UNIVERSITY OF TORONTO
ST GEORGE CAMPUS

The Canadian Society of Plant Biologists

Eastern Regional Meeting

November 20 - 21, 2015

Organizing Committee

Darrell Desveaux (Co-Chair), Dept. of Cell & Systems Biology, University of Toronto

Daphne Goring (Co-Chair) , Dept. of Cell & Systems Biology, University of Toronto

Nicholas Provart (Co-Chair) , Dept. of Cell & Systems Biology, University of Toronto

Dinesh Christendat, Dept. of Cell & Systems Biology, University of Toronto

Peter McCourt, Dept. of Cell & Systems Biology, University of Toronto

Eiji Nambara, Dept. of Cell & Systems Biology, University of Toronto

Keiko Yoshioka, Dept. of Cell & Systems Biology, University of Toronto

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Acknowledgments

The members of the 2015 CSPB/SCBV ERM organizing committee gratefully acknowledge Michael Stasiak for designing the meeting website. We also are very grateful to Lynn Gole, Asher Pasha, and all our volunteers for their assistance in organizing this meeting. Finally, we would like to thank Gale Bozzo and Ingo Ensminger for passing on very helpful advice from their previous experiences in organizing CSPB/SCBV ERMs.

Program-at-a-Glance

Friday evening events take place in the Ramsay Wright Building and Saturday events take place in the Earth Sciences Centre on the University of Toronto, St. George campus (refer to map on next page).

Friday, November 20th

Ramsay Wright Building, Rm 010 (basement), 25 Harbord St., University of Toronto

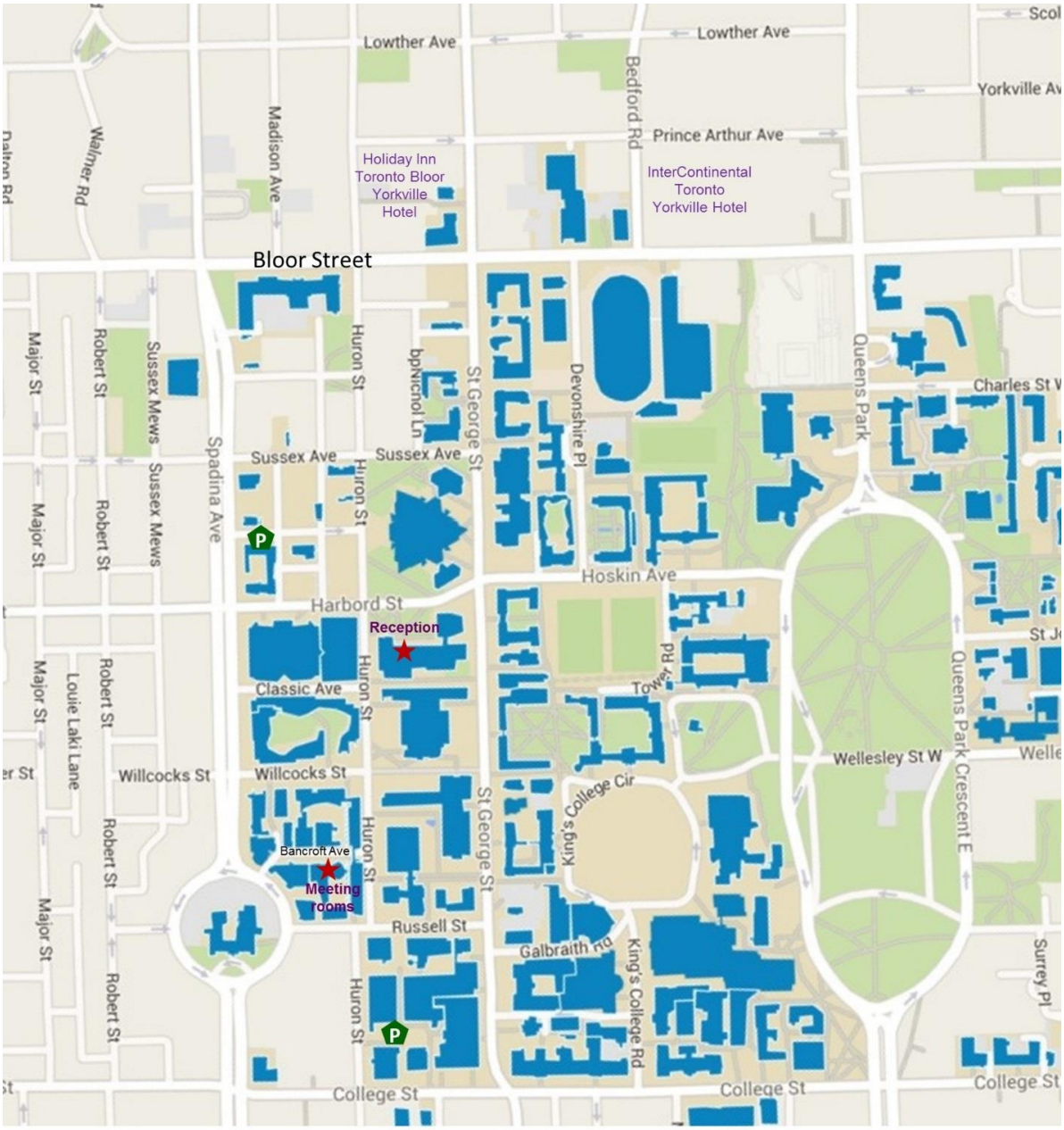
6:30 - 9:00 pm	Registration
6:30 - 8:00 pm	Mixer

Saturday, November 21st

Earth Sciences Centre, 5 Bancroft Ave. entrance, University of Toronto

7:30 - 9:30 am	Registration
7:30 - 8:15 am	Poster Setup
8:15 - 8:30 am	Conference Welcome
8:30 - 9:00 am	Plenary Lecture (Anja Geitmann, McGill University)
9:00 - 9:30 am	Plenary Lecture (Peter McCourt, University of Toronto)
9:30 - 10:15 am	Refreshment Break
10:15 - 11:55 am	Concurrent Session 1: " Abiotic and Biotic Interactions I" Concurrent Session 2: " Cell and Developmental Biology I" Concurrent Session 3: " Photosynthesis and Metabolism I"
12:00 - 1:00 pm	Lunch
1:00 - 2:30 pm	Concurrent Session 4: " Abiotic and Biotic Interactions II" Concurrent Session 5: " Cell and Developmental Biology II" Concurrent Session 6: " Photosynthesis and Metabolism II"
2:30 - 2:45 pm	Short Break (coffee/tea available)
2:45 - 4:15 pm	Concurrent Session 7: " Abiotic and Biotic Interactions III" Concurrent Session 8: " Cell and Developmental Biology III" Concurrent Session 9: " Photosynthesis and Metabolism III"
4:15 - 5:15 pm	Refreshments and Poster Viewing (Presenters should be at their posters by 4:30 pm)
5:30 pm	Presentation of Student Awards & Conference Closing

University of Toronto, St. George Campus Map



Parking Garages ★ Locations for Reception and Meeting Rooms

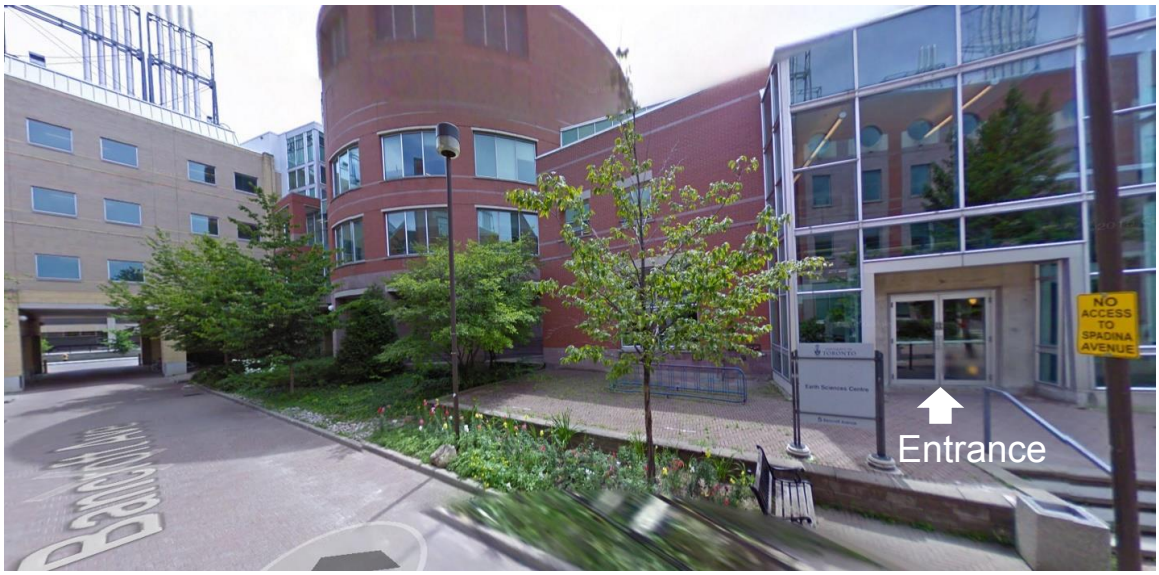
All 2015 SCPB/SCBV ERM events are scheduled to take place in the:

- Ramsay Wright Building, 25 Harbord St. (Friday evening registration and mixer)
- Earth Sciences Centre, 5 Bancroft Ave. entrance (Saturday all day)

There are two parking garages on campus that are nearby and have reasonable weekend rates:

- Graduate House Garage (17 Glen Morris St)
- Bahen Centre for Information Technology (B.C.I.T.) Parking Garage (213 Huron Street)

Saturday: Earth Sciences Centre, 5 Bancroft Ave



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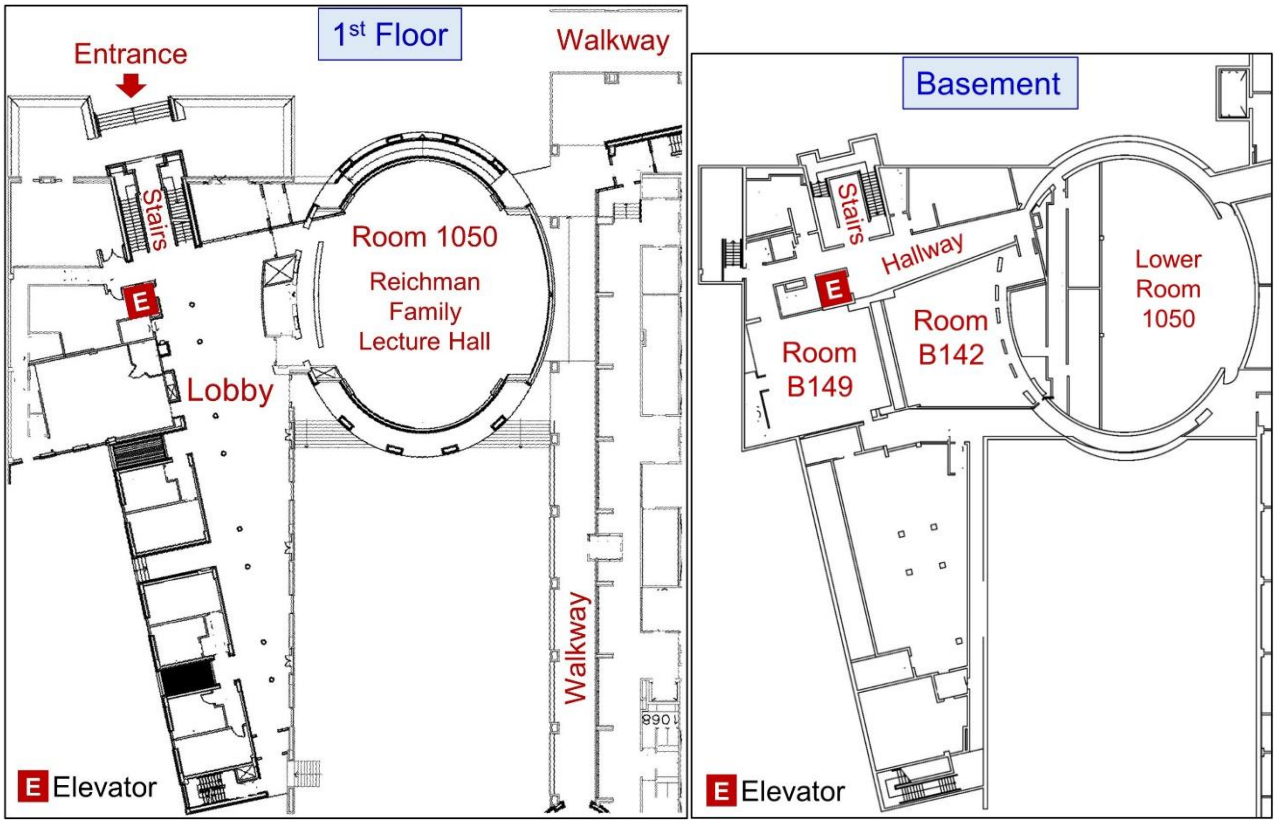

2015 CSPB/SCBV ERM Program

Friday, November 20 *Ramsay Wright Building, Rm 010 (basement), 25 Harbord Street*

