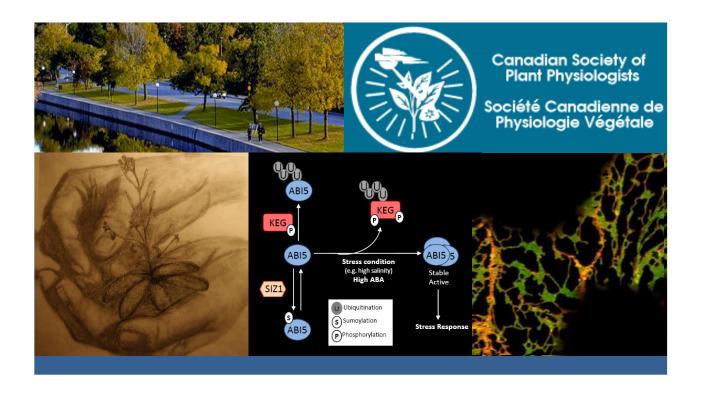
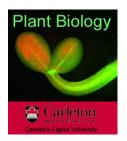
Proceedings of the Canadian Society of Plant Physiologists Eastern Regional Meeting & Plant Development Workshop





Carleton University Ottawa, Ontario December 2-3, 2011

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

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Welcome/Bienvenue

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

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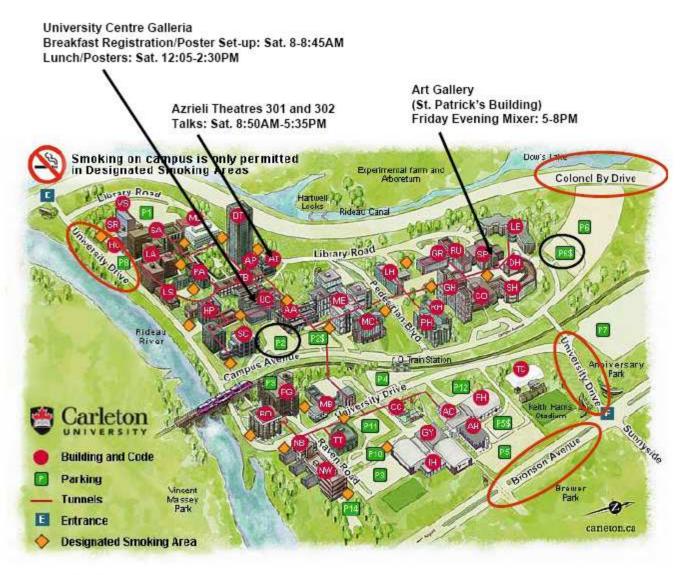
Eastern Regional Meeting/Congrès Régional de l'Est

Carleton University, Ottawa, Ontario, Canada

December 2nd and 3rd, 2011

Local Organizing Committee:
Owen Rowland
Shelley Hepworth
Gopal Subramaniam

Cover Art: Drawing of hands with plant - Sheryl Beauchamp; Ubiquitin pathway - Sophia Stone Lab; GFP image - Federica Brandizzi Lab; Composite - Shelley Hepworth



Parking:

Friday Evening – park in Parking Lot 6 near to Art Gallery (\$6 for the evening) Saturday Day – park in Parking Lot 2 in front of UniCentre (\$2 for the day)



We thank the following **SPONSORS** for their support of the 2011 Eastern Regional Meeting of the Canadian Society of Plant Physiologists:

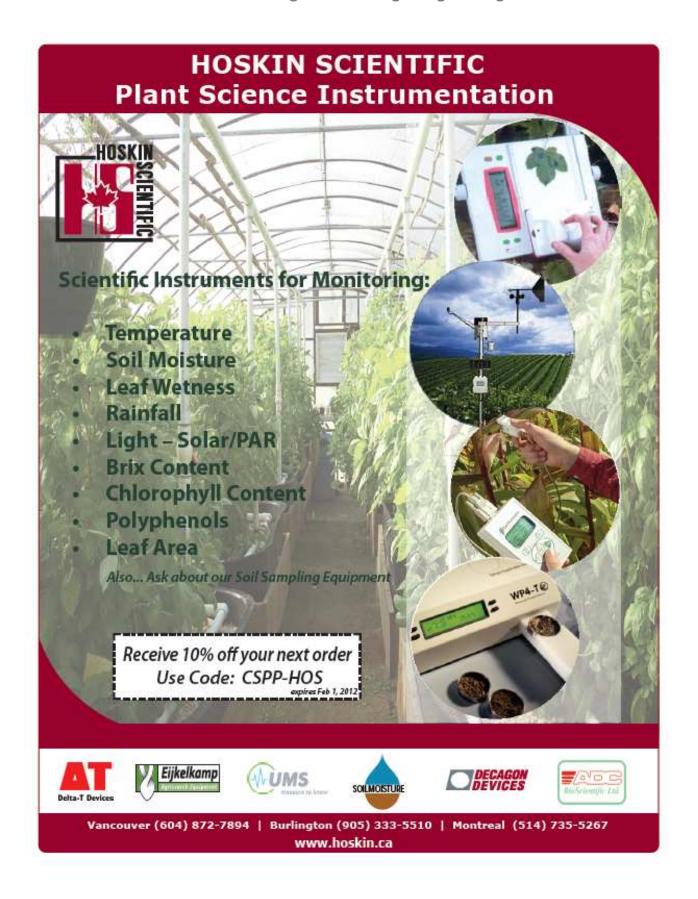




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2011 CSPP-ERM Program Overview

Friday, December 2nd, 2011 - Registration and Mixer

Carleton University Art Gallery, St Patrick's Building: 5:00-8:00 PM

| Saturday, December 8:00 - 8:45 AM | Registration, Breakfast, and Poster Set-up Location: University Centre Galleria |
|-----------------------------------|--|
| 8:50 - 10:30 AM | Opening Ceremonies and Plenary Talks Location: Azrieli Theatre 302 |
| 8:50 - 9:00 AM | Opening Ceremonies |
| 9:00 - 9:45 AM | Federica Brandizzi, Michigan State University "Functional and morphological analyses of the early plant secretory pathway" |
| 9:45 - 10:30 AM | Sophia Stone, Dalhousie University "Function and regulation of E3 ubiquitin ligases in hormone signaling" |
| 10:30 - 10:50 AM | Coffee/Tea Break (Azrieli Theatre, Upstairs Concourse) |
| 10:50 am – 12:05 pm | Speaker Sessions A and B run concurrently: Session A (<i>Azrieli Theatre 301</i>): Biotic Interactions Session B (<i>Azrieli Theatre 302</i>): Metabolism |
| 12:05 - 2:30 РМ | Lunch Buffet and Poster Viewing (including judging of student posters) Location: University Centre Galleria 12:30-1:30 PM: Odd Number Poster Presentations 1:30-2:30 PM: Even Number Poster Presentations |
| 12:05 - 1:35 РМ | CSPP Executive Meeting (Executive members only) Location: Tory Building, Room 234 |
| 2:35 - 3:50 PM | Speaker Sessions C and D run concurrently: Session C (<i>Azrieli Theatre 301</i>): Genomic Approaches Session D (<i>Azrieli Theatre 302</i>): Cell Biology |
| 3:50 - 4:10 PM | Coffee/Tea Break (Azrieli Theatre, Upstairs Concourse) |
| 4:10 - 5:25 PM | Speaker Sessions E and F run concurrently: Session E (<i>Azrieli Theatre 301</i>): Abiotic Interactions Session F (<i>Azrieli Theatre 302</i>): Plant Development Workshop |
| 5:25 - 5:35 PM | Student Award Presentations, Concluding Remarks Location: Azrieli Theatre 302 |

Detailed Scientific Program on Saturday, December 3rd, 2011

8:00 - 8:45 AM Registration, Breakfast, and Poster Set-up

Location: University Centre Galleria

8:50 - 9:00 AM Opening Ceremonies

Location: Azrieli Theatre 302

Welcome from Owen Rowland, Carleton University

9:00 - 10:30 AM Plenary Talks

Chairperson: Anja Geitmann, Université de Montréal

9:00 - 9:45 AM Plenary Talk 1

Federica Brandizzi, Michigan State University

Location: Azrieli Theatre 302

PT-1

Functional and morphological analyses of the early plant secretory pathway

Federica Brandizzi

Michigan State University-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA

A fundamental question in eukaryotic cell biology is how cells maintain efficient compartmentalization and control the delivery and integration of biomolecules into specialized organelles. In our lab, we address these questions using the plant secretory pathway as a model. This pathway is vital to the inner workings of the cell and for communicating with the external environment during growth and in response to stress; it consists of several organelles that synthesize, shuttle, and store a large part of the cell's proteins, lipids, and sugars. In plants, the activities of the endoplasmic reticulum (ER) and Golgi apparatus, the initial organelles of the secretory pathway, are also fundamental for the synthesis and deposition of the building blocks of energy-rich compartments such as the cell wall and storage vacuole. Both organelles have unique architecture and functions, which are maintained despite exchange of membranes and lumenal contents with other organelles. Efforts in our lab focus on identifying the mechanisms governing the morphology and function of the ER and the Golgi and defining the extent to which their architecture influences their function. To address these questions we have carried out forward genetic screens based on confocal microscopy analyses of Arabidopsis seedlings expressing ER and Golgi markers. The screens are designed to identify mutants with aberrant distribution of the ER and Golgi markers compared to non mutagenized seedlings. Through the characterization of these mutants we are identifying novel genes and mutations that uncover new information on the mechanisms for the integrity of the ER and the Golgi with respect to other organelles, cytoskeleton and flow of biosynthetic cargo. Our most recent findings will be presented in this talk.

9:45 - 10:30 AM Plenary Talk 2

Sophia Stone, Dalhousie University

Location: Azrieli Theatre 302

PT-2

Function and regulation of E3 ubiquitin ligases in hormone signaling Sophia Stone

Department of Biology, Life Science Centre, Dalhousie University, 1355 Oxford Street, Halifax, NS B3H 4R2, Canada

Plant growth and development is largely influenced by ubiquitin-mediated regulation of protein stability. Specificity of the ubiquitination pathway is governed mainly by the substrate-recruiting E3 ubiquitin ligases, and consequently, E3 ligases regulate numerous cellular processes. Recent work has demonstrated that E3 ligases are an integral part of multiple hormone-signaling pathways. Main points of regulation include ubiquitin-mediated degradation of proteins involved in hormone production and/or response to hormonal stimuli, specifically transcription factors which mediate cellular responses. Despite our understanding of the role of E3 ligase during various hormone signaling events, our knowledge of how these E3 ligases are themselves regulated is still very limited. To address this issue we have focused our attention Keep on Going (KEG), a RING-type E3 ligase that negatively regulates Abscisic acid (ABA) signaling. We have shown that ABA increases the abundance of ABI5, an ABA-responsive transcription factor, by targeting KEG for proteasomal degradation via self-ubiquitination. However, the mechanism by which ABA promotes KEG self-ubiquitination over substrate ubiquitination is not known. One prominent mode of regulating ubiquitination is via phosphorylation of the E3 ubiquitin ligase and/or substrate. We have taken a number of approaches to determine if ABA-induced phosphorylation can influence KEG E3 ligase activity, regulate target availability or recognition. Our results suggest that target availability plays a major role in KEG's ability to ubiquitinate its substrate.

Speaker Sessions for the CSPP-ERM and PDW $\,$

| The presenting author's name is <u>underlined</u> An asterisk (*) denotes a student presenter being considered for an award | | |
|--|---|--|
| 10:30 - 10:50 AM | Coffee/Tea (Azrieli Theatre, Upstairs Concourse) | |
| 10:50 - 12:05 PM | Speaker Sessions A and B run concurrently | |
| | Session A. Biotic Interactions (Azrieli Theatre, 301) Chairperson: Darrell Desveaux, University of Toronto | |
| 10:50 SA-1 | Coordination of a mitochondrial superoxide burst during the hypersensitive response to bacterial pathogen in <i>Nicotiana tabacum</i> Marina Cvetkovska* and Greg C. Vanlerberghe Department of Biological Sciences and Department of Cell and Systems Biology, University of Toronto Scarborough, Toronto, ON | |
| 11:05 SA-2 | The mycorrhizal phenotype of a low nodulating pea mutant E132 (sym21) Christian Huynh* and Frédérique C. Guinel Department of Biology, Wilfrid Laurier University, ON | |
| 11:20 SA-3 | Recruitment of ESCRT to mitochondria by carnation Italian ringspot virus replicase proteins Lynn G.L. Richardson* and Robert T. Mullen Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON | |
| 11:35 SA-4 | Investigation of DIR1 movement during Systemic Acquired Resistance Marisa Melas*, Marc Champigny, Philip Carella, and Robin Cameron Department of Biology, McMaster University, Hamilton, ON | |
| 11:50 SA-5 | Two-spotted spider mite induced transcriptional responses of MicroTom Ingrid Fung*, Mike Grbic, and Vojislava Grbic Department of Biology, University of Western Ontario, London, ON | |

Session B. Metabolism (Azrieli Theatre, 302) Chairperson: Isabel Molina, Algoma University

10:50 SB-1 MicroRNA156: A new player in regulating carotenoid accumulation in plants

Abdelali Hannoufa¹, Shu Wei^{2,3}, Margaret Gruber³, George G. Khachatourians⁴, Ying Wang¹, Dwayne Hegedus³, and Isobel Parkin³

¹Agriculture and Agri-Food Canada, London, ON; ²Anhui Agricultural

University, Hefei, China; ³*Agriculture and Agri-Food Canada, Saskatoon, SK;* ⁴*University of Saskatchewan, Saskatoon, SK*

11:05 SB-2 Dissecting the complex phenotypes associated with 5'-methylthioadenosine nucleosidase deficiency

<u>Ishari Waduwara-Jayabahu</u>*¹, Natasha Peer¹, Zachary, T. Hull¹, Jun-Ichi Kakehi², Markus Wirtz³, Taku Takahashi², Rüdiger Hell³, and Barbara A. Moffatt¹

¹Department of Biology, University of Waterloo, Waterloo, ON; ²Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan; ³Heidelberg Institute of Plant Sciences (HIP), University of Heidelberg, Heidelberg, Germany.

11:20 SB-3 Advances in the characterization of threonine synthase from the pulse crops *Pisum sativum* (field pea), *Cicer arientinum* (chickpea), and *Lens culinaris* (lentil)

<u>Dominique J.K. Morneau</u>* and Susan M. Aitken Department of Biology, Carleton University, Ottawa, ON

11:35 SB-4 Quantum dot production by the cyanobacterium Synechococcus leopoliensis Antoine Hnain*, Caleb van Ry, Chad Edwards, and Daniel D. Lefebvre Department of Biology, Queen's University, Kingston, ON

11:50 SB-5 A micromolar concentration of lipo-chitooligosaccharide (Nod Bj V [C18:1, MeFuc]) enhances self-pollinated rapid cycling canola (*Brassica napus* [L.]) emergence and seed productivity

<u>Timothy D. Schwinghamer</u>*, Alfred Souleimanov, Pierre Dutilleul, and Donald L. Smith

Department of Plant Science, McGill University, Ste Anne de Bellevue, OC

12:05-2:30 PM Lunch Buffet and Poster Viewing (including judging of student posters)

Location: University Center Galleria

12:30-1:30 PM: Odd Number poster presentations 1.30-2.30 PM: Even Number poster presentations

12.05-1.35 PM CSPP Executive Meeting (Executive members only)

Location: Tory Building, Room 234

2:35 - 3:50 PM Speaker Sessions C and D run concurrently

Session C. Genomics Approaches (Azrieli Theatre, 301) Chairperson: **Gopal Subramaniam**, Agriculture and Agri-Food Canada

2:35 SC-1 Chemical interrogation of seedling sugar responses uncovers crosstalk between nutrient and hormone signalling in *Arabidopsis*

<u>Michael E Stokes</u>*¹, Abhishek Chattopadhyay¹, Olivia Wilkins¹, Eiji Nambara^{1,2}, and Malcolm M. Campbell^{1,2}.

