent analysis of isoflavonoid levels in transgenic hairy roots.

B5. Plants have two COG0354 proteins with folate-dependent functions in the metabolism of iron-sulfur clusters

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COG0354 proteins have been implicated in synthesis or repair of iron/sulfur (Fe/S) clusters in all domains of life, and those of bacteria, animals, and protists have been shown to require a tetrahydrofolate to function. Two COG0354 proteins occur in Arabidopsis and many other plants, the first (At4g12130) related to those of α -proteobacteria and predicted to be mitochondrial, the second (At1g60990) related to those of cyanobacteria and predicted to be plastidial. Grasses and certain other taxa lack the latter. The subcellular locations of the Arabidopsis proteins were validated by green fluorescent protein fusions and in vitro import assays. An At4g12130 insertional mutant was embryo lethal, whereas inactivation of At1g60990 appears to exhibit a normal phenotype. Deletion of the COG0354 in *Synechococcus* impaired photoautotrophic growth in iron-limiting or oxidative stress conditions and caused accumulation of reactive oxygen species. These data establish that COG0354 proteins have a tetrahydrofolate-dependent function in mitochondria and plastids. This function almost certainly involves Fe/S cluster metabolism, and probably dates back to their respective bacterial progenitors.

C1. Siderophore-Independent Iron Uptake in the Cyanobacterium *Anabaena flos-aquae*

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Iron acquisition by iron-limited cyanobacteria is typically considered to be mediated mainly by siderophores, iron-chelating molecules released by iron-limited cyanobacteria into the environment. In this set of experiments, iron uptake by iron-limited cells of the cyanobacterium *Anabaena flos-aquae* was investigated in cells resuspended in siderophore-free medium. Removal of siderophores decreased iron uptake rates by approximately 60% compared to siderophore-replete conditions, however substantial rates of iron uptake remained. In the absence of siderophores, Fe(III) uptake was much more rapid from a weaker synthetic chelator (HEDTA; log K = 19.7) than from a very strong chelator (HBED; log K = 39.0), and increasing chelator:Fe(III) ratios decreased the Fe(III) uptake rate; these results were evident both in short-term (4 h; absence of siderophores) and long-term (116 h; presence of siderophores) experiments. The results of the short-term experiments are consistent with a Fe(III) binding and uptake mechanism associated with the cyanobacterial outer membrane that operates independently of extracellular siderophores. Iron uptake was inhibited by metabolically compromising the cells with diphenyliodonium; this indicates that the process is dependent on active metabolism to operate and is not simply a passive Fe(III) binding mechanism. Overall, these results point to a siderophore-independent iron acquisition mechanism by cyanobacterial cells.

C2. Examining the role of the TOC complex in selective protein import into dimorphic chloroplasts in the single-cell C₄ species *Bienertia sinuspersici*

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Bienertia sinuspersici is one of the three terrestrial plants that have been discovered to perform C₄ photosynthesis in single chlorenchyma cells, contradicting the dual-cell arrangement in typical C₄ plants. The single-cell C₄ systems feature two distinct chloroplast types in separate cytoplasmic compartments. While anatomical and biochemical data aided with the identification of a novel single-cell C₄ cycle via subcellular compartmentation, a rationale behind the differential localization of the key photosynthetic enzymes to the dimorphic chloroplasts is currently lacking. To investigate the selective targeting of proteins into chloroplasts of *B. sinuspersici*, we characterized the translocon at the outer envelope membrane of chloroplasts (TOC). Biochemical properties of Toc159 receptors implicate their roles in governing substrate specificity of the translocon complexes. Expression of Toc159 isoforms is differentially regulated during the development of chlorenchyma cells. Transient expression of fluorescent protein-tagged Toc159 isoforms and immunogold electron microscopy revealed their unique subcellular localization distinct from other chloroplast envelope proteins. Current research aims at elucidating the roles of these Toc receptors in the differential import of proteins into dimorphic chloroplasts in the single-cell C₄ system.

C3. Expression, purification, and biophysical analysis of Tic20, the putative pre-protein channel of the chloroplast inner envelope membrane

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Plant cells use sophisticated protein targeting and import machinery to deliver essential nuclear-encoded proteins to chloroplasts. When pre-proteins reach the chloroplast, the Tic-Toc (translocon of the inner/outer chloroplast membrane) machinery mediates transit peptide-dependent protein import across the chloroplast double membrane. Research on Tic20, a candidate for the Tic translocation channel, has been limited because the mature form of Tic20 from *Arabidopsis thaliana* (m-atTic20) had yet to be heterologously expressed in *E. coli*. The objectives of this study were to express and purify recombinant m-atTic20 and perform in vitro analyses to determine whether Tic20 could constitute part of the Tic translocation channel. cDNA corresponding to m-atTic20 was amplified by PCR and cloned into the pET21a expression vector. The recombinant protein was then expressed using the BL21 Codon-Plus (DE3)-RIPL strain of *E. coli*. Recombinant m-atTic20 was purified using Ni-NTA chromatography, reconstituted using detergents or lipids, and analyzed using BN-PAGE and CD-spectroscopy. m-atTic20 reconstituted in a mild detergent possessed 34.5% α -helical character with a propensity towards forming multi-subunit structures. These findings are consistent with the notion that Tic20 could be the channel protein across the inner membrane, and lay groundwork for additional experiments aimed at determining the native structure and function of Tic20.

C4. Preprotein specificity of the Toc159 family of chloroplast protein import receptors

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The Toc159 family of proteins serve as the primary receptors for preprotein import into chloroplasts. A working model states that Toc159 and Toc132 are receptors that preferentially recognize photosynthetic and non-photosynthetic preproteins, respectively. Relatively few chloroplast preproteins have been assigned as substrates for particular members of the Toc159 family, which has limited the proof for the hypothesis. Our study aims to expand the number of known preprotein substrates for each member of the Toc159 receptor family using a split-ubiquitin membrane-based yeast two-hybrid system starting with the Toc159 G-domain (Toc159G) as the bait. cDNA library screening shows that Toc159G is sufficient for preprotein recognition, but it alone does not confer specificity for photosynthetic or non-photosynthetic preproteins. To shed more light on the question of substrate specificity, the same assay was performed using the Toc132 G-domain as the bait. Toc132G interacted with all the proteins that were recognized by the Toc159G, but the strength of individual interactions was preprotein specific for both the baits. We are currently expanding the study to assess the role of other domains of Toc159 (i.e. the A-domain) in preprotein recognition. In total, the study will contribute to our understanding of how preprotein substrate selectivity of Toc159/Toc132 is achieved.

C5. Investigating the genetic basis for naturally occurring primary root growth variation in *Zea mays*

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Genetic variation in root growth rates is continuous and highly sensitive to environmental perturbation, making it difficult to partition the variance to causative loci. To investigate the genetic basis of plant root growth variation, we examined 206 inbred lines genotyped at over 1300 markers. These lines were derived from a cross of two parents, B73 and Mo17, which have different rates of primary root growth. We identified nine loci that influence primary root growth. To confirm the effects of these loci we introgressed two B73 alleles from chromosome 1 and chromosome 5 into Mo17. Both introgressed segments, Mo17-B1.03 and Mo17-B5.06, enhanced root growth. To investigate the molecular basis for the Mo17-B1.03 phenotype, we identified genes differentially expressed between Mo17-B1.03 and Mo17 without the introgressed segment (Mo17-M1.03) in control and osmotic stress conditions. A number of genes were found to be differentially expressed 1) between Mo17-B1.03 and Mo17-M1.03 within the same conditions and 2) between the two conditions within the same genotype. Genes associated with oxidative stress are differentially expressed suggesting a role for free radical scavenging in explaining genetic diversity for root growth.

D1. Genetic variation for *Arabidopsis thaliana* heterotic responses is in large part explained by the epistatic interaction of flowering time genes FRIGIDA and Flowering Locus C.

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Heterosis is widespread in nature and of critical importance in agriculture. A diallel analysis using five parental ecotypes (Col, Ler, Cvi, Ws and C24) was performed to investigate inheritance of 12 traits in *Arabidopsis thaliana* hybrids. Of the 20 hybrids, only hybrids of C24 with Col, Cvi and Ws were highly heterotic for traits related to flowering time while C24 and Ler were weakly heterotic. C24 hybrids also had significant heterosis for other traits including biomass and yield. C24 has an active FRIGIDA allele, while Col, Cvi and Ws have strong Flowering Locus C alleles. FRI and FLC act epistatically to delay flowering, suggesting this epistasis explains the high heterosis. Interestingly, Col and C24 hybrids had high heterosis for biomass but low seed yield. Two ecotypes having every combination of functional and non-functional FRI and FLC alleles were evaluated and crossed with C24 to determine their effects on traits. Lines containing functional FRI and FLC had larger trait values for flowering and biomass related traits, suggesting that the heterosis observed in the diallel hybrids for these traits could be largely explained by the epistatic interaction. FRI and FLC also explain the poor seed yield in the C24 and Col hybrids.