6:30 - 8:00 PM **Registration: *Ramsay Wright Building,*** *Rm 010 (basement), 25 Harbord St.*

6:30 - 9:00 PM **Opening Mixer: *Ramsay Wright Building,*** *Rm 010 (basement), 25 Harbord St. Delegates are invited to attend the opening mixer where a variety of hors d'oeuvres and both alcoholic and non-alcoholic refreshments will be served.*

Saturday, November 21 *Earth Sciences Centre, 5 Bancroft Ave entrance*

7:30 - 9:30 AM **Registration** (Lobby, ESC 1st floor, 5 Bancroft Ave)

7:30 - 8:15 AM **Poster Setup** (Lobby, ESC 1st floor, 5 Bancroft Ave)

8:15 - 8:30 AM **Conference Welcome** (1050 Reichman Family Lecture Hall, ESC 1st floor)

8:30 - 9:00 AM **Plenary Lecture I – Peter McCourt**, University of Toronto
(ESC1050 Reichman Family Lecture Hall)
"Parasitic plants, strigolactones, and chemical genomics"

9:00 - 9:30 AM **Plenary Lecture II - Anja Geitmann**, McGill University
(ESC1050 Reichman Family Lecture Hall)
"Light and the life sciences - how light-based technologies have revolutionized our understanding of biological processes"

9:30 - 10:15 AM **Refreshment Break** (Lobby, ESC 1st floor)

Concurrent Session 1: Abiotic and Biotic Interactions I (B142 Placer Dome Classroom, ESC basement)

Chair: Thomas DeFalco, University of Toronto

Session 1

1.1	10:15 - 10:30 AM	**Arabidopsis MYB92 and MYB93 transcription factors are positive regulators of suberin biosynthesis.** Jhadeswar Murmu, Bailey Paterson, Fakhria Muhammad Razeq, Nayana de Silva, Denise Chabot, Dylan Kosma, Owen Rowland
1.2	10:30 - 10:45 AM	**Eutrema salsugineum: Extreme capacity for stress tolerance may involve modest capacity for change.** Vera Marjorie Velasco, Amanda Garvin, Caitlin Simopoulos, Peter Summers, Elizabeth Weretilnyk
1.3	10:45 - 11:00 AM	**"Lost in Translation" ABA Signaling Networks and Abiotic Stress.** Shelley Lumba, Shigeo Toh, Alan Moses, Darrell Desveaux, Peter McCourt
1.4	11:00 - 11:10 AM	**Investigating Suberin Biosynthesis in Poplar Bark: Candidate Genes and Chemical Composition.** Meghan Rains, Sharon Regan, Isabel Molina
1.5	11:10 - 11:20 AM	**Investigating the role of XERICO in GA-ABA hormone crosstalk during plant stress response.** Eliana Vonapartis, Carina Carianopol, Sonia Gazzarrini
1.6	11:20 - 11:30 AM	**Using chemical genomics to identify genes involved in Fusarium graminearum spore germination.** Christopher Mogg, Gopal Subramaniam, Darrell Desveaux
1.7	11:30 - 11:40 AM	**High Throughput Screen for Enhanced HopZ1b Effector Recognition in Arabidopsis thaliana.** Amy Xin Wei Zhang, Caressa Tsai, Darrell Desveaux, David S Guttman
1.8	11:40 - 11:50 AM	**The role of the reactive oxygen species hydrogen peroxide in high temperature induced failure of male reproduction in rice.** Shaheen Bagha, Tammy Sage

Concurrent Session 2: Cell & Developmental Biology I (B149 C-I-L Classroom, ESC basement)

Chair: Katharina Brautigam, University of Toronto

Session 2

2.1	10:15 - 10:30 AM	**Thermoinhibition Uncovers a Role for Strigolactones in Arabidopsis Seed Germination.** Shigeo Toh, Duncan Holbrook-Smith, Peter McCourt
2.2	10:30 - 10:45 AM	**5'-methylthioadenosine induced polyamine deficiency leads to defective embryogenesis.** Asuramuni Nimhani Perera, Edward Yeung, Maye Saechao, Ishari Waduwara-Jayabahu, Barbara Moffatt
2.3	10:45 - 11:00 AM	**Expression and localization of AKIN10 during seed development and germination in Arabidopsis.** Aaron Chan, Sonia Gazzarrini
2.4	11:00 - 11:10 AM	**Yeast one-hybrid approach to isolate FUSCA3 upstream regulators.** Jian Wu, Nobutaka Mitsuda, Mingfang Yi, Sonia Gazzarrini
2.5	11:10 - 11:20 AM	**VIP1 is a regulator of seed mucilage accumulation during seed development in Arabidopsis.** Lisza Duermeyer, Eiji Nambara
2.6	11:20 - 11:30 AM	**Interaction between SnRK1 and ABA signaling pathways in Arabidopsis thaliana.** Carina Carianopol, Shelley Lumba, Peter McCourt, Sonia Gazzarrini
2.7	11:30 - 11:40 AM	**GLUCAN SYNTHASE-LIKE 8 Regulates Intercellular Communication during Early Seedling Development in Arabidopsis.** Behnaz Saatian, Ryan S. Austin, Susanne Kohalmi, Yuhai Cui
2.8	11:40 - 11:50 AM	**Understanding selection on gene coexpression in maize (Zea mays) with RNA sequencing.** Shuhua Zhan, Jane Tosh, Cortland Griswold, Lewis Lukens

Concurrent Session 3: Photosynthesis & Metabolism I (1050 Reichman Family Lecture Hall, ESC 1st floor)

Chair: Dinesh Christendat, University of Toronto

Session 3

3.1	10:15 - 10:30 AM	**Cross-site comparison of temperate evergreen and mixed deciduous forests using paired optical (NDVI and PRI) and photosynthesis data.** Christopher Wong, Altaf Arain, Ingo Ensminger
3.2	10:30 - 10:45 AM	**Warming delays autumn declines in photosynthetic capacity in a boreal conifer, Norway spruce (Picea abies).** Joseph Stinziano, Norman Huner, Danielle Way
3.3	10:45 - 11:00 AM	**Singlet oxygen plays a central signalling role during soybean-weed competition.** Andrew Mckenzie-Gopsill, Sasan Amirsadeghi, Hugh Earl, Elizabeth Lee, Lewis Lukens, Clarence Swanton
3.4	11:00 - 11:10 AM	**The study of variegation in Nicotiana tabacum through Plastoquinol Terminal Oxidase (PTOX) inhibition.** Hillary Peon, Allison McDonald
3.5	11:10 - 11:20 AM	**Alternative oxidase respiration in the light maintains chloroplast energy homeostasis during drought stress.** Keshav Dahal, Greg Vanlerberghe
3.6	11:20 - 11:30 AM	**Photosynthesis shows little acclimation to warming and elevated CO2 in an Antarctic grass.** Vi Bui, Carolina Sanhueza, Leon Bravo, Danielle Way
3.7	11:30 - 11:40 AM	**Economics of root phenology and turnover in wetland plants along a gradient of growing season length; or What Lies Beneath: Discovering the hidden story of plant roots.** Kathleen Cote, Peter Ryser
3.8	11:40 - 11:50 AM	**Proto-Kranz in Neurachne - a cellular and physiological case for photorespiration as a "bridge" to C4 photosynthesis in a grass lineage.** Roxana Khoshravesh, Tammy Sage, Florian Busch, Matt Stata, Nicole Dakin, Montserrat Saladie, Joanne Castelli, Harmony Clayton, Terry MacFarlane, Rowan Sage, Martha Ludwig

12:00 - 1:00 PM **Lunch** (Boxed lunches & refreshments, Lobby, ESC 1st floor, 5 Bancroft Ave)

CSBP Executive Meeting & Lunch (Rm 3087, Earth Sciences Centre)

Concurrent Session 4: Abiotic and Biotic Interactions II (B142 Placer Dome Classroom, ESC basement)

Chair: Wolfgang Moeder, University of Toronto

Session 4

4.1	1:00 - 1:15 PM	**Emerging parallels between Ca2+/calmodulin regulation of cation channels in plants and animals.** Thomas A. Defalco, Christopher B. Marshall, Kim Munro, Wolfgang Moeder, Mitsuhiko Ikura, Wayne A. Snedden, Keiko Yoshioka
4.2	1:15 - 1:30 PM	**Comparative Proteomics of Arabidopsis Phloem During the Induction of Systemic Acquired Resistance.** Philip Carella, Juliane Merl-Pham, Daniel Wilson, Sanjukta Dey, A. Corina Vlot, Robin K. Cameron
4.3	1:30 - 1:45 PM	**Identification of Arabidopsis innate immunity receptors involved in the recognition of novel phytopathogen immune elicitors.** Adam Mott, Shalabh Thakur, Pauline W. Wang, Darrell Desveaux, David S. Guttman
4.4	1:45 - 2:00 PM	**Elucidating the genetic requirements underlying the Pseudomonas syringae effector HopF2PtoT1 immune response in Arabidopsis.** Timothy Lo, Noushin Koulena, Derek Seto, Wenwan Lu, Alexandra Menna, Pauline Wang, David Guttman, Darrell Desveaux
4.5	2:00 - 2:10 PM	**Involvement of Arabidopsis triphosphate tunnel metalloenzymes in distinct biological processes -senescence and pathogen defense.** Purva Karia, Huoi Ung, Wolfgang Moeder, Kazuo Ebine, Takashi Ueda, Keiko Yoshioka
4.6	2:10 - 2:20 PM	**Identification of Novel Resistance Protein Specificities in the Arabidopsis thaliana/Pseudomonas syringae pathosystem.** Nina Kirischian, Andre Santos-Severino, Darrell Desveaux, David S. Guttman
4.7	2:20 - 2:30 PM	--

Concurrent Session 5: Cell & Developmental Biology II (B149 C-I-L Classroom, ESC basement)

Chair: Sonia Gazzarrini, University of Toronto

Session 5

5.1	1:00 - 1:15 PM	**The Arabidopsis SWI2/SNF2 Chromatin Remodeler BRAHMA Regulates Polycomb Function during Vegetative Development and Directly Activates the Flowering Repressor Gene SVP.** Chenlong Li, Chen Chen, Lei Gao, Songguang Yang, Vi Nguyen, Xuejiang Shi, Katherine Siminovitch, Susanne Kohalmi, Shangzhi Huang, Keqiang Wu, Xuemei Chen, Yuhai Cui
5.2	1:15 - 1:30 PM	**Investigating the roles of stigma-expressed RLCKs in compatible pollen signalling.** Jennifer Doucet, Daphne Goring
5.3	1:30 - 1:45 PM	**Small molecule antagonists of germination of the parasitic plant Striga hermonthica.** Duncan Holbrook-Smith, Shigeo Toh, Peter McCourt
5.4	1:45 - 2:00 PM	**Protein-protein interaction exploration based on proteome-wide tertiary structure prediction and further in vitro validation.** Michael Dong, Nicholas Provart
5.5	2:00 - 2:10 PM	**The nitrogen responsive transcriptome in potato (Solanum tuberosum L.) reveals significant gene regulatory motifs.** Jose Hector Galvez Lopez, Helen H. Tai, Bernie Zebarth, Martin Lague, Martina Stromvik
5.6	2:10 - 2:20 PM	**Autophagy's Link to Self-Incompatibility in Arabidopsis species.** Daniel Johnson, Daphne Goring
5.7	2:20 - 2:30 PM	**Investigating the functional role of Shikimate kinase-like 1 in Solanum lycopersicum through virus-induced gene silencing.** Michael Kanaris, Dinesh Christendat

Concurrent Session 6: Photosynthesis & Metabolism II (1050 Reichman Family Lecture Hall, ESC 1st floor)

Chair: Dario Bonetta, University of Ontario Institute of Technology

Session 6

6.1	1:00 - 1:15 PM	**How to Make a C4 Plant.** Rowan Sage, Tammy Sage, Matt Stata, Sarah Covshoff, Steve Kelly, Julian Hibberd
6.2	1:15 - 1:30 PM	**Forward Screening for CBI Resistance in the Monocot Crop Species, Triticum durum (Durum Wheat).** Elysabeth Reavell-Roy, Dario Bonetta, Julian Northey, Isaac Shim
6.3	1:30 - 1:45 PM	**Proanthocyanidin metabolism is critical for seed coat darkening in cranberry bean.** Jose A. Freixas-Coutin, Seth Munholland, Anjali Silva, Sanjeena Subedi, William L. Crosby, K. Peter Pauls, Gale G. Bozzo
6.4	1:45 - 2:00 PM	**Photosynthetic capacity of tropical montane tree species in relation to leaf nutrients, successional strategy and growth temperature.** Mirindi Eric Dusenge, Goran Wallin, Johanna Gardesten, Felix Niyonzima, Lisa Adolfsson, Donat Nsabimana, Johan Uddling
6.5	2:00 - 2:10 PM	**Genetic Control of Alternate Chloroplast Patterns in C4 Bundle Sheath and Mesophyll Cells.** Matt Stata, Sarah Covshoff, Julian Hibberd, Gane Ka-Shu Wong, Rowan Sage, Tammy Sage
6.6	2:10 - 2:20 PM	**Post-Translational Regulation of Starch Synthase 2 in Arabidopsis thaliana.** Jenelle Patterson, Ian Tetlow, Michael Emes
6.7	2:20 - 2:30 PM	**A systematic characterization of the isoflavonoid-specific prenyltransferase gene family in Soybean.** Arjun Sukumaran, Sangeeta Dhaubhadel