¹Department of Cell & Systems Biology, ² Centre for Analysis of Genome Evolution & Function, University of Toronto, Toronto, ON

2:50 SC-2 GLK transcription factors - an integral component of plant defense in *Arabidopsis*

<u>Jhadeswar Murmu</u>¹, Jas Singh¹, Mike Wilton², Ghislaine Allard¹, Christina Kirkham¹, Radhey Pandeya¹, Darrell Desveaux², and Gopal Subramaniam¹ ¹ECORC, Agriculture & Agri-Food Canada, Ottawa, ON; ²Department of Cell & Systems Biology, University of Toronto, Toronto, ON

3:05 SC-3 The relationship between intra-specific variation in the *Populus* transcriptome, stomatal development, and the metabolome in response to drought

<u>Hamanishi, E.T.</u>*¹, Raj, S.², Wilkins, O.², Thomas, B.R.^{4,5}, Mansfield, S.D.⁶, Plant, A.L.⁷, and M.M. Campbell^{2, 3}

¹Faculty of Forestry, ²Centre for the Analysis of Genome Evolution & Function, and ³Dept of Cell & Systems Biology, University of Toronto, Toronto, ON; ⁴Alberta-Pacific Forest Industries Inc., Boyle, AB; ⁵Department of Renewable Resources, University of Alberta, Edmonton, AB; ⁶Department of Wood Science, University of British Columbia, Vancouver, BC; ⁷Department of Biological Sciences, Simon Fraser University, Burnaby, BC

3:20 SC-4 Identification of carbohydrate-binding ability based on proteome-wide tertiary structure prediction: *in vitro* validation

Shaowei Dong*1, Andrew Doxey2, and Nicholas Provart1

¹Department of Cell & System Biology, University of Toronto, Toronto, ON; ²Department of Developmental Biology, Stanford University, Stanford, California, USA

3:35 SC-5 Next-generation mapping of disease resistance genes in *Arabidopsis*: Exploiting natural variation

<u>Timothy Lo</u>*¹, Steven P. Chatfield¹, Ryan S. Austin³, Jennifer Lewis¹, Robert Breit², Andre Santos-Severino², Nicholas J. Provart^{1,2}, Pauline Fung², Pauline W. Wang^{1,2}, David S. Guttman^{1,2}, and Darrell Desveaux^{1,2}

¹Department of Cell & Systems Biology, University of Toronto, Toronto, ON; ²Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto, ON; ³Agriculture and Agri-Food Canada, London, ON

Session D. Cell Biology (*Azrieli Theatre*, 302) Chairperson: **Tamara Western**, McGill University

2:35 SD-1 Dynamics of cell plate formation during plant cytokinesis

Chloë Triplet-van Oostende¹, Dominique Guillet², and Paul Wiseman², and <u>Anja</u> Geitmann¹

¹Institut de recherche en biologie végétale, Département de sciences biologiques, Université de Montréal, QC; ²Physics Department, McGill University, Montréal, OC

2:50 SD-2 Turnip mosaic virus alters the host cell secretory pathway and remodels the endomembrane network

Romain Grangeon*¹, Jun Jiang¹, Maxime Agbeci¹, Huanquan Zheng², and Jean-François Laliberté¹

¹INRS-Institut Armand-Frappier, Institut national de la recherche scientifique, Laval, QC; ²Developmental Biology Research Initiatives, Department of Biology, McGill University, Montréal, QC

3:05 SD-3 Identification of CGI-58-binding proteins: The potential role of CGI-58 in the regulation of triacylglycerol metabolism and lipid signaling pathways in plant cells

Nicholas Khuu*¹, Sunjung Park², Satinder Gidda¹, Patrick Horn³, Chris James³, Kent Chapman³, John Dyer², and Robert Mullen¹

¹ Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON; ²USDA-ARS-ALARC, Maricopa, AZ, USA; ³University of North Texas, Denton, TX, USA

3:20 SD-4 A novel approach for the derivation of intrinsic mechanical properties for the cell wall of *Arabidopsis thaliana*

Robert Palin^{1,2}, Jeremy Pritchard², and Colin Thomas³

¹Institut de recherche en biologie végétale, Département de sciences biologiques, Université de Montréal, QC; ²School of Life and Environmental Sciences, University of Birmingham, UK; ³School of Engineering, University of Birmingham, UK

3:35 SD-5 Localizing arogenate dehydratase from Arabidopsis thaliana

Susanne E. Kohalmi¹, Crystal D. Bross^{1,2}, and Danielle M. Styranko^{1,3}

Department of Biology, University of Western Ontario, London, ON;

Current address: Department of Biological Sciences, University of Calgary, Calgary, AB;

Current address: Faculty of Medicine, University of Saskatchewan, Saskatoon, SK

3:50 – 4:10 PM Coffee/Tea Break (*Azrieli Theatre, Upstairs Concourse*)

| 4:10 - 5:25 PM | Speaker Sessions E and F run concurrently |
|----------------|---|
| | Session E. Abiotic Interactions (Azrieli Theatre, 301) Chairperson: Leonid Savitch, Agriculture and Agri-Food Canada |
| 4:10 SE-1 | Reductive iron acquisition by algal cells interacts with the nature of the ferric chelators Mathew B. Sonier and Harold G. Weger Department of Biology, University of Regina, Regina, SK |
| 4:25 SE-2 | Intraspecific variation of drought stress tolerance in Douglas-fir Laura Verena Junker* ^{1,2} , Henning Wildhagen ¹ , and Ingo Ensminger ^{1,2} Department of Forest Ecology, Forest Research Institute Baden-Wuerttemberg, Freiburg, Germany; Department of Biology, University of Toronto at Mississauga, Mississauga, ON |
| 4:40 SE-3 | The path to restoration is stress-free Maye Saechao*, Barbara Moffatt, and Susan Lolle. Department of Biology, University of Waterloo, Waterloo, ON |
| 4:55 SE-4 | UV-B adaptation in Arabidopsis thaliana ecotypes D.K. Biswas ¹ , B.L. Ma ¹ , G.M. Jiang ² , and M.A.K. Jansen ³ Agriculture and Agri-Food Canada, Ottawa, ON; Institute of Botany, Chinese Academy of Sciences, Beijing, P.R. China; Department of Zoology, Ecology and Plant Sciences, University College Cork, Cork, Ireland |
| 5:10 SE-5 | Photosystem I photoprotection Lindsay Berry ¹ , Ricarda Bentham ¹ , and Ken Wilson ¹ Department of Biology, University of Saskatchewan, Saskatoon, SK |

| | Session F. Plant Development Workshop (Azrieli Theatre, 302) Chairperson: Shelley Hepworth, Carleton University |
|-----------|--|
| 4:10 SF-1 | Arabidopsis seed coat mucilage structure is regulated via the FEI2 receptor-like kinase/SOS5 fasciclin-like arabinogalactan protein pathway and CELLULOSE SYNTHASE 5 Heather E. McFarlane ¹ , Smadar Harpaz-Saad ² , Shouling Xu ² , Uday K. Divi ¹ , Bronwen Forward ¹ , Joseph J. Kieber ² , and Tamara L. Western ¹ Biology Department, McGill University, Montreal, QC; Biology Department, University of North Carolina, Chapel Hill, North Carolina, USA |
| 4:25 SF-2 | An insight into the function of the <i>HUA2</i> gene family Preetam Janakirama*, Sathya Sheela Jali, Uday Sajja, Sarah Rosloski, and Vojislava Grbic University of Western Ontario, London, ON |
| 4:40 SF-3 | AKIN10 and FUSCA3 interact to control lateral organ development and phase transitions in Arabidopsis Allen Yi-Lun Tsai* and Sonia Gazzarrini Department of Biological Sciences & Department of Cell and Systems Biology, University of Toronto, Toronto, ON |
| 4:55 SF-4 | Auxin Response Factor functions in plant regeneration - molecular control of de novo shoot formation Wenzislava Ckurshumova, Naden T. Krogan, George Stamatiou, Tatiana Smirnova, Adriana E. Caragea, and Thomas Berleth Department of Cell and Systems Biology, University of Toronto, Toronto, ON |
| 5:10 SF-5 | The role of ARC1 in the self-incompatibility pathway in A. lyrata Emily Indriolo, Pirashaanthy Tharmapalan, and Daphne Goring Department of Cell & Systems Biology, University of Toronto, Toronto, ON |
| 5:25 PM | Student Award Presentations, Concluding Remarks Location: Azrieli Theatre, 302 |
| 5:35 PM | Conclusion of Meeting |

Sessions A-F, Oral Presentation Abstracts

Session A. Biotic Interactions

SA-1

Coordination of a mitochondrial superoxide burst during the hypersensitive response to bacterial pathogen in *Nicotiana tabacum*

Marina Cvetkovska* and Greg C. Vanlerberghe

Department of Biological Sciences and Department of Cell and Systems Biology, University of Toronto Scarborough, Toronto, Ontario, Canada

Alternative oxidase (AOX) is a non-energy conserving branch of the mitochondrial electron transport chain, the activity of which may reduce superoxide (O_2) generation by the chain. Any O_2 that is generated can be scavenged and converted to H_2O_2 by a matrix manganese superoxide dismutase (MnSOD). We characterized multiple responses of *Nicotiana tabacum* to different pathovars of the bacterial pathogen *Pseudomonas syringae*. These included a compatible response associated with necrotic cell death (pv tabaci), an incompatible response that included hypersensitive response (HR) cell death (pv maculicola) and an incompatible response that included defenses but lacked the HR (pv phaseolicola). We show that signaling molecules known to induce the tobacco Aox1a gene (salicylic acid [SA], nitric oxide [NO], H_2O_2) accumulated preferentially during the HR response. Despite this, expression of Aox1a is suppressed during the HR response, while strongly induced during the defense response. Also, MnSOD activity declined in response to pv maculicola. Finally, we show that the HR response specifically, is accompanied by an early mitochondrial O_2 burst prior to cell death. We propose that a coordinated response of key ROS-avoiding (AOX) and ROS-scavenging (MnSOD) components in the mitochondrion is important in the determination of cell fate during different responses to pathogen.

SA-2

The mycorrhizal phenotype of a low nodulating pea mutant E132 (sym21)

Christian Huynh* and Frédérique C. Guinel

Department of Biology, Wilfrid Laurier University, Ontario, Canada

The regulation of legume-microbe interactions is important to maintain the symbiotic status of these relationships. Legumes employ systemic negative feedback systems to control nodule and mycorrhizae formation. E132 is a low nodulating pleiotropic mutant of *Pisum sativum*. The shoot-controlled nature of E132 nodulation phenotype suggests that this mutant may be a useful tool in studying the shoot-derived components of the regulation of nodulation. The signals regulating nodule and mycorrhizae formation have been suggested to overlap. Therefore elucidation of E132 mycorrhizal phenotype could provide further evidence for a common pathway. The objective of this study was to elucidate E132 mycorrhizal status. Plants were grown in vermiculite-TurfaceTM (1:1, v:v) containing *Glomus irregulare*-infected leek inoculum (1:10, v:v). Lateral roots were sampled, cleared, and stained with a Schaffer[®] ink-vinegar solution 30 days after planting. The roots were scanned for mycorrhizal structures and successful symbiosis determined by quantifying extraradical hyphae, hyphopodia, epidermal and cortical entry, and arbuscule formation. E132 was found not to differ from wild-type Sparkle in number of mycorrhizal associations indicating that its mutation may not affect a symbiotic regulatory signal common to the two mutualisms. Our results will be discussed in the context of other nodulation mutants.

SA-3

Recruitment of ESCRT to mitochondria by carnation Italian ringspot virus replicase proteins

Lynn G.L. Richardson* and Robert T. Mullen

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

Carnation Italian ringspot virus (CIRV) is a positive-strand RNA virus that assembles its membrane-bound replication complexes at mitochondria, a process that is accompanied by the inward invagination of the mitochondrial outer membrane, and the eventual formation of spherules that serve as the site of viral RNA synthesis. Recent evidence has suggested that a virus belonging to the same family as CIRV uses the host-cell ESCRT (Endosomal Sorting Complex Required for Transport) machinery – a multi-protein complex normally involved in late endosome maturation – for the analogous formation of spherules at the peroxisomal boundary membrane. Interestingly, in mammalian cells, ESCRT is also exploited by HIV to facilitate budding from the plasma membrane. Here, we investigated the potential role of ESCRT in CIRV replication complex assembly by examining whether ESCRT components re-localize from late endosomes to mitochondria in cultured plant cells transiently co-expressing CIRV replicase proteins. Our results indicate that the CIRV replicase proteins recruit certain ESCRT components to mitochondria, and we have identified regions of the replicase proteins involved in this recruitment. The implications of these results for understanding the role of ESCRT in positivestrand RNA virus replication in general, as well as normal ESCRT function in non-infected plant cells are discussed.

SA-4

Investigation of DIR1 movement during Systemic Acquired Resistance

Marisa Melas*, Marc Champigny, Philip Carella, and Robin Cameron Department of Biology, McMaster University, Hamilton, Ontario, Canada

Systemic Acquired Resistance (SAR) is a plant defense response induced by an initial infection that leads to production of a long distance signal(s). This mobile signal is perceived by distant leaves resulting in resistance to normally virulent pathogens. DIR1 is involved in SAR long distance signaling as demonstrated by the presence of DIR1 in petiole exudates (enriched for phloem sap) collected from SAR-induced wild type, but not mock-induced or *dir1-1* plants. Transgenic *dir1-1* plants containing an estrogen inducible DIR1-EGFP were used to visualize DIR1 movement to distant leaves. Estrogen-activated expression of DIR1 rescued the *dir1-1* SAR defect only after the same leaf was induced for SAR. DIR1 was also detected in distant leaf petiole exudates providing evidence that DIR1 does move to distant tissues during SAR. Whole mount immunohistochemistry studies are ongoing to visualize DIR1 during SAR. Studies using our *Arabidopsis*-Cucumber SAR model suggest that a functional DIR1 orthologue exists in cucumber (*CuDIR1*, 63% similar to *AtDIR1*). Transient expression of CuDIR1 in *dir1-1 Arabidopsis* leaves using *Agrobacterium* transient transformation restored the SAR response. Our data suggests that DIR1 is conserved in the Cucurbit and Brassica families and that DIR1 plays a similar role during SAR in both cucumber and *Arabidopsis*.

SA-5

Two-spotted spider mite induced transcriptional responses of MicroTom

<u>Ingrid Fung*</u>, Mike Grbic, and Vojislava Grbic

Department of Biology, University of Western Ontario, London, Ontario, Canada

The two-spotted spider mite (*Tetranychus urticae*) is a pest of cultivated tomatoes (*Solanum lycopersicum*) in green houses and hot dry climates worldwide. While tomato resistance to *T. urticae* feeding has been linked with constitutive defences, limited research has been done on the genetic basis of induced defences. The genetic model cultivar MicroTom (MT) was identified as resistant to *T. urticae* feeding through a screen of 28 tomato cultivars. There are three known mutations *dwarf* (*d*), *self-pruning* (*sp*), and *uniform ripening* (*u*) found in the MT background. Testing of near isogenic lines (NIL) with each of these mutations restored to wildtype function revealed that an increase in brassinosteroid biosynthesis increased susceptibility to spider mite feeding in a MT background. Here we present a preliminary analysis of the transcriptional responses of MT and a brassinosteroid biosynthesis-restored mutant (MT-D) to spider mite feeding. Through the analysis of these transcriptional responses, we hope to identify transcriptional programs differentially regulated between these two lines which may be associated with resistance to *T. urticae* feeding in MT.