D2. Patterns of *cis* natural antisense transcripts across stress growth conditions in *Arabidopsis thaliana* suggest RNA interference activity

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Natural antisense transcripts (NATs), divided into *cis*-NATs and *trans*-NATs, are endogenous transcripts that have sequence complementarity to corresponding sense transcripts. In the *Arabidopsis* genome, transcripts from neighbouring genes could putatively overlap and form dsRNA. NATs have been involved in a wide range of biological processes including RNA interference. However, whether mutual regulation of *cis*-NATs expression is a common or an exceptional phenomenon is unknown. To test the hypothesis that if two *cis*-NATs are co-expressed and inversely expressed, then they are candidates for producing siRNAs, we analysed 53.44 million RNA-seq microreads from plants subjected to six different abiotic stresses. Analysis of this RNA-seq data revealed that the median expression levels and expression breadths of *cis*-NAT genes are significantly higher than non-*cis*-NAT genes. The percentage of *cis*-NAT gene pairs with genes that are both expressed across samples is significantly higher than expected by chance (89% versus 80%, $P=3.33e-16$). Furthermore, *cis*-NAT pairs with genes inversely expressed occur significantly more frequently than expected by chance (62% vs 56%, $P=1.3e-5$). The inversely expressed *cis*-NAT pairs also tended to be highly co-expressed. Our results suggest that co-expressed and inversely expressed *cis*-NATs are more likely siRNA targets than other genes.

D3. Ultrastructural analysis of compatible and self-incompatible pollinations in *Arabidopsis* spp.

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Our overall research interest is studying the molecular and cellular mechanisms of polarized exocytosis during pollen-pistil interactions in plants. The exocyst, an evolutionary conserved protein complex consisting of eight subunits, has been shown to involve in polarized secretion in yeast and mammalian systems, where it mediates the tethering of secretory vesicles to the plasma membrane. Recently, we have identified one predicted subunit of the exocyst, Exo70A1, to be involved in compatible pollen response in *Brassica napus* and *Arabidopsis thaliana*. With the discovery that Exo70A1 is required in the stigmatic papillae for the compatible pollen response, we hypothesize that Exo70A1 functions as part of the exocyst complex to tether secretory vesicles to the plasma membrane at the pollen contact site. This is thought to then result in water transport to the pollen grain for hydration as well as the expansion of the papillar cell wall to promote pollen tube penetration and subsequent fertilization following compatible pollination. Using transmission electron microscope (TEM), we are examining the presence or absence of secretory vesicles in different Brassicaceae species following compatible or self-incompatible pollinations. Our preliminary results show the presence of secretory vesicles at the plasma membrane for compatible pollinations while absent for self-incompatible pollinations. As well, *exo70A1-1 A. thaliana* mutants appear to have secretory vesicles accumulated in the stigmatic papilla cytoplasm as seen for yeast exocyst mutants.

D4. Arabidopsis Basic Leucine-Zipper Transcription Factors TGA9 and TGA10 Interact with Floral Glutaredoxins ROXY1 and ROXY2 and Are Redundantly Required for Anther Development

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ROXY1 and ROXY2 are CC-type floral glutaredoxins with redundant functions in *Arabidopsis thaliana* anther development. We demonstrated that plants lacking the basic leucine-zipper transcription factors TGA9 and TGA10 have defects in male gametogenesis that are strikingly similar to *roxy1 roxy2* mutants. In *tga9 tga10* mutants, early steps in anther development are blocked in adaxial lobes. In abaxial lobes, microscope development proceeds to the production of inviable pollen grains within nondehiscent anthers. Histological analysis shows multiple defects in the anther dehiscence program, including abnormal stability and lignification of the middle layer, and defects in septum and stomium function. *TGA9/10* are expressed throughout early anther primordia but resolve to the middle and tapetum layers during meiosis of pollen mother cells. Several lines of evidence suggest that ROXY promotion of anther development is mediated in part by TGA9/10. First, *TGA9/10* expression overlaps with *ROXY1/2* in developing anthers. Second, TGA9/10 and ROXY1/2 operate downstream of SPOROCTELESS/NOZZLE, where they positively regulate a common set of tapetal development genes. Third, TGA9/10 directly interact with ROXY proteins in yeast and plant cell nuclei. These findings suggest that activation of TGA9/10 transcription factors by ROXY-mediated modification of cysteine residues promotes anther development in *Arabidopsis*.

D5. Utilizing synteny between common bean and soybean for molecular characterization of key genes for folate synthesis pathway in common bean.

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Common beans (*Phaseolus vulgaris*) are excellent sources of dietary folates, but levels of these compounds can vary among varieties. Previous results showed that high levels of folate content in bean varieties are correlated with high levels of expression of dihydroneopterin aldolase (DHNA) and aminodeoxychorismate synthase (ADCS) in the folate synthesis pathway. ADCS gene fragments were obtained by PCR with genomic DNA from core map parents Bat 93 and Jalo EEP558. Full-length DHNA sequences were obtained by screening BAC libraries from OAC Rex and G19833 genotypes and 454 sequencing. Only one single-nucleotide polymorphism was observed in the coding region between the DHNA sequences in OAC Rex and G19833. The translated sequences are identical between the two cultivars. A 157 kb contig assembled from two G19833 BAC clone sequences contained approximately 20 genes (including DHNA) which had an order that was highly syntenic with a segment of soybean chromosome 3 that is linked to (500 Kb away) but does not include DHNA. The DHNA and ADCS genes were mapped on chromosome 1 and 7 in *P. vulgaris*, respectively, by both a synteny analysis approach and by conventional mapping with core map RILS.

E1. Newly developed *in vivo* vector system to analyze promoter activities

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¹These authors contributed equally to this work

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The transcriptional activation of a promoter::reporter construct by an environmental cue or specific transcription factor can be analyzed by transient expression after agroinfiltration in tobacco leaves. However, comparing expression levels obtained with different promoter constructs, or with the same promoter construct induced by different transcription factors (TFs), can be a challenge because of differences between samples in infiltration efficiency or in the ratio of delivered reporter plasmid (harbouring the promoter) and effector plasmid (expressing the transcription factor). To account for these differences we have developed a system whereby the expression from the promoter of interest, measured as *Renilla* luciferase activity, can be normalized with the constitutive expression of firefly luciferase from the reporter plasmid, and with the constitutive expression of β -glucuronidase from the effector plasmid. Also, there is no background reporter gene activity from contaminating bacteria because all 3 reporter genes contain introns. We show the applicability of this system in 2 different experiments. First we identified a region in the *Vitis riparia* *CBF4* (*VrCBF4*) promoter which has a major effect on the level of gene expression. Next we show that the transcription factors VrCBF4 and VrCBF1 have different preferences for binding sites differing in only 1 nucleotide.

E2. The Plant U-box/ARM E3 ligases as potential signalling proteins for S-Domain Receptor Kinases

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The Plant U-box/ARM proteins are a group of E3 ubiquitin ligases defined by the presence of a U-box followed by an armadillo (ARM) repeat domain. One of the most well characterized members in the Brassicaceae is *Brassica napus* ARC1, which is necessary for the rejection of self-pollen in the self-incompatibility response. This recognition event is mediated by the binding of the SCR/SP11 pollen ligand to the stigma-specific S Receptor Kinase (SRK) that then activates a signalling pathway in the stigmatic papilla to cause pollen rejection. ARC1 functions downstream of SRK, binding to SRK through its ARM repeat domain. ARC1 is proposed to promote ubiquitination of Exo70A1 during the self-incompatibility response. In addition to the U-box and the ARM repeat domain, ARC1 contains the U-box N-terminal Domain (UND) that may mediate Exo70A1 binding. Large gene families with sequence similarity to the SRK (S-Domain-1 Receptor Kinases) and ARC1 (UND/U-box/ARM) have been identified in plant genomes. The predicted Arabidopsis genes show a variety of expression patterns, and may participate in other regulatory signalling pathways. We have found that several of the ARC1-related proteins can interact with S-Domain-1 Receptor Kinases, and using a number of genomic resources, we are investigating these gene families to determine their potential signalling pathways and biological functions.

E3. Alternative Oxidases of Non-Angiosperm Plants.

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Alternative oxidase (AOX) is a mitochondrial inner membrane protein that introduces a branch point in the respiratory electron transport chain at ubiquinol. AOX bypasses two sites of proton translocation across the inner mitochondrial membrane resulting in a lower ATP yield per oxygen consumed. Despite the fact that AOX seems energetically wasteful, AOX transcripts, protein levels, and enzymatic activity increase during environmental stress. In *Arabidopsis thaliana*, tobacco, soybean, and rice, much is known about AOX multigene families, gene expression, and post-translational regulation of the enzyme. Given the data available for angiosperm AOXs, it is surprising that it has not been studied in non-angiosperm plants as a logical starting point for comparative studies. Our results show that AOX is present in a moss, liverwort, fern, lycopod, and several species of conifers. We are confirming the expression of AOX genes using reverse transcriptase PCR in these species, as well as members of other non-angiosperm plant lineages (e.g. cycads, ginkos, etc.) for which molecular database information is currently unavailable. An analysis of AOX protein sequences from these species indicates that they are likely active quinol terminal oxidases, but predicts that they will exhibit a different mode of post-translational regulation compared to angiosperm AOX proteins.

E4. Arabidopsis RING E3 Ligase XBAT32 Regulates Lateral Root Production through Its Role in Ethylene Biosynthesis.

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XBAT32 is a member of the RING-type E3 ligase family in *Arabidopsis*. E3 ligases facilitate the transfer of ubiquitin to a target protein and the most commonly understood function of ubiquitination is degradation through the 26S proteasome. Previous research has shown that XBAT32 is required for lateral root production; however, the molecular role XBAT32 plays during lateral root production is unknown. We are currently investigating a model in which XBAT32 regulates ethylene/auxin control of lateral root production. We found that *xbat32* mutants overproduce ethylene. Inhibition of ethylene biosynthesis or perception significantly increases *xbat32* lateral root production. We provide yeast two hybrid evidence that XBAT32 is able to interact with two ethylene biosynthetic enzymes (1-aminocyclopropane-1-carboxylate synthase 4 (ACS4) and ACS7). We demonstrate *in vitro* that XBAT32 is capable of catalyzing the attachment of ubiquitin to both ACS4 and ACS7. We propose a model in which XBAT32 negatively regulates ethylene biosynthesis by modulating the abundance of ACS proteins. The protein levels of these two ethylene biosynthetic enzymes are stabilized in the *xbat32* mutant and the proposed effect is an overproduction of ethylene. We are currently investigating if an overproduction of ethylene is altering auxin transport in the root tissue of *xbat32* mutants.