2:30 - 2:45 PM **Short Break** (Coffee & Tea, Lobby, ESC 1st floor, 5 Bancroft Ave)

Concurrent Session 7: Abiotic and Biotic Interactions III (B142 Placer Dome Classroom, ESC basement)

Chair: Jhadeswar Murmu, Carleton University

Session 7

7.1	2:45 – 3:00 PM	**Identification of gene sets with roles in foliar browsing and heartwood rot defenses in western redcedar by comparative RNA-seq analysis.** Jim Mattsson
7.2	3:00 - 3:15 PM	**Towards guard cell-specific transcriptomics during drought - development of a guard cell-INTACT in Arabidopsis thaliana.** Anna van Weringh, Nicholas Provart
7.3	3:15 - 3:30 PM	**The relationship between genome, epigenome, and physiology in poplar in response to drought.** Katharina Brautigam, Yunchen Gong, Stefan Schreiber, Pauline Wang, Barb Thomas, Uwe Hacke, Malcolm Campbell
7.4	3:30 - 3:45 PM	**Regulation of Quinate Metabolism in Listeria monocytogenes.** Stephanie Prezioso, Dinesh Christendat
7.5	3:45 - 3:55 PM	**Characterization of a Putative Gene Operon Implicated in Pseudomonas Putida Rhizosphere Interactions.** Sophie Qin, Stephanie Prezioso, Dinesh Christendat
7.6	3:55 - 4:05 PM	**Differential Temperature Response of Wetland Monocots with Contrasting Root Strategies.** Jacob Porter, Peter Ryser
7.7	4:05 - 4:15 PM	**The Effect of High Temperature on Pollen Maturation and Germination in Diverse Taxa.** Haley A. Branch, Reginald McDonald, Tammy L. Sage, Rowan F. Sage

Concurrent Session 8: Cell & Developmental Biology III (B149 C-I-L Classroom, ESC basement)

Chair: Annette Nassuth, University of Guelph

Session 8

8.1	2:45 – 3:00 PM	**Lipid droplet-associated proteins (LDAPs) are required for the dynamic regulation of lipid droplets in vegetative plant cells.** Michal Pyc, Satinder Gidda, Kent Chapman, John Dyer, Robert Mullen
8.2	3:00 - 3:15 PM	**Possible role of a grape DREB2 transcription factors in stomatal development.** Chevonne E. Carlow, Christine Lee, M. Atikur Rahman, Annette Nassuth
8.3	3:15 - 3:30 PM	**HSP90C is a chloroplast chaperone that assists in the stromal targeting of PsbO1 in Arabidopsis thaliana.** Tim Jiang, Lingjie Zhang, Rongmin Zhao
8.4	3:30 - 3:45 PM	**Mitochondrial pleomorphy is mediated by contiguous ER dynamics.** Ashley Jaipargas, Kiah Barton, Neeta Mathur, Jaideep Mathur
8.5	3:45 - 3:55 PM	**'Piggy-back' transport of AROGENATE DEHYDRATASE 5 into the nucleus in Arabidopsis thaliana using IMPORTIN a isoform 6.** Sara Abolhassani Rad, Susanne Kohalmi
8.6	3:55 - 4:05 PM	**Epidermal pavement cells of Arabidopsis have chloroplasts.** Kiah Barton, Martin Schattat, Torsten Jakob, Gerd House, Christian Wilhelms, Jaideep Mathur
8.7	4:05 - 4:15 PM	**The localization of carbon-phosphate translocators on the inner plastid envelope membrane exhibits a stochastic relation to stromules.** Michael Wozny, Kate Delfosse, Neeta Mathur, Jaideep Mathur

Concurrent Session 9: Photosynthesis & Metabolism III (1050 Reichman Family Lecture Hall, ESC 1st floor)

Chair: Gale Bozzo, University of Guelph

Session 9

9.1	2:45 – 3:00 PM	**Combining EMS mutagenesis and enhanced screening identifies novel mutants in monoterpenoid indole alkaloid (MIA) biosynthesis in Catharanthus roseus (Madagascar periwinkle).** Trevor Kidd, Michael Easson, Paulo Cazares, Kyung-Hee Kim, Yang Qu, Vincenzo De Luca
9.2	3:00 - 3:15 PM	**Biochemistry-enabled genomics: the Ca2+-dependent protein kinase RcCDPK1 phosphorylates bacterial-type PEP carboxylase at serine-451 in developing castor oil seeds.** Sheng Ying, Allyson Hill, Michal Pyc, Erin Anderson, Wayne Snedden, Robert Mullen, Yimin She, William Plaxton
9.3	3:15 - 3:30 PM	**K+ Dependent and K+ Independent Asparaginase from Common Bean (Phaseolus vulgaris): Mechanism of Activation by K+.** Ebenezer Ajewole, Frederic Marsolais, Mariusz Jakolski, Agnieszka Pajak
9.4	3:30 - 3:45 PM	**Arabidopsis thaliana BGLU15 is required for flavonol catabolism in plants.** Jonathon Roepke, Gale Bozzo
9.5	3:45 - 3:55 PM	**The role of quinate biosynthesis in plants.** Artyom Gritsunov, James Peek, Dinesh Christendat
9.6	3:55 - 4:05 PM	**Molecular cloning and characterization of a phenylpropanoid methyltransferase with unusual para-methylating activity.** Brent Wiens, Razvan Simionescu, Vincenzo De Luca
9.7	4:05 - 4:15 PM	**Altered MTA metabolism affects lipid homeostasis and vascular development.** Benjamin Tremblay, Ishari Waduwara-Jayabahu, Hitoshi Sakakibara, Maye Saechao, Barbara Moffatt

4:15 - 5:15 PM **Refreshments and Poster Viewing.** (Lobby, ESC 1st floor, 5 Bancroft Ave) **Presenter should be at their posters by 4:30 pm.**

5:30 PM **Presentation of Student Awards & Conference Closing** (Lobby, ESC 1st floor, 5 Bancroft Ave)

List of Posters

Abiotic and Biotic Interactions:

P1. The negative effect of high temperature stress on reproduction in Arabidopsis thaliana. Vanessa Lundsgaard-Nielsen, Dinesh Christendat, Tammy L Sage

P2. A long non-coding RNA associated with sulfur nutrition in Eutrema salsugineum: An example of local adaptation? Amanda Garvin, Caitlin Simopoulos, Wilson Sung, Brian Golding, Elizabeth Weretilnyk

P3. Differentiating viable/non-viable and mature/immature Plasmodiophora brassicae resting spores using propidium monoazide-assisted qPCR. Fadi Al-Daoud, Bruce Gossen, Mary Ruth McDonald

P4. Do plant growth-promoting rhizobacteria increase plant growth and reduce the production of ethylene in Arabidopsis under cadmium and copper stress? Joshua Frank, Sheila Macfie

P5. Evaluating the roles of seed coat constituents in protecting embryos from chromium toxicity in Arabidopsis thaliana. Nayana De Silva, Celine Boutin, Anna Lukina, Isabel Molina, Owen Rowland

P6. Protein content and protease activity in senescing roots and leaves of wetland monocot species with contrasting root turnover strategies. Mona Alsahame, Peter Ryser

P7. Comparative transcriptomics of allopolyploid Glycine dolichocarpa and its diploid progenitors in response to rhizobial inoculation. Adrian Powell, Jeff Doyle

P8. Adaptation of two-spotted spider mite, Tetranychus urticae, to Arabidopsis indole glucosinolates. Golnaz Salehipour-Shirazi, Kristie Bruinsma, Huzefa Ratlamwala, Vojislava Grbic

P9. Investigation of the importance of various VrICE2 domains and the effect of cold treatment on their posttranslational modifications effect activity as a transcription factor. Rob Brandt, Scott Hughes, Annette Nassuth

P10. New insights into the spider mite mouthparts, their mode of action and their effect on plant tissues associated with feeding. Nicolas Bensoussan, Vladimir Zhurov, Maria Estrella Santamaria, Vojislava Grbic

P11. An in silico analysis of fatty acid QTL and related genes in soybean. Yarmilla Reinprecht, K. Peter Pauls

P12. Attenuation of tomato induced transcriptional response upon herbivory by adapted two-spotted spider mites. Nicky Wybouw, Vladimir Zhurov, Catherine Martel, Kristie Bruinsma, Frederik Hendrickx, Vojislava Grbic, Thomas Van Leeuwen

P13. Investigating the role of plant mitochondria in NO synthesis and signalling. Nicole Alber, Greg Vanlerberghe

P14. Translational regulation via codon usage bias during cold acclimation in Vitis. Trent Faultless, Sanjeena Dang, Annette Nassuth

P15. The experimental evolution of Pseudomonas syringae pv. phaseolicola 1448A within the non-host Arabidopsis thaliana. Andrew Jamnik, David Guttman

Cell and Developmental Biology:

P16. High throughput chemical screening using an Arabidopsis ShHTL7 system to identify strigolactone signaling agonists. Asrinus Subha, Shigeo Toh, Duncan Holbrook-Smith, Peter McCourt

P17. The effect of APK1A and APK1B knockout in reinstated self-incompatibility of Arabidopsis thaliana. Hyun Kyung Lee, Rajiv Rampersaud, Daphne Goring

P18. Characterizing an alternative targeting signal to the chloroplast outer membrane in Arabidopsis. Nicholas Grimberg, Matthew Smith, Simon Chuong

P19. The Arabidopsis HOTHEAD gene is involved in regulating seed size. Pearl Pei-Chun Chang, Susan J Lolle

P20. Stability of VNI2 protein is regulated by an interacting protein. Masatoshi Yamaguchi, Junko Kitagawa, Hirofumi Uchimiya, Maki Kawai-Yamada, Taku Demura

P21. Stigma-specific kinase knockdown lines show reduced compatible pollen acceptance in Arabidopsis thaliana. Nethangi Udugama, Jennifer Doucet, Daphne Goring

P22. Characterizing the roles of Arabidopsis calmodulin-like protein, CML39, in hormonal regulation of early seedling development and fruit formation. Ubaid Midhat, Wayne Snedden

P23. Arabidopsis At3g11620 encodes an evolutionarily conserved protein involved in the intracellular positioning of cytosolic lipid droplets. Samantha C. Watt, Satinder K. Gidda, John M. Dyer, Joe Hull, Robert T. Mullen

P24. Identifying the genetic requirements for recognition of a Pseudomonas syringae HopF effector in Arabidopsis. Derek Seto, Noushin Koulena, Timothy Lo, David Guttman, Darrell Desveaux

P25. Determination of cellulose synthase complex composition using BioID. Sara Behnami, Dario Bonetta

P26. A genetic screen for a suppressor of the irrepressible variant of AUXIN RESPONSE FACTOR 5/MONOPTEROS. Adriana Caragea, Thomas Berleth

P27. Clade I TGA bZIP factors are essential for BLADE-ON-PETIOLE-dependent regulation of flowering and inflorescence architecture in Arabidopsis thaliana. Ying Wang, Brenda C. Salasini, Madiha Khan, Bhaswati Devi, Mike Bush, Shelley R. Hepworth

Photosynthesis and Metabolism:

P28. Can we develop an assay to determine arogenate dehydratase activity in vivo? Emily Clayton, Susanne Kohalmi

P29. Chilling out: The evolution of psychrophily in algae. Marina Cvetkovska, David R. Smith, Norman P.A. Huner

P30. Characterization of the anatomy, development, and transcriptome of C3 and C4 Atriplex species. Stefanie Sultmanis, Matt Stata, Udo Gowik, Andrea Brautigam, Andreas Weber, Rowan Sage, Tammy Sage

P31. The Role of SNF1-Related Protein Kinase 1 (SnRK1) in Carbohydrate Metabolism in Arabidopsis thaliana. You Wang, Ian Tetlow, Michael Emes

P32. Effects of heat waves on photosynthetic performance in Douglas-fir. Andre G. Duarte, Nadine Ruehr, Genki Katata

P33. Biochemical characterization of AtPirin1: a flavonol degrading enzyme. Ashley Crews, Jonathon Roepke, Jose A. Freixas-Coutin, Gale G. Bozzo

P34. Altered leaf starch synthesis results in increased rates of photosynthesis in A. thaliana, before flowering. Noel Mano, Fushan Liu, Malgre Micallef, Michael Emes, Ian Tetlow

P35. Branching Enzymes as Agents for Modifying Glucan Structure in Industrial Processing. Lily Nasanovsky, Ian Tetlow

P36. Interaction of curculin-like lectin with AtPAP26-CW2, a purple acid phosphatase glycoform upregulated by phosphate-starved Arabidopsis thaliana. Mina Ghahremani, , Hue Tran, H. Del Vecchio, M. Choka, Erin Anderson, Yi-Min She, Robert Mullen, William Plaxton

Sponsors

Concurrent Oral Session Abstracts

Session 1. Abiotic and Biotic Interactions I

1.1 Arabidopsis MYB92 and MYB93 transcription factors are positive regulators of suberin biosynthesis

Jhadeswar Murmu[1], Bailey Paterson[1], Fakhria Muhammad Razeq[1], Nayana de Silva[1], Denise Chabot[2], Dylan Kosma[3], Owen Rowland[1]
[1]*Carleton University, Canada*, [2]*Agriculture and Agri-Food Canada, Canada*, [3]*University of Nevada Reno, United States of America*

Suberin is a highly complex cell wall-associated polymer composed of lipid and phenolic monomers as well as glycerol. Suberin is deposited in the cell walls of specialized boundary tissue layers such as root endodermis and periderm. A central function of suberin is to control the movement of water, solutes, and gases. It is also a stress inducible barrier that protects plants against abiotic stresses (e.g. drought, high salt, toxic metals) and pathogens. We recently reported that Arabidopsis thaliana (At) MYB41, an R2-R3 MYB transcription factor (TF), is a transcriptional regulator of suberin biosynthesis under abiotic stress conditions. In the present study, we provide evidence that AtMYB92 and AtMYB93 regulate suberin biosynthesis under normal development in the root endodermis. We first identified AtMYB92 and AtMYB93 as positive regulators in a transient assay screen using leaves of Nicotiana benthamiana. Analysis of their gene expression patterns using promoter:GUS fusions revealed that AtMYB92 and AtMYB93 have overlapping expression in root endodermis even under non-stress conditions. Further, loss-of-function single and double mutants of AtMYB92 and AtMYB93 exhibit dramatic reduction in root endodermis suberin and display reduced transcript levels of suberin biosynthetic genes in roots. The myb92 myb93 double mutant plants are not compromised in abiotic stress induction of suberin. Our results provide insight into the molecular genetic regulation of a key protective biopolymer. The identification of master regulators of suberin provides the means to generate plants with enhanced barrier properties.