Session B. Metabolism

SB-1

MicroRNA156: A new player in regulating carotenoid accumulation in plants

Abdelali Hannoufa¹, Shu Wei^{2,3}, Margaret Gruber³, George G. Khachatourians⁴, Ying Wang¹, Dwayne Hegedus³, and Isobel Parkin³

The *Arabidopsis thaliana* microRNA156 (miR156) regulates plant growth and development by suppressing eleven plant-specific transcription factor *SPL* (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE) genes. We characterized a new *Arabidopsis* T-DNA activation tagged mutant, sk156, with altered morphology and increased levels of the seed carotenoids violaxanthin, lutein, zeaxanthin and β -carotene compared to wild type (WT). We found that enhanced miR156b expression due to a T-DNA insertion was responsible for these phenotypes. Using 5'-RACE analysis, we determined SPL15 as one of the targets of miR156. Expression analysis of carotenoid catabolism *CCD* and *NCED* genes in sk156 revealed that miR156b and SPL15 increase levels of seed carotenoids by attenuating carotenoid catabolism rather than by enhancing de *novo* biosynthesis. We also overexpressed miR156b gene in Brassica napus under the 35S and napin promoters. Our results demonstrated that constitutive expression of miR156 in B. napus resulted in enhanced levels of seed lutein and β -carotene and a two-fold increase in the number of flowering shoots, whereas miR156 driven by the napin promoter did not affect these traits. These data suggest that miR156 could be applied in plant breeding initiatives for enhancing carotenoid production in B. napus and other economically important crops.

¹Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON, Canada;

²Anhui Agricultural University, Hefei 230036, China;

³Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, Canada;

⁴University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada

SB-2

Dissecting the complex phenotypes associated with 5'-methylthioadenosine nucleosidase deficiency

<u>Ishari Waduwara-Jayabahu</u>*¹, Natasha Peer¹, Zachary, T. Hull¹, Jun-Ichi Kakehi², Markus Wirtz³, Taku Takahashi², Rüdiger Hell³, and Barbara A. Moffatt¹

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Arabidopsis thaliana contains two genes encoding methylthioadenosine nucleosidase (MTN) MTN2.MTN1. At4g38800 and At4g34840. This enzyme Methylthioadenosine (MTA) produced by nicotianamine, polyamine, and ethylene biosynthesis to methylthioribose and adenine. Mutants that have disruptions in both MTN genes have a myriad of developmental defects, including abnormal vasculature and reproductive problems. The goal of this research was to define the fundamental abnormal trait associated with reduced MTN activity. To this end, a series of MTN-deficient lines with differing residual MTN activity were developed. These mutant lines were further assessed and compared with the WT with respect to their MTN activity and Met-related metabolite content. Their residual MTN activity correlated with the severity of their phenotype: (1) a complete loss-of-function MTN mutant was embryo lethal, (2) the most severe viable mutant which has 14 % of the WT MTN activity is associated with a complex pleiotropic phenotype, and (3) a less severe mutant with 28 % of the MTN activity of the WT has a delayed transition to flowering (bolting) along with the xylem proliferation defects associated with most severe mutant. Based on these analyses we conclude that the delayed bolting is the fundamental trait associated MTN deficiency.

SB-3

Advances in the characterization of threonine synthase from the pulse crops *Pisum sativum* (field pea), *Cicer arientinum* (chickpea), and *Lens culinaris* (lentil)

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The methionine and threonine biosynthetic pathways of plants diverge from the common substrate, O-phosphohomoserine (OPHS). Threonine synthase (TS) catalyzes the formation of threonine (Thr) from OPHS, releasing phosphate as a by-product. Biochemical investigations of TS have relied on HPLC-mediated separation and quantification or the discontinuous malachite green assay, which monitors the release of phosphate. We have cloned TS from the pulse crops Pisum sativum (field pea), Cicer arientinum (chickpea), and Lens culinaris (lentil), as well as the two TS homologues from Arabidopsis thaliana, and confirmed their function via the complementation of a Thr-auxotrophic Escherichia coli strain. The plastid targeting sequence of pulse TS has been verified through fusion with green fluorescent protein (GFP) and fluorescence imaging. We have also designed a continuous TS assay using threonine deaminase (TD) and hydroxyisocaproate dehydrogenase (Ho-HxoDH). The latter reduces α -ketobutryate, the product of TD, with concomitant oxidation of NADH to NAD⁺, monitored as decrease in absorbance at 340 nm. This assay was validated by the characterization of E. coli TS. The kinetic parameters $(k_{cat} = 4 \text{ s}^{-1}, K_m = 0.34 \text{ mM})$ and the determined pH optimum of 8.7, measured using the continuous assay, are consistent with values reported for this enzyme based on the discontinuous malachite green assay.

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SB-4

Quantum dot production by the cyanobacterium Synechococcus leopoliensis

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Aerobically grown cyanobacteria biotransform Hg(II) into HgS. This occurs with great rapidity $(K_{1/2} = 20 \text{ min at } 1,000 \text{ ppm Hg})$. These results lead to the investigation of the biotransformation of Cd(II) and Zn(II), both of which were also biotransformed into sulphides. The yields of CdS and ZnS after 48 h exposures to Cd(II) or Zn(II) were as high as 10 g per Kg dry weight of cells and this was enhanced by pretreatments with sulphate. Therefore, an investigation into the synthesis of the semiconducting nanoparticles, quantum dots (QDs), composed of zinc and the sulphur analogue, selenium was undertaken. Quantum dots are a special class of materials with utility in optical and electronic applications. Simultaneous incubation of *Synechococcus leopoliensis* with 1 mM $ZnCl_2$ and 5 mM Na_2SeO_3 revealed fluorescence in response to 350 nm incident illumination. After 48 hours blue emission spectra indicated that QDs were small - less than 2 nm diam. After 120 hours QDs emitted in the blue-green indicating a 3 nm diam. size. After 192 hours green-yellow emissions revealed 4 nm QDs. These findings indicate a clear potential for S. *leopoliensis* in photobioreactor-based QD production.

SB-5

A micromolar concentration of lipo-chitooligosaccharide (Nod Bj V [C18:1, MeFuc]) enhances self-pollinated rapid cycling canola (*Brassica napus* [L.]) emergence and seed productivity

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Using rapid cycling *Brassica napus*, we investigated the applicability of lipochitooligosaccharide (LCO) as a crop treatment. Seed treatment with 10 mL of 1 μ M LCO solution improved *B. napus* seedling emergence, and increased seed production and seed weight per plant. Seed treatment with 10 mL 1 μ M LCO solution, supplemented with 0.5 mL of 1 μ M LCO foliar spray at the flowering stage of plant development, also increased seed production, seed weight per plant, and single seed weight. Field experiments with agricultural cultivars will be necessary to ascertain whether or not this treatment could increase the productivity of agricultural ecosystems.

Session C. Genomic Approaches

SC-1

Chemical interrogation of seedling sugar responses uncovers crosstalk between nutrient and hormone signalling in *Arabidopsis*

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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 folate biosynthesis in plants, to restrict hypocotyl elongation in a sucrose-dependent fashion. Transcriptome analysis identified a small set of transcripts that show altered response to the compound when administered in the presence of sucrose, including multiple components of auxin signal transduction. Chemical inhibition of polar auxin transport ameliorates the inhibitory effect of the sulfonamide, further implicating auxin signalling as underpinning plant responses to the compound. Complementary reverse- and forward-genetic screens are currently being pursued to unveil the mechanisms through which nutrient cues and auxin signalling converge to shape seedling development.

SC-2

GLK transcription factors - an integral component of plant defense in Arabidopsis

<u>Jhadeswar Murmu</u>¹, Jas Singh¹, Mike Wilton², Ghislaine Allard¹, Christina Kirkham¹, Radhey Pandeya¹, Darrell Desveaux², and Gopal Subramaniam¹

Arabidopsis Golden2-like (GLK) genes, GLK1 and GLK2, are members of the GARP domain superfamily of transcription factors (TF). GLK-TFs play a redundant role in chloroplast assembly in a cell autonomous manner. The glk1 glk2 double mutants are pale green in color with fewer grana in chloroplasts than wild-type (WT), but otherwise exhibit normal developmental growth characteristics. In pathology studies, we observed that glk1 glk2 double mutants exhibit increased resistance to the biotroph Hyaloperonospora arabidopsidis (Hpa) compared to WT Arabidopsis plants. In contrast, plants overexpressing GLK1 (35S:GLK1) are highly susceptible to Hpa infection. Microarray analyses of infected Arabidopsis seedlings showed that in contrast to the WT and 35S:GLK1 plants, defense genes associated with JA/ET signalling pathway as well as basal defense genes were highly upregulated in glk1 glk2 double mutants. Epistasis analyses with genes involved in the SA, JA/ET signalling pathways suggested that GLK1 mediated disease susceptibility is partially dependent on SA accumulation. Additional pathology studies with Pseudomonas syringae, suggests that GLK1 is involved in basal defense. Together, these results suggest that Arabidopsis GLK1 acts as a negative regulator of plant basal defense.

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SC-3

The relationship between intra-specific variation in the *Populus* transcriptome, stomatal development, and the metabolome in response to drought

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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; therefore, understanding the mechanisms by which these economically and ecologically important forest trees respond to drought is of paramount importance. The ability of *Populus* trees to contend with drought is dependent on the responsiveness of the genome, and in turn, the ability of the transcriptome to appropriately remodel growth, metabolism and development. Amassing evidence indicates that different species of *Populus* have divergent mechanisms and adaptations to contend with drought stress; however, individuals within a given species also display divergent drought responses. In order to investigate the intra-specific variation underpinning the drought response, we examined six genotypes of P. balsamifera. Using Affymetrix Poplar GeneChips, we found a positive correlation between the magnitude of drought-induced changes in the transcriptome and the capacity of the genotype to maintain growth. Surprisingly identifiable differences at the transcriptome were observed, and similar responses were observed within the metabolome. Although common drought responses could be identified within the species, the complexities of these responses must be taken into consideration when defining species- or genus-level drought responses.

SC-4

Identification of carbohydrate-binding ability based on proteome-wide tertiary structure prediction: *in vitro* validation

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The interactions between carbohydrates and proteins are very important in many biological functions such as cell adhesion and signal transduction. Carbohydrate-binding proteins are very diverse in structure, and aromatic residues (e.g. Tyr and Trp) play a significant role in ligand binding. In my study, I will test the carbohydrate-binding ability of the proteins with aromatic residues predicted to form a surface where the two aromatics form a flat binding site or a sandwich structure where there is enough room in between the aromatic side chains to fit a carbohydrate. The clones of the candidate proteins from *Arabidopsis thaliana* were transferred into *Nicotiana benthamiana* and after a tag-based purification, the proteins will be hybridized to glycan arrays from the Consortium for Functional Gylcomics. Concurrently, *Arabidopsis thaliana* cell wall fractions will be presented to the proteins and the binding abilities of the proteins to them will be assayed.

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SC-5

Next-generation mapping of disease resistance genes in *Arabidopsis*: Exploiting natural variation

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The ability to recognize and defend against pathogens is crucial for the survival of all organisms. For plants, key players in this recognition are resistance (R) proteins that recognize the action of specific pathogen effector proteins and trigger a robust resistance response termed effector-triggered immunity. Nearly ~170 R proteins have been identified from the *Arabidopsis thaliana* Col-0 ecotype, however only a handful have been functionally characterized. Defining the resistance specificities of plant R proteins continues to be a major challenge in the study of plant immunity. We are exploiting the natural variation of R proteins between ecotypes of *A. thaliana* to define their specificities using Next Generation Mapping (NGM), which provides a rapid and robust alternative to classical genetic mapping techniques. NGM allows us to efficiently map polymorphisms among R proteins that contribute to their functional diversity. We will present our current efforts to map the suite of R proteins that recognizes the bacterial pathogen *P. syringae* pv. maculicola ES4326. We anticipate that this approach will not only increase our knowledge of the molecular mechanisms of plant immunity but can also be applied to defining R protein specificities in agronomically-important crop species.

Session D. Cell Biology

SD-1

Dynamics of cell plate formation during plant cytokinesis

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Cell division in plants implicates the assembly of new cell wall surface between the two daughter cells. The targeted deposition of the material necessary for this assembly - cell wall polymers and membrane is therefore a critical process the regulation of which is pivotal for successful cytokinesis and consequently organ development. The intracellular delivery of building material to the cell plate is performed by vesicles transported along the cytoskeletal elements forming the phragmoplast. How the logistics of this transport process is regulated in space and time is poorly understood, however. Monitoring and quantifying the dynamics of transport vesicles in the living cell is severely challenged by the small size of these organelles which is below the diffraction limit of the optical microscope. Particularly in situations when vesicles move rapidly and/or in dense clouds, conventional particle tracking methods are therefore powerless. By combining high temporal and spatial resolution confocal laser scanning microscopy with advanced imaging techniques originally developed for the analysis of molecular movements (STICS, spatio-temporal image correlation spectroscopy), we monitored the intracellular dynamics of vesicles during cell plate formation in dividing BY-2 cells. We used these motion data to generate a dynamic profile of cell plate formation and associated vesicle dynamics that will be useful to study the effects of mutations interfering with transport or cell wall assembly in this system.

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SD-2

Turnip mosaic virus alters the host cell secretory pathway and remodels the endomembrane network

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Turnip mosaic virus (TuMV) is a member of the genus *Potyvirus*, the largest one among plant viruses. TuMV has a positive-strand RNA genome of approximately 10 kb long, with a viral genome-linked protein (VPg) covalently linked to the 5' end and a poly(A) tail at the 3' end. The TuMV RNA is translated into a long polyprotein of 358 kDa and is processed into at least 10 mature proteins by viral proteases. Viral replication occurs in virus-induced membrane vesicles derived from the endoplasmic reticulum (ER). In the present study, we used a secreted Green Fluorescent Protein (secGFP)-based assay to investigate the impact of TuMV infection on the host early secretory pathway. We found that TuMV infection prevents ER export of secGFP, and viral protein 6K2 was responsible for this inhibition. We carefully observed the distribution of organelle markers in infected and mock-infected of Nicotiana benthamiana leaves and we noted that a remodelling of the endomembrane network occurs during TuMV infection. We observed the formation of a perinuclear aberrant structure containing an amalgam of viral factories, ER, COPII vesicles and Golgi. FRAP was used to reveal the dynamics of this cluster of membranes. We found that this structure was connected to the Golgi network and probably to the ER, but was self-sustained for viral components. Using Arabidopsis thaliana mutant plants and BFA treatment, we demonstrated that an intact endomembrane system is not required for viral factory formation but is required for cell-to-cell virus movement. Finally we performed TEM observations to analyze the membrane modifications at the submicron resolution during TuMV infection. This work is supported by NSERC and FQRNT.