POSTER ABSTRACTS

1) The Use of Various Microscopy Techniques to Image Bacteria-Root AssociationsMelanie Columbus^{1*}, Sheila M. Macfie¹, Gordon Southam²¹Department of Biology, University of Western Ontario, London, ON²Department of Earth Sciences, University of Western Ontario, London, ON

Many of the mechanistic hypotheses for how plant growth-promoting bacteria (PGPB) are able to maintain plant growth under stress conditions depend on a direct association between the plant root and bacteria. For example, the ability of the bacterial enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase to outcompete the plant enzyme ACC oxidase for the ethylene precursor ACC requires a close association between bacteria and plant tissue. It is therefore important to confirm physical proximity when studying this system. This study will use three microscopy techniques to analyze the association between two bacteria (the ACC deaminase producing bacterium UW4 and an ACC deaminase minus mutant) on *Arabidopsis thaliana* roots grown hydroponically. First, the fluorescence-based assay *BacLight*TM will be used in combination with confocal microscopy to determine the amount and location of live and dead bacteria on the root surface. Then, samples will be imaged using scanning electron microscopy to visualize bacterial association on the root surface. Finally, transmission electron microscopy will be used to analyze the thickness of biofilms growing on the root as well as any bacterial penetration into the root. Further studies could include different types and combinations of PGPB as well as roots isolated from soil.

2) Histochemical distribution of cadmium in lettuce (*Lactuca sativa*) and barley (*Hordeum vulgare*) roots

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Cadmium (Cd) is a non-essential trace element for lettuce (*Lactuca sativa*) and barley (*Hordeum vulgare*). Previous experiments have determined that approximately 80% of the total Cd is translocated to leaves of lettuce, whereas only 20% of the total Cd is translocated to barley leaves. Preferential retention of Cd in the cell walls of barley roots would explain this difference. The distribution of Cd in the cell walls (apoplast) and in the intracellular spaces (symplast) of roots of lettuce and barley was determined using a Cd-specific histological technique. Plants were grown in nutrient solution containing 0 or 1.0 μM CdCl_2 for 28 days. Before harvest, root samples from control and Cd-treated plants were collected for histochemical analysis of Cd. Dithizone, an organic dye, was used to detect Cd in cross sections of roots. A light microscope was used to take digital images of stained sections to locate the distribution of Cd and subsequent quantification was performed by analyzing images with Adobe Photoshop. While Cd was located both in the apoplast and in the symplast, apoplastic Cd was more prevalent compared to symplastic Cd in both species and the intensity of staining was higher in barley compared to lettuce.

3) Isolation of antibacterial compound from rhizobacteria antagonistic to *Clavibacter michiganensis* subsp. *Michiganensis*

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Clavibacter michiganensis subsp. *michiganensis* (Cmm) causes bacterial canker of tomato. The aim of the study was to isolate and characterize anti-cmm compound(s) produced by a novel rhizobacterial isolate antagonistic to Cmm. A novel rhizobacterial strain showing strong antibacterial activity against Cmm was isolated from the rhizosphere. Using an agar well diffusion assay, activity of the cell free supernatant was studied. The compound(s) were isolated, from bacterial culture, with n-butanol and further fractionated with HPLC. Purified fractions were subjected to SDS-PAGE analysis. Our results demonstrate that the antibacterial compound was produced during the late growth phase of the culture. Initial studies have shown that the antimicrobial compound is proteinaceous in nature and thus is a bacteriocin. SDS-PAGE of the bacteriocin shows that the molecular weight of the compound is less than 4 KDa. Further work on characterization of the compound(s) is underway. The compound may significant potential in controlling bacterial canker caused by cmm.

4) Ancient leaf beetles reflexively bleed azoxyglucosides sequestered from their host cycad plants

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Leaf beetles are not only able to detoxify defensive secondary metabolites in the leaves of their host plant but may also sequester these compounds as part of their own defense. Aulacoscelinae beetles have an ancient relationship with Cycads (Zamiaceae (Cycadophyta)) that are protected by highly toxic azoxyglucoside compounds. How these beetles, a primitive group in the Chrysomelidae superfamily, deal with host plant defensive compounds remains unknown. Adult Aulacoscelinae beetles were collected from the cycads *Zamia boliviana*, *Zamia elegantissima* and *Dioon edule* in Bolivia, Panama and Mexico, respectively. Total azoxyglucoside levels were quantified in both cycad leaves and adult beetles by HPLC. Adult beetles contained significant levels of these compounds (~0.4-1.6% FW). When disturbed, a defense mechanism of some insects is reflexive bleeding from their leg joints; the highest levels of these azoxyglucoside compounds were found in these defensive secretions. Nuclear magnetic resonance and mass spectroscopy confirmed the presence of both cycasin and macrozamin in the reflexive bleeding. This is the first account of plant-derived compounds in the reflexive bleeding of the Aulacoscelinae. The basal phylogenetic position of the Aulacoscelinae suggests that strategies such as sequestration of plant secondary metabolites may have appeared early in leaf beetle evolution.

5) Sauvignon Blanc expresses a gene highly similar to *AMAT*, responsible for the “foxy” aroma of *Vitis labrusca* berries.

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Vitis labrusca (Concord grape) wines have a characteristic and undesirable “foxy” aroma attributed to methyl anthranilate, whose formation is catalyzed by an alcohol acyltransferase, anthraniloyl-CoA: methanol anthraniloyl transferase (**AMAT**). While the Concord grape enzyme can accept various acyl-CoAs and alcohols to form a range of different volatile esters, it produces methyl anthranilate *in vivo* since ripening grapes preferentially accumulate anthranilate and methanol that supply necessary substrate precursors for this reaction. Recently, the genome of *Vitis vinifera* was published and a search for AMAT-like proteins in the database yielded a protein with 95% amino acid identity to AMAT. Further studies with different *Vitis vinifera* varieties permitted the cloning of this gene from Sauvignon Blanc grapes, while truncated forms of this gene were identified in other grape varieties. Further analysis with Sauvignon Blanc berries demonstrated that both the gene and the protein are expressed in berries. While Sauvignon Blanc berries do not produce methyl anthranilate, it is expected that the role of this AMAT-like gene is to facilitate the production of other volatile aroma compounds. Present studies are focused on functional characterization of this AMAT-like gene and on identifying the biological role in Sauvignon Blanc berries during growth and development.

6) DAT gene expression in *Catharanthus*; Reactivation and specific expression in homologous and heterologous systems

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The *Catharanthus roseus* DAT gene encodes the enzyme acetyl-CoA:deacetylindoline-4-*O*-acetyltransferase involved in the last step of vindoline biosynthesis. Vindoline production requires the participation of at least two cell types (i.e., idioblasts and laticifers) and is predominantly found in leaves, stems and young buds but interestingly it is not expressed in roots. Promoter analysis represents the best strategy to decipher its specific expression at spatiotemporal levels. PDAT 2,3 kb + *dat* was constructed by replacing the forward p35S::*Gus Plus* promoter with a 2480 bp fragment (-2431:+49) of the DAT gene promoter ligated to the *dat* gene ORF, upstream of the *GUS Plus* reporter gene to make transcriptional and translational fusion into the vector pCAMBIA1305.1. This construct and the pCAMBIA1305.1 as positive control were transformed into tobacco leaves and *Catharanthus* hairy roots. The reactivation of *dat* gene expression under its own promoter was localized into transporter tissues in *Catharanthus* hairy roots while its expression was positioned in stomata and transporter tissues in tobacco leaves. *In silico* analysis revealed putatively the presence of few binding sites for DOFs transcription factors which are known to be involved in the regulation of stomata in other species such *Arabidopsis thaliana* *TGG1* and *Brassica napus* *Myr1.Bn1* genes.

7) Screening for high and altered monoterpenoid indole alkaloid lines in EMS-treated *Catharanthus roseus* plants

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The Madagascar periwinkle (*Catharanthus roseus*) accumulates complex dimeric monoterpenoid indole alkaloids (MIAs), vinblastine and vincristine, that are produced by the enzymatic coupling of vindoline and catharanthine monomers. These powerful chemotherapeutic agents have been used extensively to treat cancers such as Hodgkin's disease and early childhood leukemia. *C. roseus* also produces the monomeric alkaloid ajmalicine, which is used as an anti-hypertensive agent, and to combat heart arrhythmias and improve blood circulation in the brain. Unfortunately, these alkaloids are produced in *C. roseus* at very low levels, and their high cost of production has impeded the development of alternative uses of these valuable products. There are still demands for developing high MIA yielding varieties of this plant since the precursors to make the dimeric alkaloids are not available from other sources. Phytochemical profiling of 3600 ethyl methane sulfonate (EMS) mutagenized lines of *C. roseus* has been performed to discover plants with high or altered MIA profiles. Rapid extraction and analysis of mutants by thin layer chromatography yielded a single plant with a unique MIA profile that was characterized by Ultraperformance Liquid Chromatography combined with Mass Spectrometry. The results obtained have validated the approach, providing important tools for pathway elucidation and manipulation.

8) Comparative metabolic and genomic analysis of monoterpenoid indole alkaloid (MIA) medicinal plants.