1.2. Eutrema salsugineum: Extreme capacity for stress tolerance may involve modest capacity for change

Vera Marjorie Velasco[1], Amanda Garvin[1], Caitlin Simopoulos[1], Peter Summers[1], Elizabeth Weretilnyk[1]
[1]*McMaster University, Canada*

Yukon Eutrema salsugineum (aka Thellungiella salsuginea) is a halophytic crucifer that thrives on soil naturally high in sulfur and low in phosphate (Pi). We exposed plants to combinations of high and low levels of sulfur and Pi to determine how this extremophile copes with P and S deficiencies. We used soil containing 200 or 5000 ppm S and treated plants for four weeks with fertilizer lacking Pi or containing 2.5 mM Pi. Leaf P and S content was positively correlated with treatment combination and RT-qPCR confirmed that plants under low S or low P conditions were responding to a deficiency by the up-regulated expression of well-characterized deficiency marker genes Induced by Phosphate Starvation 2 and Sulfur Deficiency 1. In spite of the plants registering as deficient for P and/or S, we found no significant treatment-related difference in total rosette dry biomass. A means by which plants cope with soil P deficiencies is by reallocating its use of this nutrient. One such mechanism involves membrane glycerolipid remodelling whereby phospholipids are replaced by galactolipids and sulfolipids, a response that can lead to reduced photosynthesis. Using lipid profiling we found no treatment-associated differences among the leaf phospholipid, galactolipid or sulfolipid classes and no evidence for reduced rates photosynthesis. This response indicates that the capacity for plants to undergo membrane remodelling with P limitation is not universal and suggests that Eutrema is specialized for optimum growth on low nutrient environments.

1.3. "Lost in Translation" ABA Signaling Networks and Abiotic Stress

Shelley Lumba[1], Shigeo Toh[1], Alan Moses[1], Darrell Desveaux[1], Peter McCourt[1]
[1]*University of Toronto, Canada*

Abscisic acid (ABA) mediates an assortment of responses to abiotic and biotic stresses in plants. Using systems biology approaches that focused on ABA-regulated genes, we have expanded the linear core signaling pathway of ABA to a signaling network of over 500 interactions among 138 proteins in Arabidopsis. By mapping expression data onto our interactome, we have defined critical hubs in response to different abiotic stresses. We predict that these hubs would be highly predictive of protein function. In particular, we have applied our approach to enrich for genes that confer drought protection. To mimic physiologically relevant conditions in the field, we established a moderate drought stress treatment and assay for seed yield for Arabidopsis in the growth chamber. Using these conditions, we have assayed loss-of-function and gain-of-function mutants representing hub genes for improved drought protection and seed yield. Our screening has identified both loss- and gain-of-function leads. A strong gain-of-function lead has been transformed into soybean.

1.4. Investigating Suberin Biosynthesis in Poplar Bark: Candidate Genes and Chemical Composition

Meghan Rains[1], Sharon Regan[2], Isabel Molina[3]
[1]*Queen's University, Algoma University, Canada*, [2]*Queen's University, Canada*, [3]*Algoma University, Canada*

Suberin is a plant cell wall-specific lipid polymer deposited on the inner face of certain primary cell walls and is vitally important to all land plants. Contributing to the control of water and ion fluxes, suberin represents the first barrier of defense against pathogen penetration in specific external and internal plant tissues, such as tree stem and tuber periderms. While the exact macromolecular structure of suberin is unknown, current models identify suberin as a polyester of acylglycerols cross-linked with aromatics and associated with solvent-soluble waxes. Genetic approaches have lead to the identification of genes required for suberin biosynthesis, however, the biochemical functions of many enzymes, the regulation of its synthesis, and the pathways that lead to the final assembly are poorly understood. We have performed a detailed transcriptomic and chemical analysis of hybrid poplar (P. tremula x P. alba) inner (control) and outer (cork) bark at different developmental stages. We found 1275 and 3374 differentially expressed (DE) genes at the two developmental stages studied. To improve gene annotation, we determined their Arabidopsis orthologs; only 7 % of the poplar DE transcripts were found in the ARALIP (Arabidopsis Acyl-Lipid metabolism) database, and about 11 % were related to transcriptional regulation. Functional classifications, assigned using MapMan bins, showed significant differential expression in the functional areas of cell wall, lipid metabolism, secondary metabolism, development, and transport. To link candidate catalysts and their possible biosynthetic products, the phytochemical profile of bark extracellular lipids was also investigated.

1.5. Investigating the role of XERICO in GA-ABA hormone crosstalk during plant stress response

Eliana Vonapartis[1], Carina Carianopol[1], Sonia Gazzarrini[1]
[1]*University of Toronto, Canada*

Phytohormones regulate growth and development, and allow for plants to effectively sense changes in their environment. Their signaling pathways frequently interact, together forming a complex crosstalk network that controls plant response to endogenous signals and external stressors. A well-known instance of crosstalk occurs between the two antagonistic hormones gibberellin (GA) and abscisic acid (ABA) during developmental phase transitions and unfavourable growth conditions. XERICO (XER) is a DELLA-induced putative RING E3 ubiquitin ligase that increases intracellular ABA levels, although its precise mode of action remains unexplored. Therefore, my research aims to understand the role of XER in GA-ABA crosstalk. More specifically, I am studying its function during stress response and germination, a developmental phase transition that is tightly regulated to ensure seedling survival. To elucidate XER's function in hormone signaling, we are employing a variety of in planta and in vitro molecular techniques. Localization assays suggest that this protein localizes to the cell periphery and chloroplasts. In addition, downstream targets have been isolated in yeast two-hybrid assays. To identify XER upstream regulators, we will use a yeast one-hybrid approach. Furthermore, overexpression lines have been generated to study its in planta function. Lastly, XER's role in modulating protein homeostasis will be explored through ubiquitination and degradation assays. This project will provide important insight as to how a plant E3 ligase is involved in the integration of the GA and ABA signaling pathways, and how plants coordinate their growth and development to promote survival in response to environmental stress.

1.6. Using chemical genomics to identify genes involved in Fusarium graminearum spore germination

Christopher Mogg[1], Gopal Subramaniam[2], Darrell Desveaux[1]
[1]*University of Toronto, Canada*, [2]*Agriculture and Agri-Food Canada, Canada*

Antofine is a phenanthraindolizidine alkaloid that was isolated from Vincetoxicum rossicum, an invasive species of plant belonging to the Apocynaceae family. Antofine was then discovered to inhibit spore germination in the cereal phytopathogen Fusarium graminearum. In an effort to identify the potential targets of Antofine, a library of Saccharomyces cerevisiae mutants were employed in a haploid insufficiently (HIP) assay; where Glutamate Dehydrogenase (GDH) was identified as one likely target. The F. graminearum variant of GDH was cloned, expressed, and isolated, so that the inhibitory affect of Antofine could be confirmed and compared with the inhibitory effects upon a commercially available GDH.

1.7. High Throughput Screen for Enhanced HopZ1b Effector Recognition in Arabidopsis thaliana

Amy Xin Wei Zhang[1], Caressa Tsai[1], Darrell Desveaux[1], David S Guttman[1]
[1]*University of Toronto, Canada*

Pseudomonas syringae is a Gram negative bacteria that infects many plant species. Using the type III secretion system, P. syringae can evade broad-spectrum plant defense by injecting virulence-associated effectors into the host cell. However, plants have evolved to specifically detect these effector proteins. Thus, there is selective pressure on P. syringae, to expand its effector repertoire and on plants, to evolve novel effector recognition mechanisms. Due to host selective pressure, P. syringae's HopZ family of effectors has undergone significant diversification. In particular, HopZ1a is sufficient to trigger robust recognition in soybean, tobacco and Arabidopsis thaliana when delivered by P. syringae. On the other hand, HopZ1b is weakly recognized when delivered by P. syringae and only triggers strong resistance when overexpressed directly in planta. To find means of increasing this weak resistance in the plant itself, we conducted a high throughput screen using P. syringae carrying HopZ1b and selected for resistant plants. We screened through 96 Arabidopsis ecotypes and EMS-mutagenized plants to identify natural variations or induced mutations that enhance HopZ1b recognition. Here, I present preliminary results highlighting one ecotype that recognizes HopZ1b. Subsequent mapping of associated loci could address whether this naturally occurring recognition is due to modification of the HopZ1a resistance pathway or a novel recognition mechanism.

1.8. The role of the reactive oxygen species hydrogen peroxide in high temperature induced failure of male reproduction in rice

Shaheen Bagha[1], Tammy Sage[1]
[1]*University of Toronto, Dept. of EEB, Canada*

Plant reproduction is among the most sensitive physiological components that contribute to yield. In warm and cool season crops, male reproduction is widely noted to fail completely between 30-40 degC, temperatures that promote good vegetative growth and photosynthesis. Rice (Oryza) reproduction currently occurs within a few degrees of the temperature threshold for reproductive failure. The present study examined the impact of chronic high temperature (HT, 36 degC) on male reproduction in Oryza species to determine; 1) the most sensitive stages of development, 2) the cellular processes associated with HT-induced sterility, and 3) the cell/tissue specific expression of hydrogen peroxide (H2O2), a signaling molecule known to induce programmed cell death and necrosis if homeostasis is not attainable, as in the case of chronic heat stress. Failed grain production at 36 degC in O. sativa resulted from autophagic programmed cell death of meioctyes during meiosis, whereas necrotic cell death of uninucleate microspores caused an absence of grain set in O. glaberrima, the rice species cultivated in Africa. In spite of differences in the timing of aborted pollen development, H2O2 was elevated in the nuclei of meiocytes undergoing meiosis and uninucleate microspores, prior to collapse. Levels of H2O2 were also elevated at HT in autophagosomes and lysosomes in meiocytes, and in the locule and pollen wall of uninucleate microspores. Results from this study indicate that pollen abortion at HT is correlated with enhanced H2O2, suggesting a possible signalling or functional role for this reactive oxygen species in HT induced male sterility in Rice.

Session 2. Cell and Developmental Biology I

2.1. Thermoinhibition Uncovers a Role for Strigolactones in Arabidopsis Seed Germination

Shigeo Toh[1], Duncan Holbrook-Smith[1], Peter McCourt[1]
[1]*University of Toronto, Canada*

Strigolactones (SLs) are host factors that stimulate seed germination of parasitic plant species Striga. To understand the roles of SLs in seed germination, it is necessary to develop a tractable experimental system using model plants such as Arabidopsis. We reported that thermoinhibition, which involves exposing seeds to high temperatures, uncovers a clear role for SLs in promoting Arabidopsis seed germination. Both SL biosynthetic and signaling mutants showed increased sensitivity to seed thermoinhibition. The synthetic strigolactone GR24 does not rescued max2-1 seed germination. Hormone analysis revealed that SLs alleviate thermoinhibition by modulating levels of the two plant hormones, GA and ABA. Recently, we also

reported that GR24 directly bind the HTL a/b hydrolase in Arabidopsis in vitro. Strigolactones promoted an interaction between HTL and the F-box protein MAX2 in yeast. We also found htl mutant in our GR24 insensitive screening using thermoinhibiton. These results suggest that HTL is involved in SL signaling during seed germination in Arabidopsis. Molecular analysis using a hypocotyl elongation assay showed SLs regulate the nuclear localization of the COP1 ubiquitin ligase, which in part determines the levels of light regulators such as HY5. Genetic analysis revealed that cop1 single mutant, cop1 max2 double mutant and cop1 htl-3 double mutant showed thermo-tolerant seed germination phenotype. These results indicated that COP1 is a negative regulator of seed germination and is genetically at or downstream of MAX2 and HTL in SL signaling with respect to seed germination.