SD-3

Identification of CGI-58-binding proteins: The potential role of CGI-58 in the regulation of triacylglycerol metabolism and lipid signaling pathways in plant cells

Nicholas Khuu*¹, Sunjung Park², Satinder Gidda¹, Patrick Horn³, Chris James³, Kent Chapman³, John Dyer², and Robert Mullen¹

CGI-58 (Comparative Gene Identification-58) encodes an α/β hydrolase-type protein that is involved in multiple aspects of lipid metabolism in mammals, including the breakdown of triacylglycerols (TAGs). Mutations in CGI-58 result in accumulation of lipid droplets (LDs) in non-lipid-storing tissues such as skin, liver, and blood cells. The activity of CGI-58 is determined at least in part by its interaction with other proteins including perilipin, which serves to anchor CGI-58 to the surface of LDs. Phosphorylation of perilipin releases CGI-58, allowing it to interact with Adipose Triglyceride Lipase (ATGL), thereby stimulating TAG breakdown. Similarly, disruption of the CGI-58 homolog in Arabidopsis results in the accumulation of LDs in tissues that do not normally store lipids (e.g., leaves and stems), but plants lack any apparent perilipin or ATGL homologs. Thus, to better understand the function of CGI-58 in plants, we conducted a yeast 2-hybrid assay using Arabidopsis CGI-58 as bait. Although no obvious LD-associated proteins or lipases were identified, we found several proteins known to be involved in distinct aspects of plant lipid metabolism. These and other results obtained from studies of selected CGI-58-interacting proteins and the possible role(s) of CGI-58 in TAG metabolism and lipid signaling in plant cells are presented.

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SD-4

A novel approach for the derivation of intrinsic mechanical properties for the cell wall of *Arabidopsis thaliana*

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The plant cell wall consists of a dynamic matrix of cellulose, hemi-cellulose and pectin molecules. It is known that changes in wall composition lead to altered phenotypes. However, the relationship between the wall composition and its effect on the intrinsic mechanical properties involved remains poorly understood. In this work, the effect of three mutations causing altered cell wall composition on growth behavior and mechanical properties of individual cells in of *Arabidopsis thaliana* were investigated. Shoot and root elongation were quantified to assess the effects on overall plant growth and the biomechanical properties of single cells in suspension culture were assessed using microcompression testing. The force resistance-curves were significantly different at varying deformation speeds revealing that the hydraulic conductivity of these cells was high. A speed of 3000 µms⁻¹ was required to generate sufficient force to negate water loss and measure the physical properties of wall. Mathematical modeling revealed that immediately prior to failure, *Arabidopsis* cell walls did not behave in a linear elastic, but in a plastic manner. Extraction of the strain elastic modulus from the initial, linear elastic component of the deformation revealed significant differences between the genotypes caused by the compositional changes in wall biochemistry.

SD-5

Localizing arogenate dehydratase from Arabidopsis thaliana

Susanne E. Kohalmi¹, Crystal D. Bross^{1,2}, and Danielle M. Styranko^{1,3}

Arogenate dehydrates (ADTs) are proteins that catalyze the last step in phenylalanine biosynthesis. In Arabidopsis thaliana, ADTs are encoded by six genes that have unlinked genomic loci. All six encoded proteins have a common domain structure with an N-terminal signal peptide, an internal catalytic domain and a C-terminal regulatory domain. Initial in silico analyses were inconclusive as they suggested several different subcellular locations for these proteins. To determine experimentally where these proteins are located, we amplified each ADT and cloned it as an ADT-CFP (cyan fluorescent protein) expression construct. Each ADT-CFP construct was transiently introduced into Nicotiana benthamiana leaves. Using confocal microscopy, ADT-CFPs were predominantly detected in stromules of chloroplasts. Following reports that bacterial PDTs, proteins closely related in sequence and function to plant ADTs, form homodimers, we wanted to test if *Arabidopsis* ADTs have similar interaction properties. We chose a Bimolecular Fluorescence Complementation (BiFC) assay. To do so, each ADT was cloned and transiently expressed as ADT-YFP-N or ADT-YFP-C fusion protein in tobacco in every pair-wise combination. Again, using confocal microscopy, it was determined that ADTs were able to form homo- and heterodimers and that dimers localized predominantly to stromules. Implication of these studies for the functional roles of ADTs in *Arabidopsis* will be discussed.

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Session E. Abiotic Interactions

SE-1

Reductive iron acquisition by algal cells interacts with the nature of the ferric chelators Mathew B. Sonier and Harold G. Weger

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Like the majority of vascular plants, the green alga Chlorella kesslerii uses a reductive mechanism to acquire extracellular iron. Plasma membrane ferric reductase reduces Fe(III)chelates to Fe(II), which is subsequently taken up by the cell. We demonstrate that naturallyoccurring chelators and their analogues, and also two formulations of the herbicide glyphosate. all affect iron acquisition. The ferric forms of the larger siderophores and glyphosate all supported rapid rates of ferric reductase activity, while the iron-free forms inhibited reductase activity. The smaller siderophores/siderophore precursors did not support high rates of reductase in the ferric form but did inhibit reductase activity in the iron-free form. Bioassays confirmed that ferric compounds that supported high rates of ferric reductase activity also supported a large stimulation in the growth of iron-limited cells, and that an excess of iron-free chelator decreased the growth rate. This suggests that organisms that use a reductive strategy for iron acquisition both require, and are potentially simultaneously inhibited by, ferric chelators. Furthermore, these results may provide an explanation for the frequently contradictory results of glyphosate application to crops: low concentrations of this molecule likely solubilize Fe(III), making it available for plant growth, but higher sub-lethal concentrations appear to decrease iron acquisition.

SE-2

Intraspecific variation of drought stress tolerance in Douglas-fir

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Global warming will lead to longer summer drought periods in the Black Forest, and the predominant but drought-susceptible spruce may be partly replaced. Douglas-fir (*Pseudotsuga menziesii*) may be a valuable alternative. It is common in a wide range of North America, and its provenances may vary in their drought tolerance due to local adaptation. Drought stress increases the risk of photooxidative damage as excess energy leads to the formation of reactive oxygen species (ROS) and impairs photosystems. In response, plants produce isoprenoids which mediate different photoprotective processes such as dissipation of excess energy in terms of heat and scavenging of ROS. We hypothesize that Douglas-fir provenances adapted to drought stress by enhanced photoprotective isoprenoid levels when exposed to drought stress. Carbon fixation and photoprotective capability of Douglas-fir provenances adapted to contrasting (dry or humid) environments were measured under controlled drought conditions. The humid habitat provenance maintains higher assimilation rates due to higher stomatal conductance, while the dry habitat provenance increased stomatal closure to reduce transpiration. Additionally it seems to enhance photoprotection which is revealed by isoprenoid content analysis. Therefore the humid provenance seems to be more susceptible to prolonged drought stress.

SE-3

The path to restoration is stress-free

<u>Maye Saechao</u>*, Barbara Moffatt, and Susan Lolle. *Department of Biology, University of Waterloo, Waterloo, Ontario Canada*

The reacquisition of genomic information absent from a given lineage for several generations has been documented in a number of plant species. This reappearance of lost genotypes as a real biological phenomenon, however, remains a hotly debated issue among plant scientists. In our lab we have shown that the inheritance of non-parental DNA in *Arabidopsis thaliana* occurs at low but detectable rates and manifests on single plants as genotypically distinct somatic sectors. In order to better understand this phenomenon I have tested the hypothesis that among genome changes that occur in response to stress, these putative triggers also up-regulate "restoration". My research investigates the effects of wounding and reactive oxygen species (ROS) exposure on the restoration frequency. Wild-type plants were mechanically wounded, allowed to recover and the next generation produced by these plants tested for evidence of novel genotypes. In a complementary experiment, plants were grown on ROS generating media and also tested for genotypic changes using molecular methods. There is evidence for sectoring in all groups; however, contrary to expectations, both wounding in the parental generation and ROS treatment appeared to reduce the occurrence of genotypic changes as compared to the control group.

SE-4

UV-B adaptation in *Arabidopsis thaliana* ecotypes

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A pronounced increase in UV-B radiation with increasing altitude has been reported for many regions of the world. However, little is known about UV-B protection and its relation with geographic origin of plants. Eight *Arabidopsis* ecotypes selected from different geographic locations (altitude between 32 and 3016 m) were exposed to Photosynthetic Active Radiation (PAR), PAR+ ultra violate (UV)-A and PAR+ UV-A +B for 10 days. Plant response to radiation treatments was assessed by rosette diameter, induction of total phenolics, photochemical efficiency (F_v/F_m) and the levels of chlorophyll and carotenoids. We found that plants exposed to PAR + UV-A+B showed the highest UV-B tolerance followed by those exposed to PAR + UV-A and the lowest UV-B tolerance was found in the PAR-exposed plants across all ecotypes. The observed UV-B protection in the PAR + UV-A-exposed plants was mainly mediated by carotenoid, while those exposed to PAR + UV-A+B was mediated by induction of total phenolics. There was significant positive association between constitutive UV-B protection and geographic origin of plants (altitude). Our findings indicated that *Arabidopsis* accessions from lower altitudes maintained growth in expense of higher metabolic energy for induction of UV-B protection than those of higher altitudes.

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SE-5

Photosystem I photoprotection

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When exposed to fluctuating light environments, photosynthetic organisms must maintain a balance between the energy they absorb through their light-harvesting antennae and the use of that energy by photosynthetic machinery and ultimately by cellular metabolism. Photosystem II is often thought of as a clutch. When light levels are too high, it sheds energy through nonphotochemical processes, and is even destroyed in a controlled manner. However, little is known regarding the photoprotective mechanisms which mitigate damage to photosystem I. Using a mutagenic screen we have isolated three strains of *Chlamydomonas reinhardtii*, which appear to protect photosystem I from high-light-mediated damage. The affected genes in two of the mutant strains have been identified. The first, *STT7*, encodes a light harvesting protein kinase, which protects photosystem I by reducing the amount of light it absorbs. The second, *TEF27*, encodes a novel gene product, which when disrupted leads to a pale green phenotype. The third mutant, exhibits high levels of nonphotochemical quenching, but the disrupted gene has yet to be identified. Together the phenotypes of these three mutants point to photoprotective mechanisms which divert excitation energy away from photosystem I.

Session F. Plant Development Workshop

SF-1

Arabidopsis seed coat mucilage structure is regulated via the FEI2 receptor-like kinase/SOS5 fasciclin-like arabinogalactan protein pathway and CELLULOSE SYNTHASE 5

Heather E. McFarlane¹, Smadar Harpaz-Saad², Shouling Xu^2 , Uday K. Divi¹, Bronwen Forward¹, Joseph J. Kieber², and $\underline{Tamara\ L.\ Western}^1$

Myxospermy, the production of hydrophilic mucilage that is released upon seed hydration, is a common adaptation that aids germination and dispersal. *Arabidopsis* seeds release a copious mucilage that consists of an outer, highly soluble pectin layer and an inner, strongly adherent layer that includes both pectin and cellulose. *salt overly-sensitive* 5 (sos5) mutants have altered mucilage structure in which the inner, adherent layer is reduced and the outer, soluble layer is increased in volume. *SOS5* encodes a glycophosphatidylinositol-anchored fasciclin-like arabinogalactan protein that has been shown to act in a genetic pathway with the leucine rich receptor-like kinases *FEI1* and *FEI2* that regulate cellulose synthesis in roots. Studies of *fei1* and *fei2* mutants revealed that *FEI2* also affects mucilage structure. Further, both sos5 and *fei2* mutants have a reduction in the cellulose 'rays' that span the inner mucilage layer. Analysis of seed coat phenotypes of mutants for multiple members of the *CELLULOSE SYNTHASE* (*CESA*) family identified CESA5 as the cellulose synthase responsible for the majority of cellulose production in the inner mucilage layer. Together, these data show that cellulose plays a structural role in the organization of polysaccharides in seed coat mucilage, and the regulation of its production by the *FEI2/SOS5* pathway.

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SF-2

An insight into the function of the *HUA2* gene family

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HUA2 has been shown to positively regulate two MADS box genes, FLOWERING LOCUS C (FLC) affecting flowering time, and AGAMOUS (AG) affecting floral patterning, thus having implication for the coordinate control of induction and maintenance of floral state. We have previously demonstrated that the natural variant of HUA2-Sy-0 is uncoupling the effects on FLC and AG. HUA2 is a member of a small gene family that includes HUA2-like genes HULK1, HULK2 and HULK3. Except for HUA2, single mutation in the gene family does not produce a visible phenotype, suggesting a functional redundancy within the gene family. Double and triple mutants within the gene family members result in various pleiotropic phenotypes. The quadruple mutant is lethal, suggesting that the HUA2 gene family is essential for Arabidopsis development. To gain more insight into the function of the HUA2 protein family, we have identified HUA2/HULK interacting proteins. We have shown that the members of the HUA2 protein family interact with proteins involved in pre-mRNA splicing, in both yeast two-hybrid and in vitro pull-down assay. HUA2/HULK proteins localize in the nucleus and affect the 3'-end processing of FCA, AG and U1-70K, suggesting that the members of the HUA2 protein family function in pre-mRNA processing.

SF-3

AKIN10 and FUSCA3 interact to control lateral organ development and phase transitions in *Arabidopsis*

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The SnRK1 kinases act as energy sensors in plants. Despite their role in regulating metabolic stress responses, few SnRK1 substrates have been identified. Using yeast two-hybrid screens, we have isolated the SnRK1 homologue AKIN10 as an interactor of the B3-domain transcription factor FUSCA3 (FUS3), an essential seed maturation regulator. Pull-down and bi-molecular fluorescence complementation assays confirm the interaction in vitro and in planta, respectively. In-gel kinase assays show that AKIN10 phosphorylates FUS3, while the FUS3 N-terminal domain is required for AKIN10 phosphorylation. Mutating three serines within a partial SnRK1 consensus sequence in the FUS3 N-terminal domain (fus3^{SS55A/S56A/S57A}) greatly reduces FUS3 phosphorylation by AKIN10, indicating these serines are the predominant AKIN10 targets. AKIN10 positively regulates FUS3 stability, as AKIN10 over-expression delays recombinant FUS3 degradation in a cell-free system. Plants over-expressing AKIN10 show delayed seed germination and flowering, indicating that AKIN10 promotes dormancy while antagonizing developmental phase transitions. Furthermore, AKIN10 over-expression alters cotyledon, silique and floral organ morphology, suggesting that AKIN10 regulates lateral organ development. The fus3-3 mutation partially rescues the phase transition and organ development defects from AKIN10 over-expression. Taken together, these findings indicate that FUS3 and AKIN10 physically interact and share overlapping pathways to regulate developmental phase transitions and organogenesis.

SF-4

Auxin Response Factor functions in plant regeneration - molecular control of *de novo* shoot formation

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In vitro regeneration of entire organisms from adult cell types remains one of the most fascinating properties of plants and is of great biotechnological importance. Despite recent genetic and genomic approaches, the molecular basis of regeneration is still largely unclear and many important crop plants have remained recalcitrant to regeneration. Apart from species-specific variations, the established way of inducing organogenesis in tissue culture involves a two-step process. First, exposure to a non-transported auxin induces callus formation followed by either auxin-induced root initiation or cytokinin-induced shoot generation. During the latter, several developmental stages, characterized by sets of molecular events can be distinguished. Because of the pivotal role of auxin in the regeneration process, we began a systematic evaluation of the specific roles of the evolutionary conserved Auxin Response Factor (ARF) family during in vitro organogenesis. Using loss-of function mutant, mis/overexpressor and gain-of-function genotypes, we define the functional relationship of various ARFs, and define their roles in establishing the signaling landscape during stages of in vitro organogenesis. Our data suggests that ARF genotypes have profound impact on regeneration properties in Arabidopsis and presumably in a wide variety of plant species and that ARF-derived constructs could serve as genetic tools to improve regeneration in recalcitrant species.