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Approximately 20% of plant species contain alkaloids with a broad range of physiological properties that protect them from various types of herbivores and pathogens. Within this abundant group of nitrogen containing secondary metabolites the monoterpenoid indole alkaloids (MIAs) make up the largest and most diverse class of compounds that are characteristically found within the Apocynaceae, the Loganiaceae, and the Rubiaceae plant families. Since the MIAs are among the best characterize with respect to their chemistry, biochemistry, and molecular biology, our laboratory has broadened the scope of genomic research and pathway discovery to a number of medicinally important plant species that accumulate distinct classes of these compounds. The approach being used involves metabolic profiling of each plant species in order to identify the best MIA biosynthesis tissues. Tissues identified by this process are then used to harvest mRNA enriched in MIA biosynthesis and the cDNA is produced for large scale 454 sequencing. The progress made towards these goals will be described.

9) Cloning and Characterization of Amyrin Synthase, *CrAS* Involved in Triterpenes Biosynthesis in Leaf Epidermis of *Catharanthus roseus*

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The plant kingdom produces many thousands of biologically active triterpenes that are derived from (3*S*)-oxidosqualene to generate over 80 different carbon skeletons. Different oxidosqualene cyclase (OSC) enzymes carry out the carbocation rearrangements responsible for this biological diversity. For example, *Catharanthus roseus* accumulates 2.5 % of their leaf dry weights as the α -amyrin-derived ursane-type triterpene, ursolic acid, on the leaf surface. Sequencing of a leaf epidermis enriched cDNA library generated most of the mevalonic acid pathway as well as a new OSC gene with high amino acid sequence identities to amyrin synthases (*CrAS*) from other species. Functional expression of *CrAS* in *Saccharomyces cerevisiae* resulted in the production of α -amyrin and β -amyrin in an approximate 8 to 2 ratio. Transcription analysis showed that *CrAS* is predominantly expressed in the leaf epidermis of young *Catharanthus* leaves. These results strongly suggest that triterpene biosynthesis is highly regulated during plant growth and development; that triterpenes appear to be produced in the specialized epidermis of young leaves and that synthesis is closely associated with secretion on the leaf surface where they fulfill particular biological roles.

10) ^{14}C -fixation and export of sugars and monoterpenes in leaves of two genera of the Plantaginaceae

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Antirrhinum majus L. (snapdragon) and the common weed, *Plantago lanceolata* L. (ribwort) have been re-classified within the Plantaginaceae. In addition, to labelling of starch and sucrose, *A. majus* and *P. lanceolata* both produce a prominent alcohol sugar during photosynthesis and similar monoterpenes (iridoids). In *A. majus*, mannitol was labelled but not readily exported. In comparison, in *P. lanceolata*, the alcohol sugar, sorbitol, was heavily labelled relative to sucrose during $^{14}\text{CO}_2$ -feeding and appeared to be exported to sink tissues. Interestingly, in *A. majus*, though mannitol did not appear to be heavily labelled or mobilised, two iridoids, antirrhinoside and antirrhide, were radio-labelled during photosynthesis. Furthermore, antirrhinoside was phloem-mobile accounting for 15-24% of total photoassimilate transported depending on the environmental conditions and the genetic lines tested. The second, labelled iridoid in *A. majus*, antirrhide, accumulated in the laminar tissue appearing to be less phloem mobile. Environmental conditions, such as light, temperature and CO_2 levels altered ^{14}C -partitioning and export among these iridoids, the sugars and starch in a cultivar distinct pattern. However, in comparison, in leaves of *P. lanceolata* that contain the iridoids, catalpol and aucubin, that are similar to antirrhinoside and antirrhide, respectively, the ^{14}C -labelling was primarily into sucrose and sorbitol that were exported with less partitioned to iridoids.

11) Quantitative Trait Loci (QTL) for Fiber Characteristics in Soybean Residue for Composite Materials

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The use of plant fibers in automotive parts is an attractive new market for agricultural biomass but it is limited by their poor performance in composite materials. The objectives of this research were to map QTL for fiber traits, identify genes that contribute to soybean fiber performance in composites and develop fiber gene-specific markers. Databases were searched for the genes involved in cell wall biosynthesis and modification. Gene specific PCR primers were designed and screened with genomic DNA of parents (RG10 and OX948) of a recombinant inbred line (RIL) mapping population. Gene-specific markers for key enzymes in lignin, hemicellulose, cellulose and pectin biosynthetic pathways were developed. A soybean oligo microarray was also hybridized with the genomic DNA of the parents or RILs and RNA from stem tissue to identify additional fiber genes and develop single feature polymorphism (SFP) markers. Mapping of fiber genes is underway. Fifty RILs (selected based on height/ lodging index) and parents were evaluated in three Ontario locations (Harrow, Ridgetown and Woodstock) in 2008 and 2009. Significant negative correlations were detected between lignin and cellulose and lignin and hemicellulose, while cellulose and hemicellulose were positively correlated. Some fiber QTL were associated with fiber genes. Formulation and characterization of composite materials is underway. This work will allow identification of key factors in fiber quality and the development of quick, marker-based screening method(s) to facilitate rapid introgression of genes for good fiber quality into new soybean varieties.

12) Fatty Acid ω -Hydroxylases in Soybean

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Soybean (*Glycine max*) is one of the most widely cultivated crops in the world. A substantial cause of soybean yield loss worldwide is root rot, caused by the pathogen *Phytophthora sojae* and significant effort has been expended at improving soybean resistance to this devastating pathogen. It has been established that there is a strong correlation between preformed soybean root suberin (especially the poly[aliphatic] component) and high levels of innate resistance to the pathogen *P. sojae*. Enzymes that have been shown to be of critical importance to suberin biosynthesis and in particular the poly(aliphatic) domain are ω -hydroxylases. These specific enzymes catalyze the terminal carbon hydroxylation of fatty acids that introduces a second functional group into the main monomers allowing them to be cross-linked into a polymeric matrix. Therefore to better understand the relationship between innate resistance to *P. sojae* and preformed soybean root suberin, the expression, regulation and enzyme function of ω -hydroxylases in soybean must be elucidated. To initiate this research a comparative sequence-based in silico approach, using characterized ω -hydroxylases from *Arabidopsis thaliana* and *Solanum tuberosum*, was used to identify six putative ω -hydroxylase genes in soybean.

13) Arabidopsis LONG-CHAIN ACYL-COA SYNTHETASE 1 (LACS1), LACS2, and LACS3 facilitate fatty acid uptake in yeast

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The plant cuticle is a lipid-based protective barrier that coats the aerial surfaces of plants. Cuticle precursors are mainly derived from long-chain (C16/C18) and very-long-chain (>C20) fatty acids. Arabidopsis long-chain acyl CoA synthetase (LACS) 1 and LACS2, and likely LACS3, play key roles in cuticle biosynthesis. Acyl-CoA synthetases (ACS) activate free fatty acids into acyl-CoAs for metabolic utilization. ACS proteins can also facilitate trans-membrane movement of fatty acids. A yeast *fat1Δ* mutant is deficient in both very-long chain (>C18) acyl-CoA synthetase (ACSVL) activity and exogenous fatty acid uptake. We demonstrate that heterologous expression of Arabidopsis LACS1, LACS2, or LACS3 is able complement both of these deficiencies. Furthermore, expression of these plant enzymes in yeast leads to increased uptake of a fluorescent fatty acid analogue (C1-BODIPY-C12). These findings have potential implications in the transmembrane transport and/or intracellular trafficking of plant lipids destined for export to the cuticle.

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

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Suberin is a cell wall-associated hydrophobic barrier consisting of phenolics, glycerol and a variety of fatty acid derivatives, including C18:0-C22:0 primary fatty alcohols. Suberin is deposited in various tissue layers, such as root endodermis and seed coat, serving a critical role in controlling water and ion transport. It is also deposited in response to wounding and salt stress. An eight-member gene family encoding alcohol-forming fatty acyl-CoA reductases (FARs) has been identified in *Arabidopsis thaliana*. We have found that the gene expression patterns of three of these genes, *FAR1*, *FAR4* and *FAR5*, coincide with known sites of suberin deposition. Heterologous expression of *FAR1*, *FAR4* and *FAR5* in yeast indicated that they are active alcohol-forming FARs with distinct chain length specificities ranging from C18:0 to C22:0. We found that mutants of *FAR1*, *FAR4*, and *FAR5* are each differentially affected in primary alcohol levels in root, seed coat and wound-induced leaf tissue. Specifically, C18:0-OH was reduced in *far5-1*, C20:0-OH was reduced in *far4-1*, and C22:0-OH was reduced in *far1-1*. The structural and physiological roles of suberin-associated primary fatty alcohols are currently being investigated using single, double, and triple *far* mutant lines.

15) The role of extracellular glycosidases in the *Pythium irregulare* - ginseng pathosystem

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The ginseng pathogen *Pythium irregulare* is able to selectively metabolize the 20(S) protopanaxadiol ginsenosides Rb1, Rb2, Rc, Rd, and gypenoside XVII *in vitro* via extracellular glycosidases, leading to the formation and partial assimilation of ginsenoside F2. To determine whether there is a correlation between the activity of ginsenoside metabolizing β -glucosidases and the pathogenicity of *P. irregulare* towards ginseng, the production of ginsenoside-specific glycosidases and pathogenicity of various isolates of *P. irregulare* were determined. For this, 10 isolates of *P. irregulare* were selected on the basis of their genetic variability and the host plant they were isolated from (including ginseng), and obtained from the Canadian Collection of Fungal Cultures. These isolates were cultured *in vitro*, in the presence of ginsenosides and the level of ginsenoside-specific glycosidase activity in their extracellular proteins was measured. Meanwhile ginseng seedlings were inoculated with the same suite of *P. irregulare* isolates and scored for disease symptoms to estimate the relative pathogenicity of each isolate towards ginseng plants. When combined this data shows evidence of a positive correlation between glycosidase activity in *P. irregulare* and the pathogenicity of this organism towards ginseng.