2.2. 5'-methylthioadenosine induced polyamine deficiency leads to defective embryogenesis

Asuramuni Nimhani Perera[1], Edward Yeung[2], Maye Saechao[1], Ishari Waduwara-Jayabahu[1], Barbara Moffatt[1]
[1]*University of Waterloo, Canada*, [2]*University of Calgary, Canada*

5'-methylthioadenosine (MTA) is a by-product of several reactions, such as polyamine (PA), ethylene and nicotianamine (NA) biosynthesis. As part of the Yang cycle, MTA nucleosidase (MTN) hydrolyses MTA to adenine and methylthioribose (MTR). Complete loss-of function Arabidopsis thaliana MTN mutants are lethal whereas a mutant with approximately 15% residual MTN activity (mtn1-1mtn2-1) has a complex phenotype including male and female sterility, altered polyamine profile, reduced auxin transport and vascular abnormalities. Mutants with slightly more residual activity (~28%; mtn1-1mtn2-5) are fertile and have a more moderate phenotype. Analysis of developing seeds of the severe MTN mutant revealed its abnormal embryo sac and integument formation. Reporter gene constructs for the early vascular marker (AtHB8) and auxin (DR5, MP) have been introduced into both the severe and moderate MTN mutants to determine the effect of MTN-deficiency on these key reporters. Previous experiments showed that exogenous feeding of the polyamine spermidine (Spd) can improve the fertility of the severe MTN mutant. Ongoing research is examining the effect of Spd-feeding on reporter gene expression in mutant embryos. In addition, the contribution of MTN activity in maternal embryo tissues is being evaluated. Taken together these experiments should determine the involvement of MTN activity and Spd in embryo development of Arabidopsis thaliana.

2.3. Expression and localization of AKIN10 during seed development and germination in Arabidopsis

Aaron Chan[1], Sonia Gazzarrini[1]
[1]*UTSC, Canada*

Seed development and subsequent germination are critical stages of the plant life cycle. Mutants of the SnRK1 kinase complex are known to be negatively impacted in floral and seed development. As an initial step in understanding the role of SnRK1 in seed development and germination, the temporal-spatial distribution of the most abundant SnRK1 catalytic subunit AKIN10 has been examined. Based on promoter:GUS, AKIN10-GFP and western blotting AKIN10 has been found to be ubiquitously expressed in all seed tissues throughout seed development prior to desiccation and during germination. Subcellular localization appeared to be mainly in the cytoplasm, but also occasionally in the nucleus, where SnRK1 may potentially interact with transcription factors previously reported in vitro. SnRK1 has been suggested to be involved in abscisic acid and diurnal responses, but no consistent differences in expression or localization was observed. The results suggest that AKIN10 (and by extension SnRK1) is ubiquitous and constitutive in all cells during seed development and germination. Future work with seed targeted RNAi knockdown and overexpression of AKIN10 is underway to further elucidate the role of SnRK1 in seed development.

2.4. Yeast one-hybrid approach to isolate FUSCA3 upstream regulators

Jian Wu[1], Nobutaka Mitsuda[2], Mingfang Yi[3], Sonia Gazzarrini[1]
[1]*University of Toronto, Canada*, [2]*Kanto Gakuin University, Japan*, [3]*China Agricultural University, China*

FUSCA3 is a B3 domain transcription factor (TF) that is expressed during embryogenesis to induce late embryonic development. FUSCA3 is a key regulator of hormonal pathways and is itself regulated by hormones. FUSCA3 positively regulates ABA levels while negatively regulating GA and ethylene biosynthesis and signaling pathways. These hormones then regulate FUSCA3 by unknown mechanisms. FUSCA3 expression is also regulated by other TF, master regulators of seed development. However, the complex network that regulates FUSCA3 expression is poorly understood. Yeast one-hybrid (Y1H) screening is an efficient way to identify transcription factors that interact with DNA sequences. Here, we used a prey library composed about 1500 TF cDNAs of Arabidopsis thaliana to isolate FUSCA3 upstream regulators. We isolated two MADS BOX genes (AGL75 and AGL81) that bind to the FUSCA3 promoter (~500 bp upstream of the start site). Previously, AGL15 was reported to bind the FUSCA3 promoter through ChIP assays. FUSCA3 does indeed contain AGL binding elements in its promoter. Arabidopsis microarray databases shows AGL81/15 are expressed at the early stages of seed development, similarly to FUSCA3, and they are induced by ACC. Thus, the AGL family may play an important role in regulating FUSCA3 expression. How these three genes cooperatively and specifically mediate FUSCA3 expression and function on embryo development needs further study.

2.5. VIP1 is a regulator of seed mucilage accumulation during seed development in Arabidopsis

Lisza Duermeyer[1], Eiji Nambara[1]
[1]*University of Toronto, Department of Cell and Systems Biology, Canada*

Seed mucilage is a pectinaceous-rich gel that erupts from the outer cell layer of the seed coat upon imbibition, to entirely surround the germinating seed (Western 2012). It is an adaptation that is present in many Angiosperm species, and is thought to be important for optimizing germination success in a range of conditions. It has been proposed that seed mucilage is important for adhesion of the seed to the soil, dispersal and protection of the seed to stresses, and the regulation of oxygen flow into and out of the seed. In Arabidopsis, mucilage is deposited in the epidermal layer of the seed coat during seed development. Virulence E2 interacting protein 1 (VIP1) is a bZIP transcription factor that was originally identified for its role in mediating Agrobacterium tumefaciens infection in plants. More recently it has been implicated in the turgor stress response in Arabidopsis (Tsugama et al. 2012). In addition to these roles, we have identified VIP1 as a regulator of mucilage accumulation. Our data shows that vip1 mutants have reduced mucilage, and an abnormal seed coat surface. While cellulose production appears relatively unchanged, vip1 mutants display less non-adherent and adherent mucilage. Genetic analyses are being carried out to investigate how VIP1 controls or contributes to mucilage accumulation during seed development.

2.6. Interaction between SnRK1 and ABA signaling pathways in Arabidopsis thaliana

Carina Carianopol[1], Shelley Lumba[1], Peter McCourt[1], Sonia Gazzarrini[1]
[1]*University of Toronto, Canada*

Plants have developed intricate mechanisms to enable survival and propagation under non-optimal conditions. Regardless of the type of stress, the result is energy deprivation that occurs through decreased photosynthesis and respiration rates. Energy sensors, which are conserved in yeast, mammals and plants, are activated in order to achieve energy homeostasis through metabolic compensation. In plants, the SnRK1 (Sucrose non-fermenting Related Kinase 1) members are the closest to the yeast Snf1 and mammalian AMPK energy sensors. The catalytic subunit of SnRK1, AKIN10, has been proposed to link stress, sugar and developmental signals in order to regulate plant metabolism, energy balance, growth and therefore survival of the plant under stress. Despite the important role of SnRK1 activation during stress, the mechanism of SnRK1 regulation of energy homeostasis and plant development are not fully understood. Recent work indicates SnRK1 and ABA (abscisic acid) signaling pathways intersect during stress responses and share common transcriptional targets, however, the mechanism of this interaction is unclear. The aim of this project is to investigate mechanisms of growth and development regulation by AKIN10 and ABA in Arabidopsis thaliana. A high-throughput Y2H using AKIN10 against a collection of 258 ABA-regulated genes was performed resulting in 50 putative AKIN10 interactors, 37 of which were reconfirmed by Y2H. The list includes: 5 previously characterized targets; metabolic and regulatory proteins; stress-related proteins and is enriched for transcription factors. The interactions between AKIN10 and 2 putative targets have been confirmed by BiFC assays and were chosen for further study.

2.7. GLUCAN SYNTHASE-LIKE 8 Regulates Intercellular Communication during Early Seedling Development in Arabidopsis

Behnaz Saatian[1], Ryan S. Austin[2], Susanne Kohalmi[3], Yuhai Cui[2]
[1]*Western University, Agriculture and Agri-Food Canada, Canada*, [2]*Southern Crop Protection and Food Research Center, Agriculture and Agri-Food Canada, Canada*, [3]*Western University, Canada*

Callose, a linear b-1,3-glucan polymer, accumulates at plasmodesmata (PD) where it regulates cell-to-cell communication, at the cell plate during cytokinesis, and in male and female gametophytes. Reversible deposition of callose is believed to be an important mechanism to control PD permeability and regulates PD's size exclusion limit (SEL). GLUCAN SYTHASE-LIKE (GSL), also known as CALLOSE SYNTHASE (CALS), in Arabidopsis comprises a family of 12 members. A new allele of GSL8, essp8, was identified as having seedling-lethal phenotype through forward genetic screening of an EMS mutant population of Arabidopsis showing ectopic expression of seed storage proteins (essp). Using a combination of bulked-segregant analysis, rough-mapping, and next-generation mapping, the mutation responsible for the observed mutant phenotype was detected on an intron splicing site of GSL8. essp8 seedlings exhibit several growth defects, including disruptions of root tissue patterning and morphogenesis, somatic embryo formation and incomplete cytokinesis. Histochemical detection of callose showed lack of callose deposition at the PD in essp8 roots. Cell-to-cell diffusion assay confirmed significant increase in SEL and symplastic macromolecular trafficking in the root of essp8 seedlings. Two non-cell-autonomous factors, SHORT ROOT (SHR) and microRNA165/6 (miR165/6), both required for root radial patterning, were chosen to further investigate the downstream effects of essp8 mutation on PD regulation during root development. Our findings revealed the disruption of SHR and miR165/6 movements in essp8 roots, suggesting that GSL8 is required for regulation of intercellular communication and restriction of symplastic movement.

2.8. Understanding selection on gene coexpression in maize (Zea mays) with RNA sequencing

Shuhua Zhan[1], Jane Tosh[1], Cortland Griswold[1], Lewis Lukens[1]
[1]*University of Guleph, Canada*

Within both natural and domesticated populations, gene expression genetically varies and contributes to phenotypic variation. In addition, the extent of a gene's coexpression with other genes also affects phenotypic variation. We are using maize as a model system to investigate if and how artificial selection has acted upon gene regulatory alleles. Artificial selection has generated genetically distinct maize populations for hybrid breeding, and we have analysed RNA-Seq data from a group of over 100 different maize lines derived from two individuals from two of these populations. We have calculated transcript abundances and established gene coexpression modules across this group. Expression levels of genes within coexpression modules vary across lines, and specific chromosomal loci can explain this genetic diversity. Transcript abundances of many genes are controlled by a single locus, indicating that master regulators contribute to gene expression variation. Within groups of functionally related genes, one parental allele consistently increases expression relative to the other parental allele. This result strongly suggests that positive selection on regulatory alleles has contributed to progress in maize breeding. An understanding of selection on gene expression variation and network-level connectivity variation in both breeding populations and natural populations promises to offer key insights into breeding and evolution.

Session 3. Photosynthesis and Metabolism I

3.1. Cross-site comparison of temperate evergreen and mixed deciduous forests using paired optical (NDVI and PRI) and photosynthesis data

Christopher Wong[1], Altaf Arain[2], Ingo Ensminger[1]
[1]*University of Toronto Mississauga, Canada*, [2]*McMaster University, Canada*

Evergreen conifers in boreal and temperate regions undergo strong seasonal changes in photoperiod and temperatures, which characterizes their photosynthetic activity with high productivity in the growing season and downregulation during the winter season. Monitoring the timing of the transitions in evergreens is difficult since it's a largely invisible process, unlike deciduous trees that have a visible budding and senescence sequence. To contrast a temperate evergreen and mixed deciduous forest at Turkey Point, Ontario, we utilize optical remote sensing techniques to monitor vegetation indices related to photosynthetic activity. The normalized difference vegetation index (NDVI) and the photochemical reflectance index (PRI) were monitored at the leaf- and canopy-scale starting in March 2015. Leaf chlorophyll and carotenoid pigment content was also monitored to corroborate the underlying physiology affecting the vegetation indices. Leaf- and canopy-scale photosynthesis from gas exchange and eddy covariance technique, respectively, was used to pair the optical data with photosynthesis observations. Our cross-site comparison during the spring, summer and early autumn seasons of 2015 show promising correlations between optical and photosynthetic data. Cross-site comparison shows that PRI is more suitable for annual photosynthetic activity in evergreen forests, whereas NDVI is better suited for mixed deciduous forests. These findings have

implications for using remote sensing to model carbon uptake across evergreen or deciduous forest ecosystems.