SF-5

The role of ARC1 in the self-incompatibility pathway in A. lyrata

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Self-incompatibility is a complex process in flowering plants that facilitates genetic diversity by preventing self-fertilization. In the Brassicaceae, this process is regulated by a signalling pathway initiated by the stigma-specific, *S* Receptor Kinase (SRK), following binding of a pollen-specific ligand, SCR/SP11. In *Brassica* species, downstream signalling components of the SRK pathway have been identified as the *M* Locus Protein Kinase, the ARC1 E3 ubiquitin ligase, and the Exo70A1 subunit of the exocyst complex. While the functions of SCR/SP11 and SRK are conserved in the related *Arabidopsis* species, nothing is known about the downstream signalling pathway. As a result, we set out to investigate if the role of ARC1 is conserved in regulating pollen rejection in the naturally occurring *Arabidopsis lyrata* self-incompatibility system. We have identified an *A. lyrata ARC1* homologue to *Brassica ARC1*, and have generated transgenic *A. lyrata* with an *AlARC1* RNAi construct. These plants are being tested to determine what effect knocking down AlARC1 expression has on the self-incompatibility trait. Thus, we have developed a transgenic system to investigate the self-incompatibility signalling pathway in *A. lyrata*.

Poster Presentation Abstracts

P1

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

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

P2

Mechanical and biochemical properties of the spore wall of *Glomus irregulare* determined by micro-indentation and histochemistry

Pascal Arpin¹, Robert Palin¹, Line Roy-Arcand², Anja Geitmann¹

Arbuscular mycorrhizal fungi live in symbiosis with the roots of many vascular plants, an association in which both partners exchange nutrients and minerals with each other. Fungal spores have therefore the potential to be used in agricultural applications to improve plant growth. To be able to improve spore viability and maintain spore robustness during industrial processing, we examined the biochemical composition and the mechanical properties of the spore wall. We determined the spatial distribution and temporal changes of the abundance of chitin and glomalin, two major constituents of the fungal cell wall. Furthermore, spores of different origin, age and size were subjected to microindentation to determine their stiffness. We were able to correlate parameters such as spore wall thickness and biochemical composition with the biomechanical properties.

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Proteomic analysis of the plant-pathogen interface

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Plants are constantly infiltrated by pathogens through natural openings and wounds. However, pathogenesis and virulence leading to systemic disease is rare as plants possess an active immune system to detect microbes and trigger immune responses. A virulence strategy employed by *Pseudomonas syringae* is the injection of type III secreted effectors (TTSE) directly into host cells via a molecular syringe known as the type III secretion system (TTSS). While it is clear that TTSE interact with host proteins to disable plant immunity, the precise targets of many TTSE remain unclear. One avenue of insight into host targets of TTSE is the proteomic identification of TTSE/host protein complexes. Here we describe the purification of high molecular weight HopF2^{Pto} complexes from transgenic *Arabidopsis* via gel-filtration and immuno-affinity chromatography. Liquid chromatography tandem mass spectrometry was subsequently used to identify components of HopF2^{Pto} complexes revealing novel targets of HopF2^{Pto} in *Arabidopsis*.

P4

Manipulation of auxin signaling through an irrepressible variant of Auxin Response Factor 5/MONOPTEROS

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Combinatorial interactions of AUXIN RESPONSE FACTORs (ARFs) and Aux/IAA proteins play a central role in the regulation of auxin response. Short-lived Aux/IAA proteins negatively regulate ARF activity through physical interaction, mediated by shared domains III and IV. As a new tool to explore the systems properties of this regulatory network, we generated a gain-of-function ARF genotype by eliminating domains III and IV from the functionally well-characterized ARF MONOPTEROS(MP)/ARF5. This truncated version of MP, termed MP Δ , rescued the mp loss-of-function mutant, but also displayed a number of semi-dominant traits affecting auxin signaling and organ patterning. In the absence of auxin application, the expression levels of many auxin-inducible genes were increased. Conspicuously, $MP\Delta$ leaves were narrow, pointed and filled with dense parallel veins. This phenotype originated from deregulated expression domains of PIN1, which remained unusually wide and recruited an inappropriately large fraction of leaf ground meristem cells into the midvein. An inducible variant of $MP\Delta$ allowed for the identification of direct target genes in the control of cell proliferation and temporal dissection of its influence on leaf development. A screen for suppressors of the $MP\Delta$ phenotype identifies downstream effectors of $MP\Delta$ action genetically.

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Using a steroid-induction system to identity transcriptional targets of BLADE-ON-PETIOLE1 and 2 that control inflorescence development in *Arabidopsis*

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The switch to flowering is a major developmental decision in plants, whose timing is controlled by a network of signals to ensure optimal reproductive success. In *Arabidopsis thaliana*, flowering is initiated when floral inductive signals acting on the vegetative shoot apical meristem (SAM) cause it to adopt an inflorescence meristem (IM) fate. Competence to respond to these signals requires the activities of two BELL-like homeodomain proteins expressed in the meristem: PENNYWISE (PNY) and POUNDFOOLISH (PNF), however their mode of action is unclear. Our data show that the non-flowering phenotype of *pny pnf* double mutants is caused by misexpression of genes encoding the architectural regulators, BLADE-ON-PETIOLE1 (BOP1) and BOP2, in the SAM. Compatible with this, flowering is restored in *pny pnf bop1 bop2* quadruple mutants. Presumably, BOP1/2 activate a set of genes in the SAM that block acquisition of IM fate. Through the construction of transgenic plants expressing a steroid-inducible form of BOP1, under the control of either the native BOP1 or viral 35S promoter, the identity of target genes that block flowering will be verified.

P6

A dsRNA virus factor that reduces nonself recognition-associated programmed cell death in the chestnut blight fungus, *Cryphonectria parasitica*

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The phytopathogenic fungus *Cryphonectria parasitica* devastated chestnut trees in North America in the early 1900's. More recently, the discovery of a mycovirus that converted *C. parasitica* to a hypovirulent form was investigated as a potential biological control agent. However, these efforts were thwarted since virus transmission between fungal individuals is restricted by Programmed Cell Death (PCD) that is triggered by the fungal nonself recognition system. Here we identify and characterize a virus factor, p29, that decreases nonself recognition-associated PCD in *C. parasitica*. We further investigate whether or not the presence of p29 in *C. parasitica* influences characteristics of PCD induced by various abiotic stress factors.

The role of FUSCA3 during inhibition of seed germination at high temperature in Arabidopsis

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FUSCA3 (FUS3) is a B3-domain transcription factor required for the proper development of the embryo during seed maturation. *FUS3* is involved in the accumulation of seed storage compounds, the establishment of dormancy, and desiccation tolerance. *FUS3* is expressed at high level during embryogenesis and at low level during germination and is a central regulator of hormone levels. *FUS3* is induced by auxin, negatively regulates GA level, while positively regulates ABA level. We tested the hypothesis that *FUS3* may play a role in inhibiting germination during abiotic stresses. Our results show that *FUS3* mRNA and protein levels increase in seeds imbibed at high temperature. *FUS3* induction occurs after long exposure to heat stress, suggesting that it may regulate cellular responses after prolonged exposure to high temperature. Overexpression of *FUS3* during germination

responses after prolonged exposure to high temperature. Overexpression of *FUS3* during germination confers resistance against high temperature stress by inhibiting seed germination at high temperature, and increasing seedlings survival after recovery at room temperature. Transcriptomic analysis of wild-type seeds imbibed at high temperature show a complex network of stress responses, which involve changes in the expression levels of stress-related genes, hormonal biosynthetic and signaling genes, and the activation of seed-specific programs.

In conclusion, our results expand the regulatory role of *FUS3* from late embryogenesis to germination at high temperature.

P8

Rhamnose synthase genes and exogenous protein expression in Flax (*Linum usitatissimum*) Bronwen Forward*¹, Mark Jordan², and Tamara L. Western¹

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The seed coat of linseed flax (*Linum usitatissimum l.*) secretes a thick, pectinaceous mucilage that is used as a soluble fibre, emulsifier, and substitute for animal products in food. The mucilage secretory cells (MSCs) of *Arabidopsis thaliana* are well characterized and many genes involved in mucilage synthesis and secretion have been determined. This study of flax MSC differentiation, alongside an investigation of potential RHAMNOSE SYNTHASE (RHM) genes will allow developmental staging of flax MSCs and a better understanding of genes involved in pectin synthesis, as these processes remain poorly understood in flax. We are also harnessing seed coat specific promoters to express exogenous proteins as a means of producing value-added oilseed crops. Candidate promoters for protein expression in the MSCs include GLABRA2 (GL2) from *Arabidopsis*, PINORESINOL LARICIRESINOL REDUCTASE (PLR), and a novel group of potential flax RHM genes found recently. Results for the *Gl2* promoter GUS construct shows that it targets protein secretion in the mucilage, and is excluded from the embryo as the seed matures.

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Chemical genomics: Discovery of novel fungicides and their targets in the phytopathogen *Fusarium graminearum*

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A commercial library of natural chemical compounds (TimTec NDL-3000) and crude plant extracts were screened against *Fusarium graminearum*, an economically important fungal pathogen of cereals. Of the 500 compounds screened, 35 were found to inhibit *F. graminearum* growth, four of which were selected for closer study. One compound of interest "Antofine" was purified from *Vincetoxicum rossicum*, a highly successful invasive species of plant found growing throughout Ottawa. Twenty two potential targets of Antofine were identified in a Synthetic Genome Array model, which is based on *Saccharomyces cerevisiae*. GeneMANIA (http://www.genemania.org), an online multiple association network integration algorithm was used to uncover information pertaining to genetic and physical interactions. *S. cerevisiae* mutants deficient in genes which demonstrate high levels of interaction were obtained and will be functionally complemented with their *F. graminearum* homologues.

P10

Effects of different signalling pathways on regulation of *GLK* GARP transcription factors in *Arabidopsis thaliana*

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GLK1 and GLK2 transcription factors have been suggested to be involved in chloroplast development, organic nitrogen signaling, disease resistance and circadian rhythmicity. This implies that multiple factors regulate expression of these two genes. Transcriptional regulation of GLK1 and GLK2 in Arabidopsis by various endogenous and environmental stimuli was investigated with the objective of elucidating the primary signalling pathway affecting GLK loci. The signalling pathways which were investigated with respect to GLK1 and GLK2 regulation chloroplast-to-nucleus retrograde signalling. sugars, nitrogen. phytohormones, and cold temperature. Collectively, the two genes were found to be regulated by retrograde chloroplast-to-nucleus signalling through changes in metabolite pools, reactive oxygen species (ROS) levels and redox state of the glutathione pool. GLK1 and GLK2 appear to be affected differentially by several signalling pathways, including those involving sugars and glutathione. These results confirm previous findings of regulation of GLK1 and GLK2 by retrograde chloroplast-to-nucleus signalling, as well as help unravel the complexity of regulation of these two genes by multiple environmental factors and endogenous signalling pathways.

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Structural analyses of the early stages of compatible and self-incompatible pollinations in Brassicaceae species

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In the Brassicaceae, compatible pollen-pistil interactions are believed to trigger a series of cellular events in the stigma for pollen acceptance. We are particularly interested in the role of polarized vesicle secretion and the exocyst complex in these responses. In yeast and mammalian systems, polarized secretion has been shown to be promoted through the tethering of secretory vesicles to the plasma membrane by the exocyst complex. Recently, we have identified one predicted subunit of the exocyst, *Exo70A1*, to be involved in compatible pollen response in *Brassica napus* and *Arabidopsis thaliana*. Based on this discovery, we hypothesize that Exo70A1 functions in the stigma, as part of the exocyst complex, to tether secretory vesicles to the plasma membrane at the pollen contact site. This is thought to result in water transfer to the pollen grain for hydration as well as the expansion of the papillar cell wall to promote pollen tube penetration for the subsequent fertilization. To address this, we are using transmission electron microscope to examine the presence or absence of secretory vesicles, following compatible or self-incompatible pollinations. As well, compatible pollinations are being investigated in the *A. thaliana exo70A1-1* mutant and *B. napus Exo70A1-*RNAi plants where the exocyst complex would be predicted to be disrupted.

P12

$\label{lem:continuous} \textbf{Perturbation of circadian rhythmicity in } \textbf{Arabidopsis} \textbf{ by altered GLK loci affects multiple output pathways}$

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GLK1 and GLK2 MYB transcription factors have been implicated to play a role in photosynthetic development, disease defence responses, organic nitrogen and brasinosteroid signalling. Comprehensive transcriptome analyses of Arabidopsis GLK1 OE, GLK2 OE, GLK1 KO, GLK2 KO and GLK1&2 KO identified that GLK1 and GLK2 are the circadian clock controlled morning genes with strong transcriptional and post-transcriptional co-regulation. GLK1 OE and GLK1 KO suppressed the amplitude of expression of endogenous GLK2. In contrast GLK2 OE did not affect the amplitude of expression of endogenous GLK1, but shifted the circadian rhythmicity of GLK1 by 3h into the late afternoon hours. Monitoring of circadian rhythmicity of major clock related genes identified that GLK1 OE, GLK2 OE and GLK1 KO affected differentially either diurnal amplitude or time of appearance of CCA1, LHY, APRR3, APRR5, APRR7, APRR9, TOC1, GI, PCL1, ELF3 and CHE indicating that both overexpression of GLK1 and GLK2 or knockout of GLK1 perturb circadian clocks in Arabidopsis and suggested that observed changes in circadian rhythmicity might be combinatorial effect of perturb GLK loci. Moreover, alterations in GLK loci affected diurnal changes in transcription of photosynthesis related genes, pathogenesis and defence related genes, cold-response related genes and major flowering locus related genes.

Monoterpene indole alkaloid N-methylation: Not your garden variety methyltransferase Dylan Levac* and Vincenzo DeLuca

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Monoterpene indole alkaloids (MIAs) are a remarkably diverse class of small molecules that possess potent biological activities. Our understanding of their biosynthesis comes from more than 30 years of work focusing on two non-model organisms: *Catharanthus roseus* plants and *Rauwolfia serpentina* plant cell cultures. Traditional approaches to identifying enzymes involved in MIA biosynthesis, and their corresponding genes, required laborious protein purification techniques. Recent advances in large scale DNA sequencing technology platforms (Roche 454, Illumina) has enabled candidate gene identification on a scale never seen before in plant secondary metabolism, and other fields. In this study we describe a recently discovered, emergent class of N-methyltransferases, homologous to tocopherol C-methyltransferases, and our progress towards characterizing a cryptic targeting motif.

P14

Thioesterases associated with deposition of extracellular lipid barriers in *Arabidopsis* thaliana

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Plants utilize extracellular lipid barriers for defence against environmental stresses. These barriers include cuticle, suberin, and sporopollenin, which cover aerial surfaces, root vascular cylinders, and pollen grains, respectively. Major components of these barriers are straight chain aliphatics derived from fatty acids. We have identified a four-member gene family in Arabidopsis encoding fatty-acyl thioesterases that we have called *ACYL-LIPID* THIOESTERASE1-4 (ALT1-4). The predicted proteins show strong sequence similarity to a large family of thioesterases, called hotdog-fold thioesterases, which cleave acyl-thioester bonds, releasing free fatty acids. A combination of RT-PCR, promoter::GUS experiments, and analysis of DNA microarray data show that expression of ALT1, ALT2, and ALT4 correlate with synthesis of cuticle, suberin, and sporopollenin, respectively, while ALT3 has a generalized expression pattern. All four ALT proteins have thioesterase activity when expressed in E. coli, each forming distinct products from endogenous lipids. When transiently expressed in leaves of Nicotiana benthamiana, an ALT1-GFP fusion localizes to the plastid via an N-terminal signal peptide. Taken together, ALT1-4 represent a novel thioesterase gene family, likely involved in extracellular lipid barrier deposition. Further characterization of their in vivo roles by genetic analyses holds promise to answer questions regarding the biochemistry and cell biology of extracellular lipid deposition.