16) Adenosine kinase activity contributes to intracellular cytokinin homeostasis.

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Biosynthesis, degradation and interconversion all contribute to defining the cytokinin activity of a cell. Purine salvage enzymes have long been implicated in catalyzing several of the interconversions related to reducing cytokinin activity. We are using molecular genetics to investigate the involvement of adenosine kinase (ADK) in the inactivation of cytokinin ribosides. Arabidopsis ADK-deficient lines were generated by both sense ADK1 cDNA and artificial microRNA over-expression. These ADK-deficient lines have altered branching, delayed senescence and a decreased rosette leaf and cell size. Examination of leaf sections shows they contain an increased number of cells and reduced intercellular space relative to the wild type. ADK deficiency diminishes the incorporation of radiolabeled cytokinin ribosides into the corresponding nucleotides. Cytokinin profiling by LC/MS/MS analysis of leaves of 4-week-old plants indicates ADK-deficiency leads to a significant increase in zeatin riboside levels and a small rise in CK free base and nucleotide levels. A significant increase in glucosides relative to the wild type was detected, perhaps due to the excess CK ribosides being converted to the inactive glucoside form. In addition to phenotypic and metabolic analyses, up-regulation of cytokinin signaling (ARR5 and ARR7) and cell division markers (cycB1) support the presence of elevated CK activity in an ADK-deficient background. Taken together these results indicate that ADK activity contributes to intracellular cytokinin homeostasis and modifying its expression substantially alters cellular division and plant growth. Harnessing this activity may provide a means to tailor CK profiles for improved plant productivity.

17) Accumulation of therapeutic protein IL-10 in model plant *Arabidopsis thaliana*

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Plants are one of the most economical systems for large scale production of recombinant proteins for biopharmaceutical and industrial uses. A large number of human recombinant proteins of therapeutic value have been successfully produced in plant systems. The major challenge in the field of recombinant protein production is to produce sufficient level of proteins in plants so that the production system is economically sustainable. To identify the factor (s) that regulates synthesis as well as accumulation of recombinant proteins in plant, we conducted a stepwise dissection of control of transgene expression using *Arabidopsis* as a model. EMS-mutagenized transgenic *Arabidopsis* lines, 2762 and 3262, that carries the human interleukin (IL)-10 produce relatively higher amount of IL-10 than the wild type IL-10 line (WT). The IL-10 transcripts were more stable in 2762 and 3262 than WT IL-10 line which may contribute to higher protein synthesis in these lines. To evaluate if translational regulation of IL-10 controls its synthesis in non-mutagenized WT IL-10 and higher IL-10 accumulating mutant lines, we measured the efficiency of the translational machinery. Our results indicate that the mutant lines with higher transgene expression contain more robust and efficient translational machinery compared to the control line.

18) Characterisation of the GTP binding domains of Toc159 and Toc33 using Circular Dichroism and Fluorescence Spectroscopy – A new approach to investigate structural and mechanistic details of heterodimerization

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Most chloroplast proteins are synthesised as preproteins in the cytosol and are translocated into chloroplasts by the TOC (translocon at the outer envelope of chloroplasts) complexes. The Toc GTPase families (Toc33 & Toc159) together with Toc75 form the core of the TOC complex. Several biochemical studies indicate that dimerization of the G-domains of Toc159 and Toc33 has a direct role in GTP-regulated protein import and suggest that these receptor-receptor interactions initiate preprotein translocation. To investigate the structural and functional details of the mechanism by which the G-domains dimerize and enhance import efficiency, we have begun characterizing these receptors using biophysical techniques such as Circular Dichroism (CD) and Fluorescence (FL) spectroscopy, which have been under-utilized for the study of the Toc GTPases. A series of single Trp (tryptophan) and null mutants of each of the two receptors has been prepared using site-directed mutagenesis. These proteins have been expressed in bacteria and purified in high yield. Initial characterization of Toc159G and Toc33G proteins by CD spectroscopy revealed significant α -helical content in their secondary structure. Analysis of the CD and FL spectra of Toc33G proteins suggested that Trp at positions 151 and/or 229 may be critical for functional structure integrity.

19) Cold acclimated winter cereals exhibit an enhanced CO₂ assimilation under long-term growth at elevated CO₂

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It has been suggested that the enhanced photosynthetic capacity, estimated as light saturated rates of photosynthesis, A_{sat} , exhibited by cold-acclimated (CA) winter cereals, spinach, Brassica and Arabidopsis is the result of up-regulation of photosynthetic carbon metabolism. Therefore, we hypothesized that CA (5°C) winter cereals (cv Norstar wheat; cv Musketeer rye) should maintain higher photosynthetic capacity relative to non-acclimated (NA, 20°C) controls under long-term growth and development at elevated CO₂. Furthermore, CA winter and spring cereals (cv Katepwa wheat; cv SR4A rye) should maintain a differential A_{sat} at elevated CO₂.

Long-term growth and development of plants at elevated CO₂ (700ppm) stimulated A_{sat} to a greater extent (about 60%) in CA Norstar and Musketeer relative to NA controls (30-35%). In contrast, CA Katepwa and SR4A stimulated A_{sat} by only 20-25% relative to those rates observed at ambient CO₂ (380 ppm). Compared to maximum A_{sat} observed at short-term shift to elevated CO₂, long-term growth at elevated CO₂ resulted in acclamatory loss of A_{sat} by about 30% in NA winter and spring cultivars and by about 15% in CA counterparts. The enhanced A_{sat} of CA winter cultivars at long-term elevated CO₂ was reflected into increased biomass (55%) relative to NA controls (35%) and CA spring cultivars (20%). Thus, consistent with our hypothesis, CA winter cultivars, Norstar and Musketeer exhibited higher A_{sat} relative to NA controls under long-term growth at elevated CO₂. In contrast, CA spring cultivars, Katepwa and SR4A exhibited decreased A_{sat} relative to NA controls under long-term growth at elevated CO₂. This is consistent with the thesis that cold acclimation of winter cereals does lead to the reprogramming of photosynthetic carbon metabolism.

20) Shade-Intolerant *Embothrium coccineum* (Proteaceae) exhibits plasticity with respect to photoacclimation.

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Embothrium coccineum is an endemic species of South America (35 ° S and 56 ° S). It is a shade-intolerant pioneer tree that grows on volcanic slag and exhibits root adaptation to poor soils (cluster roots, CR). We studied the plasticity of *E. coccineum* to changes in light availability and the trade-off between photosynthesis and CR formation. Control plants were germinated and grown at 15°C, 200 $\mu\text{mol photons/m}^2/\text{sec}$, and a 16/8 h light/dark cycle. Subsequently, plants were shifted to either high light (HL= 700 $\mu\text{mol photons/m}^2/\text{sec}$) or low light (LL = 50 $\mu\text{mol photons/m}^2/\text{sec}$). Total plant biomass, plant height, maximum photosynthetic rate (A_{max}) and water use efficiency (WUE) differed significantly according to their origin. Andes Mountain seedlings (Curacautín, 38° S) exhibited lower height, higher A_{max} and higher WUE than plants from the other mesic and humid origins (Puerto Montt 40° S and Chiloé 42° S). Exposure to a shift in irradiance from either LL to HL or HL to LL, resulted in 100% survival. Curacautín exhibited the lowest level of leaf damage. CR formation was observed only on Chiloé LL seedlings. Thus, the plasticity of *E. coccineum* to photoacclimation may explain its wide distribution and high capacity to colonize harsh environments.

21) Non-Photochemical Quenching of Chlorophyll Fluorescence Among Marine Diatoms.

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Diatoms (Bacillariophytes) are an extremely successful group of phytoplankton, act as carbon sinks in the environment, and are responsible for some 25% of global primary production. Diatoms dominate over other phytoplankton in photodamage conducting light environs where there are extreme fluctuations in irradiance over short time scales. Unlike most photosynthetic organisms, diatoms do not have the state transition to compensate for short-term irradiance fluctuations. Their intrinsic resistance to photodamage has been linked to their ability to perform powerful NPQ (Non-Photochemical Quenching of chlorophyll fluorescence). We surveyed several marine diatom species on their ability to induce and recover from NPQ. Time course and fast kinetic measurements of variable chlorophyll fluorescence in tandem with either DCMU (blocks the electron donor site of photosystem II) or NH₄Cl (proton gradient uncoupler) treatments showed us that there were inter-species differences on the dependence of NPQ on photosystem II linear electron transport and the build up of the trans-thylakoid proton gradient. All species showed a strong NPQ dependence on the proton gradient; however, at least one species also exhibited a strong dependence on alternate electron transport. We suspect that the differences in NPQ strategies among the species parallel with the light environs of their natural habitats.

22) Growth of Green Arabidopsis thaliana Cell Cultures Occurs Independently of Photosynthesis.

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Addition of an exogenous carbon source, such as sucrose, represses photosynthetic gene expression and chloroplast development. Typically, plant cell cultures grown in the presence of sucrose exhibit a non-green phenotype. Here we show that Arabidopsis thaliana cell cultures remain green in sucrose concentrations as high as 15% (w/v) and, in fact, require sucrose for growth. In the dark and presence of sucrose, A.thaliana cell cultures lack Chl but are able to grow at rates comparable to green cultures in the light. Light response curves for O₂ evolution indicate that light-saturated rates of photosynthesis never exceed the rates of respiration. Thus, these green cells grow below their light compensation points. However, increasing sucrose concentration from 3 to 15% inhibits both the apparent quantum yield for O₂ evolution as well as the photosynthetic capacity. Immunoblots indicate that the major components of the photosynthetic apparatus are present in these green cells when grown in the light in the presence of sucrose. Furthermore, these green cells exhibit a reversible photoacclimation in response to changes in their light environment. Initial results for energy partitioning measured by Chl a fluorescence indicate that the green cells dissipate absorbed light energy by both antenna and constitutive quenching mechanisms.