3.2. Warming delays autumn declines in photosynthetic capacity in a boreal conifer, Norway spruce (Picea abies)
Joseph Stinziano[1], Norman Huner[1], Danielle Way[1]
[1]*The University of Western Ontario, Canada*
Climate change, via warmer springs and autumns, may lengthen the carbon uptake period of boreal tree species, increasing the potential for carbon sequestration in boreal forests, which could help slow climate change. However, if other seasonal cues such as photoperiod dictate when photosynthetic capacity declines, warmer autumn temperatures may have little effect on when carbon uptake capacity decreases in these species. We investigated whether autumn warming would delay photosynthetic decline in Norway spruce (Picea abies (L.) H. Karst.), by growing seedlings under declining weekly photoperiods and weekly temperatures at either ambient temperature or a warming treatment 4 degC above ambient. Photosynthetic capacity was relatively constant in both treatments when weekly temperatures were >8 degC, but declined rapidly at lower temperatures, leading to a delay in the autumn decline in photosynthetic capacity in the warming treatment. The decline in photosynthetic capacity was not related to changes in leaf nitrogen or chlorophyll concentrations, but was correlated with a decrease in the apparent fraction of leaf nitrogen invested in Rubisco, implicating a shift in nitrogen allocation away from the Calvin cycle at low autumn growing temperatures. Our data suggest that as the climate warms, the period of net carbon uptake will be extended in the autumn for boreal forests dominated by Norway spruce, which could increase total carbon uptake in these forests.

3.3. Singlet oxygen plays a central signalling role during soybean-weed competition
Andrew Mckenzie-Gopsill[1], Sasan Amirsadeghi[1], Hugh Earl[1], Elizabeth Lee[1], Lewis Lukens[1], Clarence Swanton[1]
[1]*University of Guelph, Canada*
Far-red (FR) light reflected off neighbouring weeds significantly compromises soybean (Glycine max) fitness through an as of yet unknown mechanism. The antioxidant, photosynthetic and carbon partitioning responses of soybean at the unifoliate stage have been compared under far-red-enriched (FR-E) and far-red-depleted (FR-D) light using a biological weedy system that eliminates direct resource competition. While FR-E light did not impact catalase activity, a decrease in superoxide dismutase (SOD) activity and increases in levels of hydrogen peroxide (H2O2) and oxidised ascorbate suggested excess formation of singlet oxygen (1O2). This was further supported by enhanced sensitivity of unifoliate leaves to cell death induced by a 1O2-generating compound and increase in activity of the 1O2-responsive gene glutathione peroxidase. FR-E light also caused significant decreases in activity of a redox sensitive Calvin cycle enzyme and photosynthesis as well as changes in biomass allocation and carbohydrate levels. We propose that one primary and fundamental impact of FR-E light reflecting from early emerging weeds is increased production of 1O2, which acts to regulate H2O2 level by decreasing SOD activity and signals a cascade of physiological events that directly impacts photosynthesis and carbon partitioning.

3.4. The study of variegation in Nicotiana tabacum through Plastoquinol Terminal Oxidase (PTOX) inhibition
Hillary Peon[1], Allison McDonald[1]
[1]*Wilfrid Laurier University, Canada*
Most plant leaves are green in colour due to the presence of chlorophyll and other carotenoid pigments. The gene IMMUTANS codes for plastoquinol terminal oxidase (PTOX) which is located in the photosynthetic electron transport chain. When IMMUTANS is mutated white sectors occur on the green leaves through a process called variegation. Variegation is thought to occur from a combination of the hyper-reduction of the photosynthetic electron transport chain and inhibition of the carotenoid biosynthesis pathway. Our study explores the hypothesis that in addition to genetic mutations variegated plants can also be generated using a combination of light regime and a direct chemical inhibitor of PTOX. Our preliminary results indicate that several light regimes in combination with a specific inhibitor concentrations cause alterations in the pigmentation of Nicotiana tabacum leaves. Our results will further the understanding of the complexity and integration of the carotenoid biosynthetic and photosynthetic pathways. Since variegated plants are valuable in the Canadian horticultural and landscaping sectors, this project has the potential application of producing variegated plants without performing the time consuming traditional breeding methods.

3.5. Alternative oxidase respiration in the light maintains chloroplast energy homeostasis during drought stress
Keshav Dahal[1], Greg Vanlerberghe[1]
[1]*Department of Biological Sciences and Department of Cell and Systems Biology, University of Toronto Scarborough, 1265 Military Trail, Toronto, ON Canada M1C1A4, Canada*
We recently reported that the non-energy conserving alternative oxidase (AOX) is an important component of mitochondrial respiration in the light (RL) during drought stress and that its activity appears critical in preventing energy imbalances in the chloroplast that can otherwise compromise photosynthetic potential. Energy imbalances in the chloroplast are often reflected by changes in the relative redox state of the plastoquinone (PQ) pool (referred to as excitation pressure [EP]). In the current study, we tested whether a relationship exists between respiration and chloroplast energy homeostasis by examining EP under a wide range of RL. Wild-type tobacco plants and transgenic plants with altered AOX protein amount (knockdown and overexpression) were grown at different irradiances and subject to different drought severities. Collectively, this resulted in plants exhibiting a wide range of RL, which was then compared to EP at growth and saturating irradiances. The results revealed that 1) AOX had little influence on the dark respiration rates under any of the growth conditions 2) AOX had little influence on RL in well-watered plants, regardless of growth irradiance. 3) AOX strongly influenced RL during drought, particularly at higher growth irradiance 4)In well-watered plants, EP was relatively stable over a wide range of RL, irrespective of growth irradiance 5) In drought-stressed plants, there was a negative correlation between RL and EP, with this correlation becoming particularly strong when combined with high growth irradiance. Overall, the results indicate that AOX plays an important role in maintaining chloroplast energy homeostasis specifically during drought.

3.6. Photosynthesis shows little acclimation to warming and elevated CO2 in an Antarctic grass
Vi Bui[1], Carolina Sanhueza[2], Leon Bravo[3], Danielle Way[1]
[1]*University of Western Ontario, Canada*, [2]*Universidad de Concepcion, Chile*, [3]*Universidad de La Frontera, Chile*
Antarctica has been the fastest-warming continent for the past 50 years, and this continuing trend, in combination with elevated atmospheric CO2, can promote growth in Antarctic vegetation. We examined the photosynthetic and growth responses of Deschampsia antarctica (Poaceae), one of the two vascular plants found in Antarctica, to warming and elevated CO2. Plants were grown in one of six climate-controlled greenhouses at a temperature of 11.5oC, 15.5oC, or 19.5oC combined with current (400 ppm) or elevated (750 ppm) atmospheric CO2. After five months, photosynthetic capacities (maximum Rubisco

carboxylation rate, Vcmax, and electron transport rate, Jmax), and net CO2 assimilation rates (Anet) were measured. Biomass, foliar carbon and nitrogen concentrations, and cross-sectional anatomy were also assessed. Overall, D. antarctica showed a lack of acclimation to elevated temperature or CO2 in Vcmax and Jmax: any improvement in photosynthetic capacities was a direct result of increased leaf temperature. Anet showed no acclimation across an 8oC range in growth temperature, and a 30% downregulation at elevated CO2, but plants grown at high temperature and CO2 concentrations had higher Anet due to the direct stimulation of photosynthesis at high leaf temperature and CO2 concentrations. Cross-sectional images revealed changes in the stomatal groove structure that suggest higher moisture stress in the warmest treatment, which was somewhat alleviated under elevated CO2. Elevated CO2 and moderate warming enhanced growth, but not extreme warming, potentially due to water stress at high temperature. Overall, D. antarctica is likely to benefit from a future climate with warmer temperature and elevated CO2.

3.7. Economics of root phenology and turnover in wetland plants along a gradient of growing season length; or What Lies Beneath: Discovering the hidden story of plant roots

Kathleen Cote[1], Peter Ryser[1]
[1]*Laurentian University, Canada*

Wetland plant species in northern Ontario either have root systems which survive the winters ("multi-season roots") or annually renewed root systems ("single-season roots"). My research question is, are species with single-season roots or species with multi-season roots more constrained by shortness of growing season? Lake Superior Provincial Park and Manitoulin Island were chosen as study locations in order to investigate root growth along a range of growing season lengths. My study species with multi-season roots are Carex lasiocarpa, Eleocharis palustris, and Trichophorum caespitosum; those with single-season roots are Rhynchospora fusca, Sagittaria latifolia, and Sparganium emersum/americanum. The length of roots that grew into steel mesh cores as well as the percentage of live and dead roots were measured over one full growing season in five harvests. Above-ground growth was estimated by collecting shoot samples and measuring dry mass. Temperature data loggers were used to monitor soil and air temperature. Preliminary findings indicate that 1) root length is higher in the north (Superior) than in the south (Manitoulin) for both multi- and single-season species; and 2) compared to the south, in the north, there is a larger increase in root length for multi-season roots than for single-season roots. This research will hopefully shed light on the root growth strategy of wetland plants in northern Ontario.

3.8. Proto-Kranz in Neurachne - a cellular and physiological case for photorespiration as a "bridge" to C4 photosynthesis in a grass lineage

Roxana Khoshravesh[1], Tammy Sage[1], Florian Busch[2], Matt Stata[1], Nicole Dakin[3], Montserrat Saladie[3], Joanne Castelli[3], Harmony Clayton[3], Terry MacFarlane[4], Rowan Sage[1], Martha Ludwig[3]
[1]*University of Toronto, Canada*, [2]*ANU College of Medicine, Biology and Environment, Australia*, [3]*University of Western Australia, Australia*, [4]*Western Australian Herbarium, Australia*

The Australian grass tribe Neurachninae is the only known monocot clade with closely-related species using C3, C4 and C2 photosynthesis. To gain insight into the evolution of C4 photosynthesis in grasses, we examined gas exchange, leaf anatomy and cellular location of glycine decarboxylase subunit P (GDC-P) in nine Neurachinae species. The apparent CO2 compensation point of photosynthesis in the absence of day respiration (C*) was typically C3 (near 60 ppm) in Thyridolepis mitchelleana, Neurachne alopecuroidea and N. queenslandica, and typically C4 (near 0 ppm) in N. munroi and Paraneurachne muelleri. C* was intermediate in N. annularis (52 ppm), N. lanigera (43 ppm) and N. minor (17 ppm). In the mestome sheath cells (MSC), there was a progressive increase in chloroplast investment as C* declined while mitochondrial size and numbers rose four-fold as C* declined from 60 to 43 ppm. Mitochondrial numbers were low in the MSC of C4 species. The MSC is the location of Rubisco in C4 species and refixation of photorespired CO2 in C2 species. GDC content in the MSC cells increased as C* declined from N. alopecuroidea to N. lanigera, and then declined in the C4 species. In the mesophyll cells, chloroplasts/cell and GDC content/mitochondria gradually decreased with declining C*. These results demonstrate progressive changes in organelle number and GDC content as C2 metabolism rises in Neurachne. Scavenging of photorespiratory CO2 within a vascular sheath tissue thus appears to be a critical early stage of C4 evolution in both grasses and eudicots.

Session 4. Abiotic and Biotic Interactions II

4.1. Emerging parallels between Ca2+/calmodulin regulation of cation channels in plants and animals.

Thomas A. Defalco[1], Christopher B. Marshall[1], Kim Munro[2], Wolfgang Moeder[1], Mitsuhiko Ikura[1], Wayne A. Snedden[2], Keiko Yoshioka[1]
[1]*University of Toronto, Canada*, [2]*Queen's University, Canada*

Ca2+ signaling is central to many aspects of plant physiology; however, the channels involved in mediating Ca2+ flux in plants remain largely elusive. This is at least partially due to the absence of plant homologs to many of the well-characterized Ca2+ channels present in animals. Instead, plants have expanded families of ligand-gated, non-specific cation channels such as the cyclic nucleotide-gated channels (CNGCs), which are hypothesized to provide Ca2+ channel function in plants. An interesting aspect of CNGC structure-function is their regulation by the ubiquitous multifunctional Ca2+ sensor calmodulin (CaM). While numerous, diverse cation channels are regulated by CaM in animals, thus far CNGCs appear to be the only channels to interact with CaM in plants. Furthermore, the specific mechanism by which CaM regulates mammalian CNGCs is well characterized, but was previously unresolved for any plant isoforms. We have recently demonstrated a novel mechanism by which CaM provides Ca2+-dependent allosteric inhibition of AtCNGC12, a positive regulator of immune responses in Arabidopsis. Herein we expand on these findings, including emerging evidence that CaM not only provides negative feedback regulation, but also positively regulates AtCNGC12 function in planta via multiple CaM-binding sites. We will also present evidence that CaM may function as a constitutive subunit of multimeric CNGC complexes, suggesting unexpected regulatory parallels between plant CNGCs and numerous animal cation channels. Finally, we will discuss the diversity and conservation of CaM interactions across the Arabidopsis CNGC family, which has implications for how isoforms may assemble as functional channels in vivo.

4.2. Comparative Proteomics of Arabidopsis Phloem During the Induction of Systemic Acquired Resistance

Philip Carella[1], Juliane Merl-Pham[2], Daniel Wilson[1], Sanjukta Dey[2], A. Corina Vlot[2], Robin K. Cameron[1]
[1]*McMaster University, Canada*, [2]*Helmholtz Zentrum Muenchen, Germany*

Systemic acquired resistance (SAR) is a plant defense response that provides long-lasting, broad-spectrum pathogen resistance to uninfected systemic leaves following an initial localized infection. In Arabidopsis thaliana, local infection with virulent or avirulent

strains of Pseudomonas syringae pv. tomato (Pst) leads to the generation of mobile SAR signals that travel from locally infected to distant leaves to initiate SAR. In this study, we took a proteomics approach to identify proteins that accumulate in phloem exudates during the induction phase of SAR. Label-free quantitative LC-MSMS (liquid chromatography, tandem mass spectrometry) was employed to identify proteins in phloem exudates collected from lmock-inoculated or SAR-induced leaves. By comparing mock- and SAR-induced exudate proteomes, we identified 16 proteins that accumulate in the phloem during SAR, as well as 42 proteins that decrease in abundance. The functional relevance of these proteins to SAR was explored by performing SAR assays on T-DNA mutants, which revealed a role for thioredoxins, a polyketide cyclase, and a light-signalling protein. The Arabidopsis SAR phloem proteome provides new insights into the dynamic nature of the phloem during stress, and our interrogation of this data set identified novel components of long-distance SAR signalling.