A novel BAHD acyltransferase required for the synthesis of alkyl hydroxycinnamates in *Arabidopsis* root waxes

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Hydroxycinnamic acid derivatives are important components of plant cell walls. Ferulic acid allows cross-linking of polysaccharides. It is also a constituent of suberin, the extracellular lipidic barrier that regulates water and solute transport and restricts pathogen entry in certain cells, such as those of root periderm. While suberin is insoluble in organic solvents, a number of soluble lipids can be extracted by rapid chloroform dipping of roots. These root-associated waxes include esters of long-chain primary alcohols and phenylpropanoids such as coumaric, ferulic, and caffeic acids. Previously we identified a member of the BAHD super-family of acyl transferases, ASFT, which transfers ferulate into aliphatic suberin in Arabidopsis. However, *asft* mutants were unchanged with respect to deposition of alkyl hydroxycinnamate esters (AHCE) in roots. Here we identify a closely related gene, *BAHD2*, that is responsible for the synthesis of a sub-set of AHCE, the alkyl caffeates. *BAHD2* was required for deposition of alkyl caffeates but not alkyl coumarates. YFP-reporter analysis demonstrated strong *BAHD2* expression in the periderm, while the acyl transferase activity of recombinant BAHD2p was highest with caffeoyl-CoA substrate. Alkyl coumarates but not alkyl caffeates are induced by salt stress in roots. Thus the physiological function of alkyl caffeates remains obscure.

P16

Regulation of secondary cell wall deposition: Not the usual suspects

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The secondary cell wall that surrounds xylem and fibre cells of vascular plants is primarily composed of cellulose, hemicellulose, and lignin. It is of high importance biologically and economically, and has been intensely studied. Nevertheless, some aspects of secondary cell wall formation remain poorly understood. To identify genes that may have uncharacterized roles in secondary cell wall formation, genes that are transcriptionally co-regulated with those encoding lignin biosynthetic enzymes were identified using the Bio-Array Resource (BAR) Expression Angler tool. *Arabidopsis thaliana* mutants with T-DNA insertions within the selected genes were then examined to test the hypothesis that such genes are involved in secondary cell wall formation. Inflorescence stems were sectioned, stained with toluidine blue and phloroglucinol, and examined using microscopy to identify secondary cell wall phenotypes. Mutants were identified that had altered xylem or interfascicular fibre cells with respect to wild type. Mutant phenotypes are being characterised at the cellular, subcellular, molecular and chemical levels, and the genes underpinning the mutant phenotypes are being examined to better understand their precise contribution to the mutant phenotype.

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Generating a genetic engineering strategy to create methionine-sufficient variety of *Cicer arietinum*: Characterizing S-adenosylmethionine synthetases

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Grain legumes, such as *Cicer arietinum* (chickpea), are deficient in methionine, from the perspective of human nutrition. Methionine deficiencies in humans lead to Protein-Energy Malnutrition, a syndrome with symptoms including lowered resistance to disease and retardation of both mental and physical development in young children. *S*-adenosylmethionine synthetase (SAMS) catalyzes the condensation of ATP and methionine to produce *S*-adenosylmethionine, the metabolite that regulates the methionine and threonine biosynthetic pathways at the *O*-phosphohomoserine branch-point. Investigations into this branch-point have identified SAMS as a target for genetic engineering strategies aimed at increasing the methionine content of grain legumes. Although characterization of SAMS in chickpea is required to generate such a strategy, a comprehensive study that includes cloning to identify putative chickpea SAMS, sequence comparison with the model plant species *Arabidopsis thaliana* and complementation to verify function has not been reported. This study has identified five putative chickpea SAMS isoforms that share 62-83% nucleotide and 77-93% amino acid sequence identities with one another, and 56-78% nucleotide and 76-93% amino acid sequence identities with the four *A. thaliana* SAMS isoforms.

P18

Impact of alternative oxidase on photosynthesis and oxidative stress under drought or combined drought and moderate light stress in *Nicotiana tabacum*

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The mitochondrial electron transport chain includes a non-energy conserving alternative oxidase (AOX) induced by stress. In response to increasing drought severity, wild-type (Wt) Nicotiana tabacum show a gradual increase of AOX transcript and protein. Induction of AOX is exaggerated further when the drought is then combined with moderate light stress. The drought stress decreases photochemical quenching (qP), operating efficiency of photosystem II (Φ_{PSII}) and CO₂ assimilation rate, while increasing non-photochemical quenching (NPQ). However, the maximum efficiency of PSII (Fv/Fm) only declines following the combined stress. Significantly, photosynthetic performance (qP, Φ_{PSII} , CO₂ assimilation rate) was more strongly impacted by drought in knockdown plants lacking AOX. Also, during the more severe combined stress, plants lacking AOX displayed enhanced oxidative stress and membrane damage, as determined by measures of H₂O₂, lipid peroxidation, electrolyte leakage and Fv/Fm. Paradoxically, at the later stages of severe stress, the knockdown plants had lower transcript level of key anti-oxidant enzymes than Wt. Further, preliminary results suggest that knockdown plants cannot as readily recover from severe stress as Wt. Our results indicate that AOX acts to optimize photosynthetic performance under moderate drought stress and that, under severe stress, it also aids in maintaining ROS homeostasis.

Corn and Soybean relay strip intercropping in China: A review

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As a result of population increase, the demand of food is ever increasing. It is important to increase the multiple crop index of land for the development of grain production in the area abundant in solar and heat energy. Relay strip intercropping of corn and soybean has been widely used by farmers in the Southwest region of China. Several experiments among years and locations were carried to select suitable cultivars, sowing date, planting arrangement, fertilizer and Uniconazole usage management, and to understand the physiology mechanism. Results showed that corn grown in relay cropping has yielded over sole cropping, while soybeans in relay strip intercropping suffer yield reductions of 5 to 20 percent. With wider strips of corn there is more increase in corn yields and less reduction in soybean yields. Changes in component crop yields also depend on sowing date, varieties; erect corn, late mature soybean, early sowing date of corn, late sowing date of soybean benefit to gain greater total yield. Results also showed that maize-soybean relay cropping provides an ecological approach to soybean aphid control and enhance diversity of soil microorganism. Relay strip intercropping corn and soybean can be highly effective over a large area and contribute to the sustainability of crop production.

P20

Effect of Thuricin 17 and LCO on crop growth

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The second half of the 20th century saw widespread deployment of environmentally problematic green-revolution technologies to agriculture; to further improve crop yields more subtle interventions are now needed. Thuricin 17 is a small bacterially produced protein that improves crop growth, particularly under stressful conditions. Considerable work remains for the full commercial development of thuricin 17. Lipo-chitooligosaccharides have shown plant growth promotion activity similar to thuricin 17 and are now in the market place. Thus, thuricin 17 was compared with LCO application in an effort to understand the potential of thuricin 17. Experiments were conducted, constituting a mix of field and controlled environment work that has provided a clearer indication of the applied potential of thuricin 17. Green house experiments and field experiments will helped us to determine the most effective exposure times, for either acute or chronic exposure. Collectively, these studies have expanded our understanding of a relatively recent and unexpected finding and shown the potential for exploitation in a crop production setting.

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Genetic and epigenetic patterns in economically important *Populus* hydrid clones Katharina Bräutigam¹, Sherosha Raj ¹, and Malcolm M. Campbell ^{1,2}

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For plants, it is of key importance to adapt to local environments. This is especially true for organisms with long generation times such as forest trees and plants with limited genetic diversity. It is well established that the genetic makeup of an organism determines its performance to a large extent. More recently, the importance of epigenetic patterns for plant growth and performance has been reported. To assess the extent and possible role of DNA methylation in poplar genotypes, genetic and epigenetic patterns in economically important poplar clones were investigated. Fingerprinting techniques revealed unique and shared genetic and epigenetic patterns among different genotypes, that point towards additional, possibly heritable, variability between polar clones. To investigate the effect of clonal history on DNA methylation patterns, individuals of the same genotype were obtained from geographically distinct locations and grown under controlled laboratory conditions. A few candidate loci with variable within-clone methylation patterns were identified, while global within-clone patterns remained similar. The findings indicate that DNA methylation might add an additional layer of variability which helps long-lived organisms to better contend with local environmental conditions. Moreover, the data provide insights into long-standing applied questions related to the nursery source of poplar clones and how that impacts on future clone performance in plantations.

P22

Molecular and functional characterization of flax SDG lignan glycosyltransferases

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Flax (*Linum usitatissimum*.) is a multi-purpose crop contributing to 1% and 3% of the world's food and fiber production, respectively, and has a wide variety of nutraceutical and health properties. Flax seeds are rich source of secoisolariciresinol diglycoside-(SDG) lignan, a precursor of the mammalian lignans enterolactone and enterodiol. SDG lignans are diphenolic nonsteroidal phytoestrogens having health benefits to human, especially with antidiabetic and anticancer properties. *In planta*, lignans are naturally found glycosylated and glycosylation affects the molecule's stability, solubility, reactivity, and thereby its bioavailability and bioactivity. Pinolariciresionol reductases (PLRs) and 1-uridine diphosphate glycosyltransferases (UGTs) act as key enzymes in lignan biosynthesis. However, less information is yet available on the flax lignan glycosylation machineries. Here, we report on the identification, molecular and functional characterization of 6 putative flax UGTs. Five of the 6 UGTs were found to be seed specific and the transcript expression patterns of two tested UGTs closely correlated with SDG lignan synthesis and accumulation in developing flax seed. The hereologous expression of these UGTs and their ability to metabolize secoisolariciresinol into SDG is discussed.

Crop responses to soil salt and water stress in arid areas

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Soil salinization and water shortage are particularly prominent in dry lands associated with rainfed agriculture. The research aims to illustrate the physiological mechanisms of oat and maize under salt and water stresses in the rainfed areas of China, which will attain the goal of increasing crop productivity of agriculture in these areas. Two studies were carried out: 1) to investigate the effect of differential soil salt contents on the growth and yield of oat, the experiment was conducted at six levels of soil salt content (0, 0.2%, 0.4%, 0.6%, 0.8% and 1%); 2) to investigate the effect of 4 water-retaining agents on the growth and development of maize. Results revealed that the soil salt content significantly affected growth and development of oat, yield decreased from 0.6% to 0.8%. It was observed that Application of PAA, PAM, Ha and Pengrun to maize plants could increase seed germination and plant growth, and reduce yield losses under drought conditions.

P24

Investigating a conserved role for *BLADE-ON-PETIOLE* genes in secondary cell wall development in *Arabidopsis thaliana* and *Populus trichocarpa*

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In Arabidopsis thaliana, the transition to flowering causes the elongation of internodes to provide a regular spiral arrangement of lateral branches and flowers on the primary stem. Subsequently, internodes become fortified through the differentiation of interfascicular fibres with secondary thickened cell walls, which provides mechanical support. The KNOX homeobox protein BREVIPEDICELLUS (BP) has been identified as a key developmental regulator of lignin biosynthesis, a major component of secondary cell wall formation in plants and wood formation in trees. We show here that antagonistic interactions between BP and a pathway containing the lateral organ boundary genes BLADE-ON-PETIOLE1/2 (BOP1/2) and KNOTTED1-LIKE FROM ARABIDOPSIS THALIANA6 (KNAT6) controls the timing and pattern of lignin deposition in Arabidopsis stems. Many of the defects in bp stems are caused by BOP1/2-KNAT6 gain-of-function. We also show that the reciprocal functions of BOP1/2 and BP in the stem are a likely consequence of their antagonistic regulation of genes that control cell differentiation, including those involved in lignin biosynthesis, which are repressed by BP and activated by BOP1/2 in stems. Two potential BOP orthologs are highly expressed in poplar xylem, which suggests a conserved role for BOP1/2 in promoting secondary wall development in trees. We are using a variety of approaches to test this model in the poplar species *Populus* trichocarpa. These experiments will provide insight into developmental regulation of wood formation in trees.

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The role of pre-mRNA splicing-related proteins in the photosynthetic adjustment and acquisition of freezing tolerance of *Arabidopsis thaliana* undergoing cold acclimation Marc Rosembert*^{1, 2}, David P. Sprott¹, Ekaterina Ponomareva^{1, 2}, Jas Singh¹, Leonid V. Savitch¹

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The molecular response to cold stress and acclimation involves the transcriptional modulation of stress related genes. Global proteome analyses of Brassica napus undergoing cold acclimation have revealed that low temperature had a prominent effect on the quantitative representation of proteins related to pre-mRNA splicing and RNA stability. Serine/Arginine-rich (SR) proteins and LAMMER-type protein kinases are the essential players involved in pre-mRNA splicing and RNA metabolism. Transcriptome analyses of B. napus undergoing cold acclimation have revealed that the expression level of 9 out of 19 SR proteins and 2 out of 7 splicing-related LAMMER-type protein kinases had increased in response to cold stress and cold acclimation. We hypothesized that this subset of SR proteins and LAMMER-type protein kinases might be involved in cold acclimation processes. The photosynthetic adjustment and the acquisition of freezing tolerance are the two major processes which occur in cold-hardy plants undergoing cold acclimation. In the present study, we evaluated the ability of Arabidopsis thaliana lines lacking cold induced SR proteins and LAMMER kinases to undergo cold acclimation. For the first time we have shown that At-AME3 LAMMER kinase and At-SCL30, At-RSZ22, At-RS40 SR proteins are indispensable in the process of cold acclimation induced photosynthetic adjustment and acquisition of freezing tolerance.

P26

Characterisation of a transcriptional circuit involving the transcription factor, *At*MYB61 Michael Prouse* 1,2, Julia Romano* 1,2, Christian Dubos*, and Malcolm M. Campbell* 1,2

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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.

P27

Effect of biochar and biofertilizer on the growth and yield of bioenergy grasses

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High yielding, fast growing, low-input perennial grasses, or their mixtures, can be better bioenergy sources than any single species. Agronomic field studies were conducted on bioenergy crop production with two model species (reed canary grass - C₃ species and switchgrass-C₄ species) using biochar (20 ton/hectare) and biofertilizers (PGPR able to mobilize soil phosphorus or supply nitrogen) as soil amendments, to maximize yield and soil carbon sequestration while reducing N fertilizer costs and GHG emissions, on two soil types (sandy and loamy) at two research sites (the warmer-McGill University and the cooler - Laval University). As the development of a viable bioenergy sector is strongly favored in Quebec due to Quebec's GHG emission reduction targets for 2020, so this study has shown the potential of biochar and biofertilizers to reduce energy inputs and increase soil carbon sequestration through an extremely efficient low-input system for producing biomass feedstock under Quebec conditions.