23) Effect of light, ABA and cytoskeletal inhibitors on the clumping of chloroplasts in leaves of *Kalanchoë blossfeldiana*, a succulent CAM plant

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The phenomenon of chloroplast clumping was previously reported in mesophyll cells of some succulent crassulacean-acid-metabolism (CAM) plants under a combination of light and water stress. The plant stress hormone abscissic acid (ABA) also induced the clumping of chloroplasts in leaves of plants under normal light conditions. In this study, we examined the effect of light intensity, light quality, and inhibitors of the cytoskeleton on the clumping of chloroplasts in leaves of the succulent CAM plant, *Kalanchoë blossfeldiana*. In well-watered plants, light intensities over $1200 \mu\text{mol m}^{-2}\text{s}^{-1}$ caused chloroplast to clump, whereas in water stressed plants, chloroplasts clumping was observed in leaves under low light intensities. When ABA-treated leaves were exposed to $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ of UV (400 nm) or blue (470 nm) light, chloroplast clumping was induced whereas exposure to $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ of red (625 nm) light did not. Treatment of ABA-treated leaves with actin-depolymerizing agent, cytochalasin B or microtubule-disrupting agent, nocodazole, completely abolished the clumping of chloroplasts induced by ABA. These findings indicate that the clumping of chloroplasts in leaves of CAM plants under drought stress is induced by UV or blue light and involves both actin filaments and microtubules.

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

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

25) *In vitro* regenerated wetland sedge *Eriophorum vaginatum* L. via callus culture is genetically stable

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Eriophorum vaginatum L. is a tussock-forming perennial sedge that can survive in cold, nutrient-poor, and heavy-metal-contaminated environments. This species could be used in phytoremediation, restoration and creation of wetlands. However, its propagation on a large scale is problematic due to seed dormancy and limitations in reproduction by rhizomes. Therefore, tissue culture is a potential alternative. *E. vaginatum* seeds were cultured on Murashige and Skoog (MS) medium supplemented with plant growth regulators (2,4-D, kinetin, BA) at different concentrations to induce callus formation and plant regeneration. Genetic stability of callus and regenerated plants was studied by flow cytometry. MS medium supplemented with 0.5 mg l⁻¹ 2,4-D and 0.5 mg l⁻¹ or 1 mg l⁻¹ kinetin for callus induction, and with 4 mg l⁻¹ BA for shoot regeneration, were the most efficient; up to 42 shoots regenerated per seed. The regenerated shoots were rooted on MS medium without growth regulators and were successfully acclimatized in the greenhouse. Flow cytometry revealed that the nuclear DNA content was similar in all plant materials and amounted about 0.8 pg/2C. A protocol for *in vitro* culture of *E. vaginatum* which allows the production of a large number of genetically stable plants in the short time is detailed.

26) Regeneration of pepper (*C. annuum* L.) haploid plants through *in vitro* androgenesis

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In vitro induced androgenesis is the formation of sporophyte from immature pollen grains in anther or isolated microspore cultures. It is a fast and efficient method of obtaining haploid plants, widely applied in breeding of many vegetable and ornamental species. Because of a single set of chromosomes, haploids are a precious material for basic genetic studies, gene mapping, mutagenesis and transformation. As a result of spontaneous or colchicine induced diploidisation of haploid embryos, fully homozygous, doubled haploid plants (DH lines) are obtained in a short period of time. The importance of DH lines comes from their usability as the parental source for F₁ hybrids. Examining the effect of genotype, stage of microspore development, media composition and the season of culture starting, we were able to induce first successful haploids in anther cultures of Polish pepper genotypes. Ploidy level of androgenic regenerants was determined cytometrically and the effective method of colchicine treatment was established. Isoenzymatic analysis confirmed microspore origin of obtained DH lines and biometrical assessment of the most desired agronomical traits allowed for selection of two superior lines to be registered as the first Polish anther-derived cultivars of *Capsicum*.

27) Comparative rooting response of chrysanthemum cuttings to short and long methods of exogenous auxin application

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Pot chrysanthemum growers report heavy shipping and handling losses during winter production. This problem was thought to be due to poor anchorage of plants in the pots. Factorial experiments were designed to compare the effects of short (3 sec. dip of the base of the cutting) and long (3 min. complete immersion of the cutting) application methods of various auxins on the rooting of tip cuttings of two chrysanthemum cultivars 'Duluth' and 'Presidio'. Both methods increased the height of root initiation in cultivar Duluth while in cultivar Presidio it first increased and then decreased with increasing concentration of IBA. In the short dip method, Duluth had significantly greater root length and root surface area with 2,000 ppm IBA while Presidio had greater total root length and root surface area with 1,000 ppm NAA compared to the control and other treatments. In the long dip method, Duluth had significantly greater root length and root surface area with 100 ppm IBA and cultivar Presidio had peaks in these parameters with 50 ppm NAA compared to the control treatment. Overall, these results indicated that the long dip method at low concentrations had similar results to those of the short dip method at high concentrations and 'Duluth' responded better to IBA while 'Presidio' to NAA.

28) Effect of light quality on total nonstructural carbohydrate (TNC) content and rooting of cuttings of chrysanthemum cultivars.

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Chrysanthemum is one of the most economically important floriculture species grown year round in greenhouses as a result of high market demand. During winter, many growers experience losses with plant falling over in the pots, especially during shipping over long distances. This problem was thought to be due to poor anchorage of the plants in the pots. Experiments were set up factorially to compare the effect of red and far-red light supplementation during stock plants production and during the rooting stage of the cuttings of 2 chrysanthemum cultivars, namely Duluth and Presidio. The two cultivars responded differently in terms of rooting to stock plant lighting. Duluth was non-responsive to the stock plant lighting while Presidio showed significant increases with red lighting in root length, root number and root surface area compared to far-red lighting. Analysis of the total nonstructural carbohydrate (TNC) content of the cuttings from stock plants indicated that TNC content significantly increased under red lighting on stock plants compared to far-red lighting and that Duluth accumulated a significantly higher concentration of TNC than Presidio. The far-red / far-red lighting combination during stock plant and rooting caused significantly more elongation of cuttings compared to the red /red lighting combination.

29) Low-temperature photosynthetic performance of two winter annual grass invaders.

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Bromus tectorum (cheatgrass) and *Bromus rubens* (red brome) are two annual grasses that are among the most destructive bioinvaders of North America. In order to understand low-temperature performance of *B. tectorum* and *B. rubens*, it is essential to determine their major photosynthetic limitations at low temperatures and to understand patterns of thermal acclimation when grown at sub-optimal temperatures. To examine the effect of acclimation on the photosynthetic response we grew *B. tectorum* and *B. rubens* in normal (22°/14°C) and cool (12°/5°C) temperature treatments. Both species *B. tectorum* and *B. rubens* acclimated to sub-optimal temperatures increasing net and gross CO₂ assimilation rates, yet no down-ward shift in the temperature optimum was detected. In both treatments, the reduction in the oxygen concentration from 21% to 2% resulted in substantial stimulation of net photosynthetic rate down to 2°C. By fitting our experimental data to the photosynthetic models we can conclude that photosynthesis of both species was limited by Rubisco carboxylation capacity from 2°C and up to 42°C.

30) Genotype by environment interaction for fibre and starch profiles of potato (*Solanum tuberosum* L.)

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Potatoes have been a staple for hundreds of years but can be relatively high in glycemic index (GI). Due to this, the potato has lost favor in many diets and the positive benefits are being outweighed by potential health problems. Potatoes contain two different types of starch: amylose and amylopectin. Structural differences between amylose and amylopectin contribute to variations in breakdown after ingestion, affecting GI. These differences contribute to three categories of starch: rapidly digestible (RDS), slowly digestible (SDS) and resistant (RS). RS is commonly associated with dietary fibre and health benefits. There are two components to fibre: soluble and insoluble, which have different functional properties in the colon. Our research examines the genotype by environment influence on fibre and starch profiles. Field trials were conducted in 2009 and 2010 with 12 potato genotypes in three locations across Southern Ontario. Analysis of fibre and starch profiles will help elucidate the genotypic and phenotypic framework through which human health is affected by what we ingest. Potatoes were analysed using protocols adapted from Sigma, Ankom, Megazyme and Englyst *et al.* (1992). Analysis from one field season indicates genotypic, environmental and genotype by environment influences on fibre and starch.

31) Photoprotective isoprenoids increase drought stress tolerance of Douglas fir

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The aim of this project is to determine the diversity of isoprenoid-related mechanisms of drought tolerance in Douglas-fir provenances. Under drought conditions, plants are particularly susceptible to photooxidative damage, as excess energy impairs the photosystems and produces reactive oxygen species (ROS). Essential isoprenoids comprise accessory pigments and antioxidants which mediate different photoprotective processes such as dissipation of excess energy in terms of heat and scavenging of ROS, whereas non-essential isoprenoids are volatile and proposed to decrease the effects of thermal stress. We hypothesize that the adaptation to drought stress is mediated by the patterns of biosynthesis or abundance of essential and non-essential isoprenoids. The relationship between drought tolerance and isoprenoid metabolism was studied in fifty-year-old trees of four provenances grown at two field sites in southern Germany. Measurements of photosynthetic capability and emission of volatiles were conducted in well-watered trees during the spring season and drought stressed trees during summer and combined with analyses of isoprenoid content in sampled needles. I will present preliminary results that demonstrate varying physiological responses to drought among Douglas fir provenances.