4.3. Identification of Arabidopsis innate immunity receptors involved in the recognition of novel phytopathogen immune elicitors

Adam Mott[1], Shalabh Thakur[1], Pauline W. Wang[1], Darrell Desveaux[1], David S. Guttman[1]
[1]*University of Toronto, Canada*

The initial steps of the plant immune response to infection involve the binding of conserved microbial features called microbe-associated molecular patterns (MAMPs) to cognate plant receptors. The importance of this recognition to plant survival is well known, yet it remains difficult to identify novel MAMPs and their host receptors. Without identifying these molecules and determining how they interact, we are unable to understand the roles they play in defining the host-pathogen relationship. Using a comparative genomics approach, we have identified new peptide MAMPs from the phytopathogenic bacterium Pseudomonas syringae and utilized a reverse genetic screen to identify Arabidopsis thaliana immune receptors required for their recognition. Using a novel high-throughput assay we have tested the activity of seven bacterial MAMPs on 187 immune receptor T-DNA insertion knockouts. This primary screen identified several lines that were unable to respond to a single MAMP, suggesting those genes may play a role in MAMP binding. We focused on the interaction between the xup25 MAMP from a bacterial xanthine/uracil permease and the Arabidopsis receptor xanthine/uracil permease sensing 1 (XPS1). We showed that xup25 treatment results in a classic immune response, including pathogenesis-related gene induction, callose deposition, and resistance to virulent bacteria, all in an XPS1-dependent manner, indicating that XPS1 is specifically required for xup25 recognition. The study demonstrates an efficient method to identify immune elicitors and the plant receptors responsible for their perception. Further exploration of these molecules will increase our understanding of plant-pathogen interactions and the basis for host specificity.

4.4. Elucidating the genetic requirements underlying the Pseudomonas syringae effector HopF2PtoT1 immune response in Arabidopsis

Timothy Lo[1], Noushin Koulena[1], Derek Seto[1], Wenwan Lu[1], Alexandra Menna[1], Pauline Wang[1], David Guttman[1], Darrell Desveaux[1]
[1]*University of Toronto, Canada*

Having caused outbreaks on kiwifruit, horse chestnut and the common bean, the Gram-negative bacterial pathogen Pseudomonas syringae is a major threat to the world's food sources. By utilizing a needle-like secretion system, P. syringae is able to inject virulence proteins termed effectors into plant host cells, where they suppress the plant's immune system and promote virulence. P. syringae is able to cause disease on numerous plant species due to the diversity of effector proteins at its disposal. Over 30 different families of effector proteins exist including the HopF family of effectors. Within the HopF family, only two family members, HopF1Pph1449B and HopF2PtoDC3000, have been well-characterized and been shown to be important in causing disease in both common bean and the model plant organism Arabidopsis thaliana. However, genome sequencing has now identified over 200 members in the HopF family that exist over a broad range of P. syringae strains. To elucidate the functional diversity within the HopF family, functional screens were conducted on Arabidopsis to identify novel functional phenotypes. One previously uncharacterized member is HopF2PtoT1, which we have identified to trigger an immune response in Arabidopsis. This study aims to identify the genetic requirements of this novel immune response and the role of the HopF2PtoT1 effector in host specificity. Understanding the molecular mechanisms responsible for restricting pathogen virulence will enhance the protection of agricultural crops to increase food security.

4.5. Involvement of Arabidopsis triphosphate tunnel metalloenzymes in distinct biological processes -senescence and pathogen defense

Purva Karia[1], Huoi Ung[1], Wolfgang Moeder[1], Kazuo Ebine[2], Takashi Ueda[2], Keiko Yoshioka[1]
[1]*University of Toronto, Canada*, [2]*The University of Tokyo, Japan*

The triphosphate tunnel metalloenzyme (TTM) superfamily comprises a group of enzymes that hydrolyze organophosphate substrates. They exist in all domains of life, yet the biological role of most family members is unclear. Arabidopsis thaliana encodes three TTM genes and unlike other members of this family, two of the Arabidopsis TTMs, AtTTM1 and 2, displayed pyrophosphatase activity, making them the only TTMs characterized so far to act on a diphosphate substrate. However, despite their high sequence identity, they were shown to have diverse biological functions. AtTTM2 plays a role in pathogen resistance whereas AtTTM1 is involved in leaf senescence. Knockout mutants of AtTTM1 exhibit delayed dark-induced and natural senescence, indicating the involvement of AtTTM1 in the core senescence pathway. The ttm1 ttm2 double mutant displayed the same degree of delayed senescence and enhanced pathogen resistance as their respective single mutants, further confirming their roles in distinct biological processes. To gain insight into the molecular mechanism underlying their biological functions, the sub cellular localization of AtTTM proteins was analyzed using confocal microscopy. Both proteins displayed a punctate signal and a detailed analysis suggests their localization at the mitochondrial outer membrane. These data suggest a novel connection between immunity-related programmed cell death and senescence through mitochondrial membrane-localized phosphatase activity.

4.6. Identification of Novel Resistance Protein Specificities in the Arabidopsis thaliana/Pseudomonas syringae pathosystem

Nina Kirischian[1], Andre Santos-Severino[2], Darrell Desveaux[3], David S. Guttman[3]
[1]*Department of Cell and Systems Biology, University of Toronto, Canada*, [2]*Centre for the Analysis of Genome Evolution and Function, University of Toronto,, Canada*, [3]*Department of Cell and Systems Biology and Centre for the Analysis of Genome Evolution and Function, University of Toronto, Canada*

Plants continue to survive in the face of perpetual pathogen attack through the detection of pathogens and the mounting defense responses. Plants have evolved resistance (R) proteins that elicit

an immune response upon recognition of secreted pathogen effectors. Plant-pathogen interactions have driven the functional diversification of R proteins in plant genomes, yet less than a dozen R proteins have been characterized. A key challenge in plant immunity research is to identify novel R protein - effector specificities. We screened multiple, genetically diverse isolates of the bacterial pathogen Pseudomonas syringae against the model plant Arabidopsis thaliana to identify strains that elicited a novel immune response. To avoid identifying previously known R protein - effector interactions, we specifically selected strains that did not carry pathogen effectors known to elicit immunity in Arabidopsis. The screen identified P. syringae pv. syringae FF5 (PsyFF5), which is pathogenic on ornamental pear, as a strong immune elicitor. To identify the genetic requirements underlying this immune response, a forward genetic screen was conducted and next-generation mapping will be utilized to identify the genes of interest. By coupling natural variation and next-generation mapping, novel R protein-effector specificities will be uncovered to further understand plant-pathogen interactions.

Session 5. Cell and Developmental Biology II

5.1 The Arabidopsis SWI2/SNF2 Chromatin Remodeler BRAHMA Regulates Polycomb Function during Vegetative Development and Directly Activates the Flowering Repressor Gene SVP

Chenlong Li[1], Chen Chen[2], Lei Gao[3], Songguang Yang[4], Vi Nguyen[2], Xuejiang Shi[5], Katherine Siminovitch[5], Susanne Kohalmi[6], Shangzhi Huang[7], Keqiang Wu[8], Xuemei Chen[3], Yuhai Cui[2]
[1]*Agriculture and Agri-Food Canada,London/Western University, Canada*, [2]*AAFC-London, Canada*, [3]*University of California, Riverside, United States of America*, [4]*South ChinaBotanical Garden, Chinese Academy of Sciences, China*, [5]*Mount Sinai Hospital, Canada*, [6]*Western University, Canada*, [7]*Sun Yat-sen University, China*, [8]*National Taiwan University, Taiwan*
The chromatin remodeler BRAHMA (BRM) is a Trithorax Group (TrxG) protein that antagonizes the functions of Polycomb Group (PcG) proteins in fly and mammals. Recent studies also implicate such a role for Arabidopsis (Arabidopsis thaliana) BRM but the molecular mechanisms underlying the antagonism are unclear. To understand the interplay between BRM and PcG during plant development, we performed a genome-wide analysis of trimethylated histone H3 lysine 27 (H3K27me3) in brm mutant seedlings by chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq). Increased H3K27me3 deposition at several hundred genes was observed in brm mutants and this increase was partially supressed by removal of the H3K27 methyltransferase CURLY LEAF (CLF) or SWINGER (SWN). ChIP experiments demonstrated that BRM directly binds to a subset of the genes and prevents the inappropriate association and/or activity of PcG proteins at these loci. Together, these results indicate a crucial role of BRM in restricting the inappropriate activity of PcG during plant development. The key flowering repressor gene SHORT VEGETATIVE PHASE (SVP) is such a BRM target. In brm mutants, elevated PcG occupancy at SVP accompanies a dramatic increase in H3K27me3 levels at this locus and a concomitant reduction of SVP expression. Further, our gain- and loss-of-function genetic evidence establishes that BRM controls flowering time by directly activating SVP expression. This work reveals a genome-wide functional interplay between BRM and PcG and provides new insights into the impacts of these proteins in plant growth and development.

5.2. Investigating the roles of stigma-expressed RLCKs in compatible pollen signalling

Jennifer Doucet[1], Daphne Goring[1]
[1]*University of Toronto, Canada*
Species in the Brassicaceae have dry stigmas, meaning pollen must first be recognized by the stigma for successful germination. Upon compatible pollen recognition, water and enzymes are transported through vesicle trafficking from the stigmatic papillae to the pollen, allowing pollen hydration, germination and pollen tube penetration for subsequent pollen tube growth and fertilization. Despite known physiological responses following compatible pollen recognition, the signalling events that trigger these responses remain unknown. We used a reverse genetics approach to identify candidate genes involved in compatible pollen acceptance signalling by searching for stigma-expressed genes in publicly-available microarray datasets. We identified two stigma-expressed Receptor-Like Cytoplasmic Kinases (RLCKs) and confirmed their expression profiles using RT-PCR. These RLCKs are conserved within the Brassicaceae, supporting their possible involvement in a family-wide compatible pollen recognition pathway. These two RLCKs were knocked-down in A. thaliana using RNA silencing though an artificial microRNA construct; CRISPR-Cas9 knockouts for both genes have also been generated. These transgenic lines are currently being assessed for altered stigmatic papillar responses to compatible pollen, and results show reduced pollen adhesion, pollen hydration, pollen tube penetration, and seed set. This project represents an exciting first step towards understanding compatible pollen acceptance signalling.

5.3. Small molecule antagonists of germination of the parasitic plant Striga hermonthica

Duncan Holbrook-Smith[1], Shigeo Toh[1], Peter McCourt[1]
[1]*University of Toronto, Canada*
Antagonists of the plant hormone strigolactone would be useful tools to disrupt the lifecycle of the agronomically devastating root parasite Striga. A chemical screen was developed to suppress strigolactone signalling in Arabidopsis and one compound, Soporidine, was shown to specifically inhibit a strigolactone receptor in both Arabidopsis and Striga. Consistent with this, Soporidine was also able to inhibit strigolactone-dependent germination in Striga hermonthica.

5.4. Protein-protein interaction exploration based on proteome-wide tertiary structure prediction and further in vitro validation

Michael Dong[1], Nicholas Provart[1]
[1]*UofT CSB, Canada*
Proteins rarely function alone, most of the times they interact with the other proteins to fulfill their functions. Protein-protein interactions are crucial for biological activities and the exploration and analysis of protein-protein interactions (PPI) are essential for our biological understanding of cellular function. In this study, a list of candidate interacting protein pairs was generated by a docking software based on their shape complementarity feature from a predicted Arabidopsis thaliana structure-ome containing around 30,000 predicted structure models. The predicted PPIs were further validated in Y2H. My project aims to (1) provide evidence that predicted protein structures can be used in protein structural and functional studies, and (2) provide an alternative method in PPIs exploration.

5.5. The nitrogen responsive transcriptome in potato (Solanum tuberosum L.) reveals significant gene regulatory motifs

Jose Hector Galvez Lopez[1], Helen H. Tai[2], Bernie Zebarth[2], Martin Lague[2], Martina Stromvik[1]
[1]*Department of Plant Science, McGill University, Canada*, [2]*Agriculture and Agri-Food Canada, Potato Research Centre, Canada*

Nitrogen (N) availability is an important abiotic factor for the growth of potato (S. tuberosum) because of its potential effects on yield. Supplementation of N through fertilization is required but excess N in the soil leaches to water systems and negatively impacts the environment; therefore, studies on N use by the plant are key. Three commercial potato cultivars (Shepody, Russet-Burbank and Atlantic) were grown under two different rates of applied N-fertilizer (0 kg N ha-1 and 180 kg N ha-1) to obtain more information on the underlying gene regulation mechanisms associated with N. Total mRNA samples from foliar tissue were taken at two different time-points during the growth season and sequenced. The results for each cultivar and time-point were analyzed separately to find differentially expressed genes. In total, thirty genes were found to be over-expressed and nine genes were found to be under-expressed in plants from all potato cultivars when they were grown with added N-fertilizer. The 1000 bp upstream flanking regions of the differentially expressed genes were analyzed to find overrepresented motifs using three motif discovery algorithms (Seeder, Weeder and MEME). Nine different motifs were found, indicating potential gene regulatory mechanisms for potato under N-deficiency.