P28

Biomechanics of stems and hypocotyls in Arabidopsis thaliana (L.) Heynh

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Biomechanical factors influence growth, morphogenesis and mechanical stress response in plants, yet the mechanics of plant structures remain largely unexplored. The stem is a hierarchical structure displaying several nested levels of organization; *Arabidopsis thaliana* is being used as a model system to investigate its mechanical properties. To better understand the significance of its architectural features, tensile, torsional and 3-point bending tests have been developed to assess the mechanical properties of the *Arabidopsis* stem. By testing a series of mutants displaying structural defects, it will be possible to elucidate the architectural contributions of the various cell wall components and tissues that form the stem. Additionally, to better understand the role of the cell wall in cell growth, tests are being performed on etiolated hypocotyls from mutant lines displaying cell wall defects. The role of cellulose microfibril angle in cell wall anisotropy is also being examined using confocal microscopy. The information collected collected through experiment and microscopy is being used to develop a multiscale mechanics model of plant stems. Currently, a Voronoi tessellation approach has been developed to model the cellular structure of the stem from a micrograph. This technique will contribute to the development of novel biomimetic structures.

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Dissecting the plant-pathogen interface

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The ability of *P. syringae* to cause plant diseases is dependent on type III effector proteins delivered into plant cells by the type III secretion system. These effectors have been demonstrated to promote pathogen growth, suppress host defenses and elicit host-specific disease symptoms by interacting and modifying targets within the host cell. However, plant resistance (R) proteins can monitor the cytosolic environment for effector-induced perturbations and activate a robust defense response that is often accompanied by a localized programmed cell-death termed the hypersensitive response.

We have identified the type III effector $HopF2_{Pto}$ as a potent suppressor of the hypersensitive response generated by the *Arabidopsis* R protein RPS2. We demonstrated that $HopF2_{Pto}$ quells this defense response by targeting a central regulator of plant immunity, RIN4. We will present our recent efforts to characterize the molecular interface between pathogen type III effector $HopF2_{Pto}$ and Arabidopsis RIN4. We have identified that $HopF2_{Pto}$ binds to the C-terminal portion of RIN4 *in vitro*. Interestingly, $HopF2_{Pto}$ ADP-ribosylates RIN4 residues adjacent to two protein sequences targeted by two, sequence-unrelated, type III effectors. Our results support observations that pathogens subvert host responses to infection by altering key regulators of immunity.

P30

Effects of a high temperature stress on isoflavones concentration and expression of key genes involved in isoflavones synthesis

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Soybean contains a range of compounds with putative health benefits including isoflavones. Studies were conducted to determine the effects of high temperature stress imposed during seed development stages [i.e., late-reproductive (R5-8)] on isoflavones concentration and expression of key genes involved in isoflavones synthesis (CHS7, CHS8, IFS1 and IFS2). Plants were grown in growth chambers set at different temperatures in order to generate contrasting treatments [i.e., stress conditions of 33/25°C (day/night temperature) and control conditions of 23/15°C]. Total isoflavones concentration in mature seeds was 79% lower in stressed plants compared to the control. The expression of CHS7, CHS8, IFS1, and IFS2 was significantly reduced in the stressed plants at the R7 stage when compared to control plants. The reduction in pods ranged from 95 to 98% for IFS1 and CHS8, respectively and ranged in seeds between 68 to 98% for IFS1 and CHS7. Response at earlier stages (i.e., R5 and R6) was small and variable. The present study demonstrates that isoflavones concentration in soybeans are reduced by temperature stress occurring during seed formation due to a reduction in the expression of key genes involved in their synthesis.

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Investigating the regulation of root symbioses using the pea mutant E151 (sym15)

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Leguminous plants form two agriculturally-important symbiotic relationships with bacteria and fungi. When associated with rhizobial bacteria, the plant forms nodules on its roots whereas it forms mycorrhizae in the presence of mycorrhizal fungi. The study of these symbioses relies heavily on mutant lines that are defective in the development, the maintenance, or the regulation of these associations. E151 (sym15) is a mutant of pea (Pisum sativum) with characteristics indicating a role played by ethylene in its nodulation phenotype. To provide evidence for the involvement of this hormone, we took two approaches. First, we treated mutant and wild-type (WT) plants inoculated with rhizobia with compounds altering ethylene biosynthesis or action. We added the immediate precursor to ethylene, aminocyclopropane (ACC), an inhibitor to ACC synthase, or the ethylene receptor antagonist Ag²⁺ in the form of Ag₂SO₄. We evaluated various morphometric parameters and nodule number 35 days after inoculation (DAI). Second, we assessed the mycorrhizal phenotype of the mutant 35 DAI and compared it to that of mycorrhizal WT treated with low levels of gaseous ethylene. Our results indicate that ethylene likely plays a role, albeit indirect, in the symbioses formed by E151. We propose that its involvement is mediated by cytokinin.

P32

Effect of plant photosynthesis under the different wavelengths of LED

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Absorption is a useful parameter in studies of plant physiological responses to different spectral qualities of light. We analyzed the photosynthesis rate verse LEDs at different wavelengths. Two plants (lettuce and petunia) and 13 specific wavelengths of LED (405, 417, 430, 449, 470, 501, 519, 575, 624, 633, 661, 680 and 700 nm) were used in this study. The intensity of the LED lighting system was calibrated and measured before and after the test at canopy level using a spectroradiometer to determine wavelength width, intensity and any changes that may occur during the test. The LED lighting system irradiated the plant with the carbon dioxide usage rate monitored until stabilization occurred. Carbon dioxide utilization rate was measure with the Li-COR LI-6400XT Portable Photosynthesis System, with photosynthesis rate normalized with leaf area. The plants were tested at irradiance levels consisting of 30 µmol m⁻² sec⁻¹. From our research we found photosynthesis, absorbance, quantum yield and action spectrum peaks in the range of 430 to 450 nm and in the range from 630 to 680 nm. This research will allow for improved selection of LEDs in the PAR spectrum.

Characterization of a negative regulator of FUSCA3 abundance in Arabidopsis

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The B3 domain transcription factor FUSCA3 (FUS3) is a short-lived protein that regulates seed development and phase transitions through the action of the hormones, abscisic acid (ABA) and gibberellins (GA) in *Arabidopsis thaliana*. Recently, it has been shown that FUS3 contains a PEST domain in the C-terminal region resulting in its instability and subsequent targeting for degradation by the 26S proteasome. Although FUS3 is targeted for degradation by the 26S proteasome, the mechanism of post-translational regulation is still unknown. Here, we show that FUS3 interacts with the *FUS3-interacting protein* 2 (*FIP2*), an E3 ligase, and that the interaction is predominantly nuclear. Expression studies using the *AIP2:GFP* reporter indicate that the E3 ligase is expressed throughout embryogenesis, germination, and vegetative growth; showing preferential expression in the epidermis, similar to *FUS3*. Cell-free degradation assays also show GST-FUS3 to be more abundant in *fip2* lysate compared to wild type. Furthermore, plants overexpressing *FUS3* (*ML1:FUS3*) show delayed germination and late flowering phenotypes, both of which can be greatly rescued by overexpression of *FIP2*. Collectively, these results indicate that FIP2 plays a major role in the negative regulation of FUS3 levels in *Arabidopsis*.

P34

Arabidopsis fatty acyl reductases that generate fatty alcohols associated with root and seed coat suberin

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Suberin is found in the cell wall of certain tissue layers and has important roles in controlling water and nutrient transport, as well as protection against pathogens. Suberin is a polymer consisting of phenolics, glycerol and a variety of fatty acid derivatives, including even chainlength primary fatty alcohols. An eight-member gene family encoding alcohol-forming fatty acyl reductases (FARs) has been identified in Arabidopsis thaliana. Three of these genes, FAR1, FAR4 and FAR5, are transcriptionally induced by salt stress and wounding, and are specifically expressed in root endodermal cells and in the mycropyle region of the seed, which are known sites of suberin deposition. Analysis of suberin composition of single mutants with T-DNA insertions in FAR1, FAR4, or FAR5 showed that suberin composition was modified in each far mutant; specifically, C18:0-OH was reduced in far5, C20:0-OH was reduced in far4, and C22:0-OH was reduced in far1, indicating that FAR1, FAR4, and FAR5 each generate distinct chainlengths of fatty alcohols found in root, seed coat, and wound-induced suberin. We have further developed double and triple far mutant lines using conventional genetic crossing and artificial microRNA mediated gene silencing. The double mutants far1 far4, far1 far5, far4 far5 showed reduced levels of both respective chain length fatty alcohols and the triple mutant far1 far4 far5 showed reduced levels of all the three C18:0-C22:0 primary fatty alcohols.

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Understanding regeneration: A systems approach

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Meristem initiation and regulation affects every aspect of plant growth and development. Manipulation of these plant stem cell niches is also critical for *in vitro* regeneration and micropropagation. These processes are frequently limiting factors in transformation, genetic modification and propagation of many economically important species. Callus-based systems are often used for plant regeneration, but organogenesis from callus is relatively slow, non-synchronous, prone to somatic mutations, and as such has not proven a very tractable system for research. We have developed a rapid, synchronous and high throughput regeneration methodology, to which we have applied a systems approach to advance our understanding of plant stem-cell regulation, meristem initiation and regulation. Using global gene expression analysis, cell and domain-specific profiling, QTL analysis and a variety of bioinformatic approaches we have identified patterns associated with specific stages of regeneration, candidate regulators of the process, and putative novel down-stream targets of shoot meristematic genes. Mutant analysis and tissue culture assays have confirmed that many of these new targets are functionally required for regeneration.

P36

Glyoxylate reductase isoform 1 (GLYR1) is localized to the cytosol and not peroxisomes in plant cells

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Glyoxylate reductase (GLYR) is a key enzyme in plant metabolism which catalyzes the detoxification of both photorespiratory glyoxylate and succinic semialdehdye, an intermediate of the γ-aminobutyrate (GABA) pathway. Two isoforms of GLYR exist in plants, GLYR1 and GLYR2, and while GLYR2 is known to be localized in plastids, GLYR1 has been reported to be localized in either peroxisomes or the cytosol, Here, we reappraised the intracellular localization of Arabidopsis GLYR1 using both transiently-transformed suspension cells and stably-transformed plants, in combination with fluorescence microscopy. The results indicate that GLYR1 is localized exclusively to the cytosol regardless of the species, tissue and/or cell type, or exposure of plants to environmental stresses that would increase flux through the GABA pathway. Moreover, the C-terminal tripeptide sequence of GLYR1, -SRE, despite its resemblance to a type 1 peroxisomal targeting signal, is not sufficient for targeting to peroxisomes. Collectively, these results define the cytosol as the intracellular location of GLYR1 and provide not only insight to the metabolic role(s) of GLYR1 and the compartmentation of photorespiratory and GABA pathways in plant cells, but also serve as a useful reference for future studies of proteins proposed to be localized to peroxisomes and/or the cytosol.

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Functional characterization of novel cytochrome P450 genes involved in medicinal monoterpenoid indole alkaloid pathways

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The monoterpenoid indole alkaloids (MIAs) of Madagascar periwinkle (*Catharanthus roseus*) are known to be among the most important sources of anticancer drugs. MIAs are derived by combining the iridoid secologanin with tryptamine to form the central precursor strictosidine that is then converted to most known MIAs, such as catharanthine and vindoline that dimerize to form anticancer vinblastine. Recent large scale sequencing activities with a number of MIA producing species combined with comparative bioinformatic tools has allowed the identification of candidate cytochrome P450 monooxygenases (CYPs) that are likely to be involved in MIA biosynthesis. This study uses Virus-Induced Gene Silencing (VIGS) to investigate the *in vivo* biological roles of candidate genes in intact Catharanthus plants. Changes in the MIA profile of VIGS silenced plants has been used to identify the reactions being affected as well as the potential substrates involved. This approach is being used to elucidate complete pathways involved in catharanthine and vindoline biosynthesis. We show that VIGS is a promising reverse genetic tool for rapid analysis of candidate MIA pathway genes whose biochemical functions can then be identified by functional expression within yeast and by biochemical assay.

P38

Biological nitrogen fixation and nodulation characteristics of various pulse species, market classes and varieties in semiarid northern latitudes

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In order to better understanding the nodulation characteristics and nitrogen fixation of various pulse species, market classes and varieties grown on the semiarid northern latitude areas, an experiment was conducted at Swift Current, Saskatchewan, from 2008 to 2010. No effect of pulse species on number of nodules and nodule mass was estimated in 2009 due to the large variation under field under relatively dry condition in the year. Pea had the highest values of nodule number and mass across the two wet years (2008 and 2010), which was 25%, 26% and 75% higher than lentil, fababean and chickpea on nodule number and similar as chickpea while 5 times as lentil and 3 times as fababean on nodule mass. Dry bean produced the least nodule number and mass. Overall, Fababean fixed most N amount in the three and dry bean fixed the least. No consistent variances were tested of any of the market classes and variety effect on nodule characteristics and nitrogen fixation suggested that the lack of genetic advances on nitrogen fixation and nodulation for the pulse varieties released in Canada and improve the genetic variance related to nitrogen fixation supposed to be the top agenda of breeding.

The exocyst: a vital role in the pollen-stigma interactions in Arabidopsis thaliana

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The exocyst complex is proposed to be a crucial component in the stigmas of *Arabidopsis thaliana* for accepting compatible pollen during the early stages of pollen-pistil interactions that include pollen adhesion, hydration, germination and pollen tube growth. The proposed role of the exocyst is to tether secretory vesicles to the stigmatic plasma membrane for vesicle fusion. We are interested in investigating the roles of each of the exocyst eight subunits individually in the compatible pollen response in *A. thaliana*. For example, previously published work from the Goring group showed that the Exo70A1 subunit was involved in compatible pollen-pistil interactions where stigmas of *Exo70A1* T-DNA knockout *A. thaliana* plants failed to permit wild-type pollen grains to either hydrate or form pollen tubes. Currently, the Sec15 subunit, represented by the gene pair, *Sec15a* and *Sec15b*, is being studied. When the more highly expressed *Sec15b* gene was suppressed with a stigma-expressed RNAi construct in Col-0, very little change was observed in the ability of the transgenic stigma to accept compatible pollen. *A. thaliana Sec15a* homozygous T-DNA knockout plants were then transformed with this RNAi construct, and preliminary results show that this strategy is more successful in compromising the compatible pollen response in the transgenic stigmas.

P40

Arabidopsis TCP8 regulates leaf epidermal and vascular development by influencing cell proliferation and differentiation

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TCP proteins regulate numerous aspects of morphogenesis and developmental timing including organ symmetry (Luo et al, 1999) and leaf curvature (Nath et al, 2003; Palatnik et al, 2003). Here we describe the role of the previously uncharacterized class I TCP factor, TCP8, in regulating leaf epidermal cell proliferation, differentiation and vascular patterning in *Arabidopsis*. tcp8 rosette leaves exhibit shape irregularities and crinkled margins. Quantitative analyses of the vasculature of rosette leaf one in wild type and tcp8 revealed significant differences in the number and spatial patterns of veins, suggesting that TCP8 serves to couple polar organ growth with vascular differentiation. Mutant cauline leaves are larger and broader than the wild type, suggesting that TCP8 functions in the restriction of leaf blade growth along the medio-lateral axis of the leaf, and in forming a smooth leaf margin, as mutants show increased serration density. Both stomatal and trichome densities are increased in the mutant cauline laves, but vascular complexity is reduced. Our findings reinforce previous studies demonstrating a role for TCP factors in the regulation of organ growth by modulating cell proliferation and differentiation in a context dependent fashion.