32) Stomatal development and drought in *P. balsamifera*

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Drought is one of the most significant factors limiting tree growth. Trees in the genus *Populus* are particularly noted for their drought sensitivity. *Populus* trees may utilise several different mechanisms to contend with water-deficit stress imposed by decreased soil water availability. For example, when *Populus* trees perceive water deficit, alterations in the transcriptome lead to changes in the physiological response, ultimately influencing plant development. Leaf physiology, growth and development can be significantly modified in this manner. Using a time-course experimental approach through leaf development over a period of water-deficit stress, variability in gene expression underlying changes in leaf development under drought stress in two different *P. balsamifera* genotypes was investigated. The *Populus balsamifera* genotypes differed in their transcriptome and physiological responses to water deficit, but both genotypes had reduced stomatal indices in leaves that had developed under water-deficit conditions relative to leaves that had developed either prior to water deficit, or comparable leaves that had developed in fully irrigated plants. The molecular mechanisms underpinning this common response were explored by interrogating the transcript abundance profiles of genes known to shape stomatal development. Ultimately, the findings further the understanding of the interplay between drought stress and molecular underpinnings of leaf development in an important tree species.

33) Inorganic carbon uptake at acid pH by the alga *Chlorella kessleri*

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Eukaryotic microalgae and cyanobacteria face several challenges in obtaining carbon dioxide from the surrounding aqueous medium to support photosynthesis. These challenges include the low diffusion rate of CO₂ in water than in air and the low affinity of the main carboxylating enzyme Rubisco for CO₂. Algae have been found to respond to these conditions by means of a carbon concentrating mechanism (CCM) that consists of active uptake of carbon dioxide, bicarbonate or both, elevating CO₂ concentrations around Rubisco. The ability of the freshwater alga, *Chlorella kessleri*, to maintain a carbon concentrating mechanism at low pH was investigated. Previous research has shown that *C. kessleri* has a carbon concentrating mechanism at alkaline pH. The alga grows over the pH range 4.0 to 9.0 and was found to take up bicarbonate and CO₂ actively at pH 6.0. At acid pH (below 5.0), *C. kessleri* does not have active bicarbonate or CO₂ uptake, but relies solely on the diffusion of CO₂ for photosynthesis. Therefore, the alga does not have a carbon concentrating mechanism when grown at acid pH but maintains an adequate supply of CO₂ by diffusive uptake.

34) Inorganic carbon acquisition in the acid-tolerant alga *Stichococcus bacillaris*

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The processes of CO₂ acquisition were characterized for the acid-tolerant, free-living chlorophyte alga *Stichococcus bacillaris*, CPCC 177. The alga grows over a wide pH range of pH 3.0 to 9.0. External carbonic anhydrase (CA) was detected in cells grown above pH 5.0, with the activity increasing substantially from pH 6.0 to 9.0. The capacity for HCO₃⁻ uptake of cells treated with the membrane impermeable CA inhibitor acetazolamide (AZA), was investigated by comparing the calculated rate of uncatalyzed CO₂ formation with the rate of photosynthesis. Active bicarbonate transport was found in cells grown in media above pH 7.0. Monitoring CO₂ uptake and O₂ evolution by membrane-inlet mass spectrometry demonstrated that air-grown, AZA-treated cells caused a rapid drop in extra-cellular CO₂ concentration to a CO₂ compensation concentration of 18 - 19 μM at pH 8.0; this CO₂ concentration is above the equilibrium CO₂ concentration at this pH, indicating that the cells do not exhibit active uptake of CO₂. O₂ evolution continued when cells reached CO₂ compensation point, confirming the capacity of these cells for active bicarbonate uptake. These results indicate that *Stichococcus bacillaris* possesses a CCM dependent on active HCO₃⁻ transport.

35) Mapping and characterization of a developmental mutant in *Zea mays*

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A number of single gene mutations have had a major impact on agriculture due to their potential to increase yield. For example, dwarfism alleles were introduced to rice and wheat with great success resulting in the so called "Green Revolution". By examining a maize, *Zea mays*, population derived from the B73 inbred treated with EMS (Ethyl Methane-Sulphonate), our lab identified a developmental mutant exhibiting some positive attributes. The mutant and wild type plants segregate 1:3 within an F2 population indicating that the mutant is caused by a single recessive mutation. We assayed chromosomal DNA from 72 individuals of an F2 population at 100 Single Nucleotide Polymorphisms spread across the 10 maize chromosomes and found a region that co-segregates with the observed developmental phenotype at $p < 0.0001$. The same chromosomal region was found again to co-segregate with the mutation by use of Bulk Segregant Analysis. We plan to use more molecular markers in the chromosomal region in order to fine map the mutation and identify the gene affected by the mutation. To characterize the pleiotropic effects of the mutation, we measured a number of plant traits at flowering time. We also identified the developmental window in which the mutant phenotype is expressed. Additionally, longitudinal and transversal sections of the stalk were examined in order to find differences in the cell architecture and the vascular system between mutant and wild type plants. Further experiments include the use of exogenous applications of hormones involved in plant growth and development.

36) Genetic and physiological characterization of a water stress sensitive *Zea mays* mutant

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Mutant characterization provides novel information about gene function at the molecular and whole plant level. Here we describe the initial stages in characterizing an ethyl methanesulfonate (EMS) induced maize mutant. Originally identified through decreased root growth rate under osmotic stress, the mutant under field conditions exhibits a water stress phenotype. Interestingly, the mutant phenotype is conditional. When grown under greenhouse conditions, the mutant develops normally with no evidence of water stress. We found the mutant and wild type phenotype segregate 3:1 (wild type:mutant) in an F2 population, suggesting a single recessive gene is responsible for the phenotype. This F2 population was genotyped with 69 single nucleotide polymorphism (SNP) markers distributed throughout the genome and the gene lies within a 23 Mb chromosomal segment. Fine mapping with more individuals ($n=985$) is ongoing. Stem cross sections of plants with the mutant phenotype have shown normally developed metaxylem elements suggesting water transport is not compromised. We believe the mutant is deficient in water uptake in the primary root and are assessing root growth rates under stress conditions.

37) Re-annotation of microarray platforms using recent genomic sequence data.

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Whole-genome sequences and genome annotation projects have advanced beyond the data available when microarray platforms were designed for their respective species. It is desirable to re-annotate these arrays with recent genomic information, to reduce redundancy (multiple array elements which represent a single transcript) and non-specificity (array elements which represent multiple transcripts), and also to relate the probe identifiers to the shared vocabulary of gene models. Since the sequence and gene-model data is continually growing, this re-annotation is not a one-time process but rather an ongoing effort with the goal of keeping pace with the state of knowledge of the genome of a given organism. Here we describe a process for re-annotating an existing microarray platform, given as inputs 1) the probe sequences on the array, 2) the EST sequences from which the probes were designed, 3) the complete genomic sequence, and 4) the gene-model sequences reported by automatic gene-calling software such as GeneBuilder or FGENESH. As an example, we present the re-annotation of the Arizona Maize Oligonucleotide Array, using Release 4a.53 of the B73 maize inbred line genome.

38) 14-3-3 proteins regulate the nuclear-cytoplasmic distribution of GmMYB176 involved in isoflavonoid synthesis in soybean

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14-3-3s are a group of proteins that are ubiquitously found in eukaryotes. Plant 14-3-3 proteins are encoded by a large multi-gene family and are involved in signaling pathways to regulate plant development and protection from stress. Recent studies in *Arabidopsis* and rice have demonstrated the isoform specificity in 14-3-3s and their client protein interactions. Here, we identified and characterized 16 14-3-3 proteins (SGF14) in soybean. Comparative analysis of SGF14s with *Arabidopsis* and rice 14-3-3s indicated that SGF14s also group into epsilon and non-epsilon classes. Subcellular localization study demonstrated that 14-3-3 proteins in soybean have isoform specificity, however, some overlaps were also observed between closely related isoforms. We have recently shown that SGF14s interact with GmMYB176, an R1MYB transcription that regulates the expression of *CHS8* gene and isoflavonoid synthesis in soybean. GmMYB176 consists of three predicted 14-3-3 binding sites. The detailed characterization of 14-3-3s interaction with GmMYB176 identified a critical motif (D2) within GmMYB176 for its interaction with 14-3-3. Deletion of D2 motif from GmMYB176 disrupts GmMYB176-SGF14 interaction and changes the subcellular localization of GmMYB176. Our results suggest that soybean 14-3-3 may regulate isoflavonoid synthesis by regulating the nuclear-cytoplasmic distribution of GmMYB176.