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Investigating the association between age-related resistance and the transition to flowering in *Arabidopsis thaliana*

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The Age-Related Resistance (ARR) response in *Arabidopsis thaliana* is a developmentally regulated disease resistance pathway. As the plant ages, it becomes increasingly resistant to normally virulent pathogens including the bacterium *Pseudomonas syringae* and the oomycete *Hyaloperonospora arabidopsidis*. A 10- to 100-fold reduction in pathogen growth is observed in mature 6 week-old ARR-competent compared to young 3 week-old ARR-incompetent plants. The onset of ARR-competence has been associated with the transition to flowering, such that plants grown in short day conditions flower and display ARR later at 6 wpg, while long day grown plants flower and display ARR earlier at 4 wpg. To determine if the transition to flowering is required for ARR competence, we assayed the ARR phenotypes of several early and late flowering mutants grown under short day conditions. Additionally, we performed ARR assays on young short day grown plants induced to flower via the photoperiodic pathway to determine if the transition to flowering induces ARR. Preliminary data from both experiments suggests that the transition to flowering is not an essential component for ARR competence. Instead it appears that plants reach a certain developmental age, in terms of rosette leaf number, before they become competent for the ARR response.

P42

Substrate specificities of a family of alcohol-forming fatty acyl-CoA reductases from *Arabidopsis thaliana*

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Primary fatty alcohols are found throughout the biological world, either in free form or in a combined state (e.g. wax esters). In plants, for example, they are components of extracellular lipid barriers (cuticle, suberin, and sporopollenin). Alcohol-forming fatty acyl-CoA reductases (FARs) are responsible for the NADPH-dependent reduction of fatty acyl-CoAs to primary alcohols via an unreleased aldehyde intermediate. The genome of *Arabidopsis thaliana* contains eight predicted *FAR* genes. We have assessed the substrate specificities of seven of these FARs by heterologous expression in *Saccharomyces cerevisiae* (FAR7 is a pseudogene). The full length open reading frames for FAR1, FAR2 (MS2), FAR3 (CER4), FAR4, FAR5, FAR6, FAR8, as well as versions of FAR2 and FAR6 with truncations of their predicted N-terminal chloroplast targeting sequences, were cloned into a modified version of pYES2 that allows for inducible expression of fusion proteins with a His6 and T7 epitope tag for protein purification and detection. The lipid contents of FAR-expressing yeast were measured by gas chromatography and the protein expression monitored by Western blot analysis. The GC analysis revealed that the FAR family can produce primary alcohols ranging from C16:0 to C26:0 in yeast, with each FAR having distinct substrate specificity.

Gene set analysis uncovers diel-mediated transcriptome pathways in datasets that can be used to test the impacts of artificial nighttime lighting in poplar

<u>Joseph Skaf</u>*^{†1}, Erin T. Hamanishi^{†2}, Katharina Braeutigam¹, OliviaWilkins¹, Sherosha Raj¹, and Malcolm M. Campbell^{1, 3}

Here, we show that gene set analysis (GSA) on existing datasets provides a way to identify robust regulation patterns and to hypothesize pathway responses for previously uncharacterized treatments. GSA was performed on a poplar microarray dataset containing predawn and midday time points. From daytime-sensitive gene sets, significant genes that were also shown to have light-sensitivity in the literature were selected for further characterization in an artificial nighttime lighting (ANL) experiment. Urban regions have high levels of illumination at night due to ANL, yet little is known about how this impacts pathway responses in plants. To investigate the effect of ANL on poplar trees, physiological measurements and RNA samples from leaves were taken across two days from two genotypes of *Populus balsamifera* trees planted in a stereotypical urban setting. The treatment trees were exposed to ANL and the control trees were not. Net leaf carbon assimilation rates were found to be lower in ANL treated trees than control trees during daytime hours. Transcript abundances of genes selected through GSA and subsequent filtering were found to have ANL-sensitivity. With pathway-associated genes being ANL-sensitive in poplar, there is reason to proceed with an ANL microarray experiment to identify ANL-sensitive pathways. Moreover, GSA was shown to be a valuable tool for the selection of promising candidate genes in transcript abundance studies.

P44

Expression of Brassica napus LEA genes in developing seeds of Arabidopsis thaliana

Sarah Amer*^{1,2}, Natalie Labbé¹, Owen Rowland², and Johann Schernthaner¹

Late embryogenesis abundant (LEA) proteins have been associated with dehydration and desiccation tolerance in many plants, animal, and bacteria. They have been shown to accumulate to high levels during the late stages of seed development. Little is known about their structure and mode of action. To better understand the temporal and spatial expression of LEA proteins, lea promoters from *Brassica napus* were fused to YFP. Transgenic seeds containing the lea promoter-YFP fusion at different developmental stages were analyzed to determine LEA expression in seeds. We show that the LEA proteins are being expressed as early as 10 days after flowering in seeds. To investigate the expression of LEA proteins during abiotic stresses, we tested the effect of salt, drought, and cold on the expression of genes in young transgenic seedlings. These transgenic seedlings containing the lea promoter-cDNA-YFP were analyzed at different time intervals to provide an overview of how proteins respond to stress. Overall the response to drought and salt stress occurred as quickly as 3 hours after stress treatment while that to cold took longer to detect, with the expression of the proteins mainly localized to the roots.

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Antagonistic interactions between BLADE-ON-PETIOLE1 and 2 and the BEL1-like homeodomain proteins PENNYWISE and POUND-FOOLISH regulate the *Arabidopsis* floral transition

<u>Paul Tabb</u>*, Alexander Edwards, Michael Bush, and Shelley R. Hepworth Department of Biology, Carleton University, Ottawa, Ontario, Canada

In flowering plants, the architecture of the inflorescence and flowering time are optimized for reproductive success. Flowering is initiated when floral inductive signals acting on the vegetative shoot apical meristem (SAM) cause it to adopt an inflorescence meristem (IM) fate. In the model plant *Arabidopsis thaliana*, this switch promotes both the production of flowers at the expense of leaves and the elongation of internodes to generate an inflorescence. Competence to respond to floral-inductive signals requires the activities of two meristem genes that encode BEL1-like homeodomain transcription factors: PENNYWISE (PNY) and POUND-FOOLISH (PNF). Inactivation of these genes blocks meristem conversion to an IM fate, but how they function is largely unknown. We show here that the ectopic activity of three sets of genes acting in a linear pathway cause the non-flowering phenotype of *pny pnf* double mutants. This pathway is defined by the lateral organ boundaries genes encoded by *BLADE-ON-PETIOLE1/2* (*BOP1/2*), *ARABIDOPSIS THALIANA HOMEOBOX GENE1* (*ATH1*) and *KNOTTED1-LIKE FROM ARABIDOPSIS THALIANA* (*KNAT6*) whose expression patterns are tightly regulated in shoot apices. We present a model and preliminary evidence to explain how misexpression of this pathway negatively regulates the floral transition.

P46

Identification of carbohydrate-binding ability based on proteome-wide tertiary structure prediction: *In vitro* validation

Shaowei Dong*¹, Andrew Doxey², and Nicholas Provart¹

The interactions between carbohydrates and proteins are very important in many biological functions such as cell adhesion and signal transduction. Carbohydrate-binding proteins are very diverse in structure, and aromatic residues (e.g. Tyr and Trp) play a significant role in ligand binding. In my study, I will test the carbohydrate-binding ability of the proteins with aromatic residues predicted to form a surface where the two aromatics form a flat binding site or a sandwich structure where there is enough room in between the aromatic side chains to fit a carbohydrate. The clones of the candidate proteins from *Arabidopsis thaliana* were transferred into *Nicotiana benthamiana* and after a tag-based purification, the proteins will be hybridized to glycan arrays from the Consortium for Functional Gylcomics. Concurrently, *Arabidopsis thaliana* cell wall fractions will be presented to the proteins and the binding abilities of the proteins to them will be assayed.

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QTLs controlling fibre properties and lignin content in *Arabidopsis* stems

<u>Arnaud Capron</u>¹, Harry Chang², Hardy Hall³, Brian Ellis³, Thomas Berleth¹, and Rodger Beatson²

Wood properties are critical to the quality of pulp used to produce paper products. Fibre properties and lignin content define strength and quality features of paper. Genes governing those traits are largely unknown, in part, because relevant woody species have not been amenable to high-resolution genetic mapping. We have used *Arabidopsis thaliana* to detect Quantitative Trait Loci (QTLs) involved in fibre properties and lignin content with the goal of identifying the relevant genes. Because of the high level of orthology between *Arabidopsis* and *Populus* genes, we expect that identified loci will have similar functions in trees. Taking advantage of an established *Arabidopsis* Recombinant Inbred Line (RIL) population, we measured fibres using a flow-through fibre quality analyzer (FQA) and lignin content using a high-throughput microscale technique. In conjunction with a high-density genetic map, linkage analysis was performed and 3 significant QTLs were identified. Our results demonstrate the feasibility of obtaining robustly heritable natural variations of fibre traits and lignin content. Next, we will seek to identify the genes responsible for the observed variation in *Arabidopsis* and then trees.

*we would like to acknowledge funding by an NSERC Strategic Project Grant and technical support by PAPRICAN's FP Innovation's Evalutree group.

P48

Thuricin 17 and lipo-chito oligosaccharide act as plant growth promoters and alleviate drought stress in *Arabidopsis thaliana*

Sowmyalakshmi Subramanian*¹, Erika Mitkus², and Donald. L. Smith¹

The use of plant growth promoting rhizobacteria is a low-input option to deal with abiotic stressors (egs. drought, salt) that can cause important crop losses. Thuricin 17 (TH17) is a class IId bacteriocin, a small protein that has been shown to stimulate plant growth in soybean. This study evaluates the effects of TH17 on the growth and physiology of *Arabidopsis thaliana*, compared with already established lipo-chitooligosaccahrides (LCOs). DAB staining and electrolyte leakage (EL) for plants treated with TH17 and LCO showed no H₂O₂ stress or increase in EL. Petriplate assays to screen for drought tolerance through PEG 8000 infusion were conducted and plant growth studied 21 days post stress by determining fresh and dry weights and differences in shoot and root growth. Plants were able to tolerate stress of up to 400 g L⁻¹ PEG, equivalent of -0.7 Mpa of drought, in petriplates. Free proline and total reducing sugar content and RT-PCR for the two most important genes up-regulated under drought stress DREB2 and RD29A were studied. Our studies suggest that chronic exposure to TH17 and LCO enhances plant growth and physiology and the effect is more pronounced when the plants are stressed.

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| Presenter | Number | Abstract Title | |
|-------------|--------|--|--|
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| Biswas | SE-4 | UV-B adaptation in Arabidopsis thaliana ecotypes | |
| Brandizzi | PT-1 | Functional and morphological analyses of the early plant secretory pathway | |
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| Capron | P47 | QTLs controlling fibre properties and lignin content in <i>Arabidopsis</i> stems | |
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| Chatfield | P35 | Understanding regeneration: A systems approach | |
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| Duong* | P33 | Characterization of a negative regulator of FUSCA3 abundance in <i>Arabidopsis</i> | |
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| Fung* | SA-5 | Two-spotted spider mite induced transcriptional responses of MicroTom | |

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| Grangeon* | SD-2 | Turnip mosaic virus alters the host cell secretory pathway and remodels the endomembrane network | |
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| Huynh* | SA-2 | The mycorrhizal phenotype of a low nodulating pea mutant E132 (<i>sym21</i>) | |
| Indriolo | SF-5 | The role of ARC1 in the self-incompatibility pathway in A. lyrata | |
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| Levac* | P13 | Monoterpene indole alkaloid N-methylation: Not your garden variety methyltransferase | |
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| Lo* | SC-5 | Next-generation mapping of disease resistance genes in Arabidopsis: Exploiting natural variation | |
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|---------------|-------|--|
| Palin | P2 | Mechanical and biochemical properties of the spore wall of |
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| Richardson* | SA-3 | Recruitment of ESCRT to mitochondria by carnation Italian |
| | | ringspot virus replicase proteins |
| Rosembert* | P25 | The role of pre-mRNA splicing-related proteins in the |
| | | photosynthetic adjustment and acquisition of freezing tolerance |
| | | of Arabidopsis thaliana undergoing cold acclimation |
| Saechao* | SE-3 | The path to restoration is stress-free |
| | | |
| Safavian* | P11 | Structural analysis of the early stages of compatible and self- |
| | | incompatible pollinations in Brassicacea species |
| Salim* | P37 | Functional characterization of novel cytochrome P450 genes |
| | | involved in medicinal monoterpenoid indole alkaloid pathways |
| Schwinghamer* | SB-5 | A micromolar concentration of lipo-chitooligosaccharide (Nod Bj |
| | | V [C18:1, MeFuc]) enhances self-pollinated rapid cycling canola |
| | | (Brassica napus [L.]) emergence and seed productivity |
| Selvaraj* | P22 | Molecular and functional characterization of flax SDG lignan |
| Dervaraj | 1 22 | glycosyltransferases |
| Shanta* | P27 | Effect of biochar and biofertilizer on the grwoth and yeild of |
| Silalita. | F21 | |
| C1 CV | D.10 | bioenergy grasses |
| Skaf* | P43 | Gene set analysis uncovers diel-mediated transcriptome pathways |
| | | in datasets that can be used to test the impacts of artificial |
| | | nighttime lighting in poplar |
| Sprott | P12 | Perturbation of circadian rhythmicity in <i>Arabidopsis</i> by altered |
| ~ r | | GLK loci affects multiple output pathways |
| Stone | PT-2 | Function and regulation of E3 ubiquitin ligases in hormone |
| Stolic | 112 | signaling |
| C4alraak | D1 | |
| Stokes* | P1 | Integration of nutrient and hormone signalling drives root |
| ~ | 7.7.1 | meristem development in <i>Arabidopsis</i> |
| Stokes* | SC-1 | Chemical interrogation of seedling sugar responses uncovers |
| | | crosstalk between nutrient and hormone signalling in Arabidopsis |
| Subramanian* | P48 | Thuricin 17 and lipo-chito oligosaccharide act as plant growth |
| | | promoters and alleviate drought stress in Arabidopsis thaliana |
| Tabb* | P45 | Antagonistic interactions between BLADE-ON-PETIOLE`1 and |
| | | 2 and the BEL1-like homeodomain proteins PENNYWISE and |
| | | POUND-FOOLISH regulate the <i>Arabidopsis</i> floral transition |
| Tsai* | SF-3 | AKIN10 and FUSCA3 interact to control lateral organ |
| 1 501 | 313 | |
| | | development and phase transitions in <i>Arabidopsis</i> |

| Vishwanath* | P34 | Arabidopsis fatty acyl reductases generate fatty alcohols associated with root and seed coat suberin | |
|------------------------|------|---|--|
| Waduwara- Jayabahu* | SB-2 | Dissecting the complex phenotype associated with 5'- methylthioadenosine nucleosidase deficiency | |
| Wang | P18 | Impact of alternative oxidase on photosynthesis and oxidative stress under drought or combined drought and moderate light stress in <i>Nicotiana tabacum</i> | |
| Wang | P38 | Biological nitrogen fixation and nodulation characteristics of various pulse species, market classes and varieties in semiarid northern latitudes | |
| Weger | SE-1 | Reductive iron acquisition by algal cells interacts with the nature of the ferric chelators | |
| Western | SF-1 | Arabidopsis seed coat mucilage structure is regulated via the FEI2 receptor-like kinase/SOS5 fasciclin-like arabinogalactan protein pathway and the CELLULOSE SYTHASE 5 | |
| Wheeler* | P16 | Regulation of secondary cell wall deposition: Not the usual suspects | |
| Wilson | SE-5 | Photosystem I photoprotection | |
| Wilton* | P29 | Dissecting the plant-pathogen interface | |
| Zayed* | P39 | The exocyst: a vital role in the pollen-stigma interactions in <i>Arabidopsis thaliana</i> | |
| Zhang* | P19 | Corn and Soybean relay strip intercropping in China: A review | |