39) Characterisation of a transcriptional circuit involving the transcription factor, *AtMYB61*

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AtMYB61, a member of the R2R3-MYB family of transcription factors in *Arabidopsis thaliana*, alters gene expression in response to sugars, resulting in pleiotropic modifications of carbon allocation throughout the plant body. *AtMYB61* transcript abundance increases in response to the major product of photosynthesis, sucrose, and is repressed in response to two major products of photorespiration, glutamate and glycine. Phylogenetic footprinting, bioinformatic, and biochemical analyses support the hypothesis that *AtMYB61* expression is de-repressed by soluble sugars in a mechanism involving intragenic sequences. Current experiments suggest the involvement of specific proteins in the regulation of *AtMYB61* expression by interaction with gene regulatory sequences embedded in an *AtMYB61* intron. The gene targets that reside downstream of *AtMYB61* have also been characterised. Putative downstream target genes of *AtMYB61* were predicted on the basis of comparative transcriptome analysis. *AtMYB61* targets include genes that encode the following proteins: a KNOTTED1-like transcription factor (KNAT7, At1g62990); a caffeoyl-CoA 3-O-methyltransferase (CCoAOMT7, At4g26220); and a pectin-methylesterase (PME, At2g45220). Statistically over-represented motifs were identified in the 5' non-coding regions of the putative target genes, and these correspond to previously characterized AC element motifs that function as R2R3-MYB targets. The consensus motif functions as a *bona fide* target for *AtMYB61* binding as determined by an electrophoretic mobility shift assay. Binding between the gene regulatory sequences of the putative target genes, which contain multiples of these motifs, was confirmed via electrophoretic mobility shift assays. Altogether these experiments provide assessment of the ability of *AtMYB61* to bind to gene regulatory sequences present in the 5' non-coding sequences of the three putative downstream targets: *KNAT7*, *CCoAOMT7* and a *PME*, substantiating its role as a potential regulator of the transcription of these genes. Together with the analysis of the regulation of *AtMYB61* expression, these studies provide insights into the entire transcriptional regulatory circuit centred around *AtMYB61*.

40) Antagonistic interaction between *BLADE-ON-PETIOLE1/2* and BEL1-like homeobox genes *PENNYWISE* and *POUND-FOOLISH* co-ordinates flowering and *Arabidopsis* inflorescence architecture

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The transition to reproductive development in *Arabidopsis* is tightly regulated by environmental and endogenous signals that converge to control the expression of *LEAFY* and *APETALA1*, key regulators of floral-meristem identity. Flowering in many species is marked by the onset of internode elongation between successive lateral organs to generate an inflorescence. Co-ordination of flowering and internode elongation is poorly understood but requires the activities of three BEL1-like homeodomain proteins. Floral evocation is blocked in plants mutant for *PENNYWISE* (PNY) and *POUND-FOOLISH* (PNF). This block is partially rescued by mutation of a third BEL1-like protein, *ARABIDOPSIS THALIANA* HOMEODOMAIN GENE1 (*ATH1*), an activator of the floral repressor *FLOWERING LOCUS C* (*FLC*). Down-regulation of *ATH1* occurs at the start of inflorescence development. Previously, we showed that co-misexpression of the lateral organ boundary-associated genes *BLADE-ON-PETIOLE1* (*BOP1*) and *BOP2* and *KNOTTED1-LIKE FROM ARABIDOPSIS6* in *pny* stems restrict growth causing compact inflorescences. We show here that *bop1 bop2* mutations fully rescue floral evocation in *pny pnf* mutants, suggesting that *BOP1/2* misexpression blocks both flowering and stem elongation via effects on *ATH1* and/or *FLC*. Mechanistic study of *BOP1/2*-PNY/PNF antagonism will therefore shed new light on how flowering is co-ordinated with changes in stem architecture at the floral transition.

41) Antagonistic interaction of *BLADE-ON-PETIOLE1* and 2 with *BREVIPEDICELLUS* and *PENNYWISE* regulates *Arabidopsis* inflorescence architecture

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BLADE-ON-PETIOLE1 (*BOP1*) and 2 encode NPR1-like transcriptional co-regulators that are expressed in lateral organ boundaries where they control the architecture of *Arabidopsis* leaves, fruits, and flowers. *BOP1/2* are negative regulators of *KNOTTED1-LIKE* (*KNAT*) homeobox genes *BREVIPEDICELLUS* (*BP*), *KNAT2*, and *KNAT6* in leaves. Here, we examine the interaction of *BOP1/2* with *BP* and the BELL1-like homeobox gene *PENNYWISE* (*PNY*), whose interacting products regulate meristem function and pattern the inflorescence and fruit. We show that overexpression of *BOP1/2* causes *bp* and/or *pny*-like inflorescence defects and that inactivation of *BOP1/2* rescues *bp* and *pny* inflorescence and fruit defects, similar to inactivation of *KNAT2* and *KNAT6*. *BOP2* expression domains are differentially enlarged in *bp* and *pny* mutants, corresponding to compact internodes, clustered or downward-oriented siliques, and ectopic stem lignification. We further show that co-misexpression of *BOP1/2* and *KNAT6* are required to restrict growth in the inflorescence. Antagonism between *BOP1/2*-*KNAT6* and *BP*/*PNY* in inflorescences is explained in part by their opposing regulation of target genes, represented by *AtPRXR9GE*, a lignin biosynthetic gene that is repressed by *BP* and activated by *BOP1/2* in stems. These data establish the molecular basis of *bp* and *pny* defects and reveal how antagonism between *BOP1/2*-*KNAT6* and *BP*/*PNY* regulates inflorescence architecture.

42) The role of the exocyst subunit, *Sec15*, in the *Arabidopsis thaliana* compatible pollen response.

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In *Arabidopsis thaliana*, the exocyst complex is proposed to have an essential role in the stigma during the early stages of pollen-pistil interactions for accepting compatible pollen. The exocyst is proposed to dock vesicles at the plasma membrane for delivering resources to the pollen grain for adhesion, hydration, germination and pollen tube growth. The core of my research involves exploring the role of one of the subunits of the complex, *Sec15*. In the *Arabidopsis thaliana* genome, there are two homologs for *Sec15*, *Sec15a* and *Sec15b*. Previously, we suppressed the expression of *Sec15b*, as it was predicted to be more highly expressed, based on the public microarray expression datasets, in *A. thaliana* stigmas. *Sec15b*'s expression was down regulated using an RNAi construct under the control of the stigma-specific *SLR1* promoter. However, these transgenic *A. thaliana* plants only showed a mild defect in pollen hydration as a result of reduced *Sec15b* expression. Thus, an alternate strategy was devised to suppress the expression of both *Sec15a* and *Sec15b* in the stigma, and these transgenic plants are currently being analyzed. Preliminary results show that this strategy is more successful in blocking the compatible pollen response in the transgenic stigmas.

43) RNA Expression Analyses of Plant U-box/ARM family members in *Arabidopsis thaliana*.

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In *Brassica* self-incompatibility, the recognition of a pollen SCR/SP11 ligand by a stigma-specific S Receptor Kinase (SRK) is proposed to trigger a signalling pathway in stigmatic papilla that culminates in self-pollen rejection. In this self-incompatibility response, an E3 ubiquitin ligase, ARC1, functions downstream of activated SRK. *Brassica* ARC1 belongs to the sub-family of class II plant U-box (PUB) proteins that contain a U-box domain, an ARM repeat domain and U-box N-terminal domain (UND). The ARM repeat domain enables ARC1 to bind to SRK while the UND domain is thought to mediate binding to ARC1's target, Exo70A1. In an effort to better understand the function of 17 related UND/U-box/ARC E3 ligases in *Arabidopsis thaliana*, we are conducting gene expression analyses using RT-PCR in different tissues from different ecotypes. These RT-PCR analyses are also being compared to the public microarray expression datasets available at the Bio-Array Resource (<http://bar.utoronto.ca>) to assess the level overlap between these two different approaches.

44) Validation of *de novo* bioinformatic predictions of *Arabidopsis thaliana* cis-regulatory elements using *in planta* GFP/GUS expression assays.

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Cis-regulatory elements (CREs) are transcription factor (TF) binding sites that allow for gene activation, enhancement, suppression, or silencing. Currently, CREs are poorly characterized with <2% known CREs for the ~1500 *Arabidopsis thaliana* TFs. Our research aims to characterize the *de novo* predictions of CREs involved in abiotic stress responses (cold, cold and evening, genotoxic, heat, osmotic, salt), hormone treatment (ABA) and tissue specific expression. The *de novo* predictions were generated using *AtGenExpress* expression compendia and custom designed expression profiles. The probabilistic and enumerative methods used for CRE prediction resulted in putative CREs for 38 conditions; 9 of which, based on CRE composition and downstream target function, are being further analyzed. These 9 CREs were stably transformed in *A. thaliana* Columbia-0 as either a native or synthetic construct with an eGFP/GUS reporter. Preliminary testing of leaves from seedlings of synthetic genotoxic and heat lines has shown GUS staining upon stress induction. Further testing of all 9 CREs on 18-day old seedlings are currently in progress to allow for verification, validation and characterization of the putative CREs. Characterization will have significant biological and industrial applications allowing for a better understanding of mechanisms governing genetic regulation.

45) Characterization of Corn Cellulose Fiber for Manufacturing Automotive Plastic Parts

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Corn production in Ontario provides a large source of natural fiber that might be used in automobiles as replacements for glass fibers in plant-based or petroleum-based plastics. However, the use of corn fiber for producing reinforced polymeric composites in automotive parts is limited by the lack of information about its functional properties, especially during mechanical stress. The objective of the current research is to determine the relationships between the genetic makeup of corn inbred lines, their fiber compositions and functional properties. Quantitative trait loci (QTL) for cellulose, lignin, and hemicellulose content as well as QTL for corn stalk fiber composition (especially ferulic acid content) will be determined using a recombinant inbred line (RIL) population that is segregating for ferulic acid content in the seed. The RILs are segregating for cellulose, hemicellulose, lignin and phenolics (free and cell wall-bound) in their stalks and cobs. Differences between parents and among RILs have been observed in FTIR spectra of stalk fibers in wavelengths associated with polysaccharide and lignin functional groups. The characterization of composite materials is underway. The study will provide an understanding of the genetic control of cell wall traits that are important for the use of corn fibers in biocomposite materials. This could identify selection criteria for corn lines/hybrids with superior fiber traits for biocomposite production.