5.6. Autophagy's Link to Self-Incompatibility in Arabidopsis species

Daniel Johnson[1], Daphne Goring[1]
[1]*University of Toronto, Canada*

Self-incompatibility (SI) in flowering plants is a reproductive barrier resulting in self-pollen rejection to prevent inbreeding depression. In the Brassicaceae, SI is controlled by the pistil through the failure to deliver factors required for pollen hydration and germination. SI is initiated by the allelic binding of the pollen S-locus Cysteine Rich/S-locus Protein 11 (SCR/SP11) ligand to the pistil S Receptor Kinase (SRK). Downstream components include the ARC1 E3 ubiquitin ligase, which is proposed to ubiquitinate and inhibit Exo70A1, following SRK activation. With compatible pollinations, Exo70A1, as part of the exocyst, is responsible for directing secretory vesicles to the stigmatic papillar plasma membrane under the pollen grain. Inhibition of Exo70A1 results in blocked resource delivery to pollen, causing pollen rejection. Recent work from our research group has implicated autophagy in the sequestration of secretory vesicles during the SI response. Thus, we hypothesize that a breakdown in autophagy will compromise SI pollen rejection. A. lyrata SCR, SRK, and ARC1 transgenes have been transformed into Arabidopsis thaliana Col-0 and autophagy mutants to reconstitute SI, and the phenotypes of these transgenic lines are currently being assessed. Autophagy and vesicle markers are also being used to visualize cellular responses following the addition of SI pollen. Overall, these studies will aid in understanding the role of autophagy in the pistil to reject SI pollen.

5.7. Investigating the functional role of Shikimate kinase-like 1 in Solanum lycopersicum through virus-induced gene silencing

Michael Kanaris[1], Dinesh Christendat[1]
[1]*University of Toronto, Canada*

The shikimate pathway represents a crucial pathway in plants that ultimately leads to the synthesis of a variety of aromatic compounds involved in plant growth and development, including lignins, hormones, pigments, and aromatic amino acids. Shikimate kinase (SK) represents one of the crucial and highly regulated enzymes involved in the pathway whereby shikimate is converted to shikimate-3-phosphate in the fifth reaction of the pathway. Exploring the evolutionary diversification of SK in plants as a result of gene duplication events has led to the identification of two clusters of SK homologs, termed shikimate kinase-like 1 and 2 (SKL1 and SKL2). Both SKL1 and SKL2 lack key domains as identified in SK and have been suggested to have undergone neofunctionalization that has allowed many plants to develop new and more complex metabolic functions. The role of SKL1 in Arabidopsis thaliana has been shown to have biological significance in the development and biosynthesis of chloroplast, whereby genomic mutations of SKL1 have led to drastic photobleached phenotypes. Present work is focused on determining the functional role of SKL1 in other plant species including Solanum lycopersicum through virus-induced gene silencing by use of tobacco rattle virus, leading to the temporary knock-down of SKL1 and determination of biological impacts.

Session 6. Photosynthesis and Metabolism II

6.1. How to Make a C4 Plant

Rowan Sage[1], Tammy Sage[2], Matt Stata[2], Sarah Covshoff[3], Steve Kelly[3], Julian Hibberd[3]
[1]*Univerity of Toronto, Canada*, [2]*University of Toronto, Canada*, [3]*Cambridge University, United Kingdom of Great Britain and Northern Ireland*

C4 photosynthesis is a CO2 concentrating mechanism that enhances the efficiency of carbon gain in terrestrial plants and supports productivity in the highest yielding crops such as maize. Most crops, however, are the less efficient C3 plants including the leading grains rice and wheat, all pulses, and Canadian crops such as rapeseed. To improve agricultural efficiency, there is renewed interest in engineering the C4 pathway into leading crops such as rice and wheat by exploiting recent advances in molecular technology, and using the natural evolution of C4 photosynthesis as a guide. Our group has helped identify many of the estimated 70 distinct evolutionary origins of C4 photosynthesis which can be studied to understand how C4 photosynthesis is naturally assembled by evolution. Comparative transcriptomics of closely related C3 and C4 species from over a dozen of these lineages grown at the University of Toronto has identified over 113 genes that increase in expression in all C4 lineages examined, and 36 whose expression declines. Most of these genes are of a metabolic nature, but a subset includes transcription factors of unknown function that may play critical roles in C4 leaf development. Current work is emphasizing the dissection of individual traits, for example, the genetic control of chloroplast division patterns that differ between C3 and C4 plants. Through this work, novel gene candidates that we identify will be passed to the molecular teams in the C4 Rice Engineering consortium (C4Rice.irri.org) for evaluation and potential engineering into a C4 Rice proto-type.

6.2. Forward Screening for CBI Resistance in the Monocot Crop Species, Triticum durum (Durum Wheat)

Elysabeth Reavell-Roy[1], Dario Bonetta[1], Julian Northey[2], Isaac Shim[1]
[1]*UOIT, Canada*, [2]*Frontier Agri-Science, Canada*

Cellulose is the primary structural component of plant cell walls, and is composed of b-1,4-glucan chains. The cellulose synthase complex is composed of cellulose synthase subunits (CESAs). Although extensively studied, cellulose biosynthesis is not fully understood. One way to elucidate the role of cellulose synthases is through their interaction with inhibitor compounds. Flupoxam is

a known cellulose biosynthesis inhibitor (CBI). The Bonetta laboratory at the University of Ontario Institute of Technology (UOIT) found flupoxam resistant Arabidopsis thaliana mutants using forward genetic screening. Flupoxam resistance was found in plants with single nucleotide polymorphisms (SNPs) in CESA1 and CESA3. Flupoxam resistant mutants demonstrated decreased cellulose crystallinity and increased accessibility of sugars upon enzyme digestion. In a larger crop plant, these characteristics would allow for cellulose to be more easily digested for production of cellulosic ethanol. The higher accessibility of sugars would allow for more easily digestible feed for ruminants. A forward genetic screen was carried out in Triticum durum (Durum wheat). Seeds were mutagenized with Ethyl methanesulfonate (EMS), producing point mutations randomly in the genome. From the M2 population, 200,000 seeds were screened with 5 uM flupoxam: 177 seeds showed resistance. M3 seeds were retested; strongly resistant mutants will be studied to determine the resistance genotype. Wheat mutants can be compared to Arabidopsis mutants to determine if the CESA-inhibitor relationship is conserved between species. If wheat mutants also demonstrate higher cellulose accessibility and reduced crystallinity, they could contribute to energy crop development or superior ruminant feed

6.3. Proanthocyanidin metabolism is critical for seed coat darkening in cranberry bean

Jose A. Freixas-Coutin[1], Seth Munholland[2], Anjali Silva[3], Sanjeena Subedi[3], William L. Crosby[2], K. Peter Pauls[1], Gale G. Bozzo[1]
[1]*Department of Plant Agriculture, University of Guelph, Canada*, [2]*Department of Biological Sciences, University of Windsor, Canada*, [3]*Department of Mathematics and Statistics, University of Guelph, Canada*

Seed coat darkening negatively impacts the aesthetic value and marketability of edible dry bean (Phaseolus vulgaris) including that of cranberry beans. Cranberry beans susceptible to postharvest darkening contain higher levels of proanthocyanidins than their non-darkening counterparts. Proanthocyanidin availability is critical for seed coat darkening in other legumes and the model species Arabidopsis thaliana. Anthocyanidin reductase (ANR) catalyzes a key step in proanthocyanidin biosynthesis by converting anthocyanidins (e.g., cyanidin) into flavan-3-ols. We tested the hypothesis that proanthocyanidin metabolism gene expression, including ANR, is involved in cranberry bean seed coat darkening. Two recombinant inbred lines (RILs) from an 'Etna' (darkening) by 'Wit-Rood' (non-darkening) cranberry bean cross were greenhouse cultivated and RNA prepared from seed coats harvested at early, intermediate and mature stages of bean development for RNAseq analysis. Differentially expressed genes between darkening and non-darkening RILs were represented by 14 separate clusters; 1 cluster contained 27 proanthocyanidin metabolism genes. A principal component analysis revealed anthocyanidin reductase 1 (PvANR1) transcript levels were highly associated with the darkening RIL. The recombinant PvANR1 was expressed in Escherichia coli and enriched by Ni2+-affinity chromatography. Following removal of the hexahistidine tag, biochemical analysis revealed that PvANR1 catalyzed the conversion of cyanidin into catechin and epicatechin. Kinetic studies indicated the substrate cyanidin serves as an inhibitor of this reductase activity. Moreover, sigmoidal kinetics were apparent when this activity was tested at varying levels of the cofactor NADPH. The results suggest that modulating the expression of PvANR1 may be a rational approach to preserve the quality of cranberry beans.

6.4. Photosynthetic capacity of tropical montane tree species in relation to leaf nutrients, successional strategy and growth temperature

Mirindi Eric Dusenge[1], Goran Wallin[2], Johanna Gardesten[2], Felix Niyonzima[3], Lisa Adolfsson[2], Donat Nsabimana[3], Johan Uddling[2]
[1]*University of Western Ontario, Canada*, [2]*University of Gothenburg, Sweden*, [3]*University of Rwanda, Rwanda*

Photosynthetic capacity of tree leaves is typically positively related to nutrient content and little affected by changes in growth temperature. These relationships are, however, often poorly supported for tropical trees, for which interspecific differences may be more controlled by within-leaf nutrient allocation than by absolute leaf nutrient content, and little is known regarding photosynthetic acclimation to temperature. To explore the influence of leaf nutrient status, successional strategy and growth temperature on the photosynthetic capacity of tropical trees, we collected data on photosynthetic, chemical and morphological leaf traits of ten tree species in Rwanda. Seven species were studied in a forest plantation at mid-altitude, whereas six species were studied in a cooler montane rainforest at higher altitude. Three species were common to both sites, and, in the montane rainforest, three pioneer species and three climax species were investigated. Across species, interspecific variation in photosynthetic capacity was not related to leaf nutrient content. Instead, this variation was related to differences in within-leaf nitrogen allocation, with a tradeoff between investments into compounds related to photosynthetic capacity (higher in pioneer species) versus light-harvesting compounds (higher in climax species). Photosynthetic capacity was significantly lower at the warmer site. We conclude that (1) within-leaf nutrient allocation is more important than leaf nutrient content per se in controlling interspecific variation in photosynthetic capacity among tree species in tropical Rwanda, and that (2) tropical montane rainforest species exhibit decreased photosynthetic capacity when grown in a warmer environment.

6.5. Genetic Control of Alternate Chloroplast Patterns in C4 Bundle Sheath and Mesophyll Cells

Matt Stata[1], Sarah Covshoff[2], Julian Hibberd[2], Gane Ka-Shu Wong[3], Rowan Sage[4], Tammy Sage[1]
[1]*University of Toronto, EEB, Canada*, [2]*Cambridge University, United Kingdom of Great Britain and Northern Ireland*, [3]*University of Alberta, Canada*, [4]*Univerity of Toronto, EEB, Canada*

C4 photosynthesis is a mechanism which concentrates CO2 into the bundle sheath (BS) around Rubisco, thereby raising photosynthetic efficiency. To effect CO2 concentration, major structural modifications are required, notably an enlarged BS and a reduction in mesophyll (M) cell numbers relative to C3 ancestors. BS chloroplasts also increase in size and number, however chloroplast changes in M cells have not been described. In this study, we observed that M chloroplast numbers were reduced in C4 species relative to close C3 relatives in a dozen independent lineages of C4 evolution, while M chloroplast size often increased. This is likely due to the need for diffusive access of CO2 to the M cytosol, the site of primary carbon fixation in C4 species. To identify potential genetic control of these chloroplast patterns, we investigated transcript levels for known plastid division and biogenesis genes in juvenile leaves of the model genus Flaveria. Four of these genes exhibited expression levels which were correlated both with C4 cycle strength and observed BS or M chloroplast patterns. Expression of these genes was not found to be correlated with photosynthetic pathway in other C4 lineages. It is therefore likely that different elements of the plastid division pathway have been modified in different C4 lineages to achieve a common phenotype. Understanding how this has naturally occurred will inform efforts to engineer the C4 pathway into important C3 crops.

6.6. Post-Translational Regulation of Starch Synthase 2 in Arabidopsis thaliana

Jenelle Patterson[1], Ian Tetlow[1], Michael Emes[1]
[1]*University of Guelph, Canada*

Starch is the most important caloric energy source to humans, and is produced in plants in both storage tissues, such as tubers and seed endosperm, and in leaves to transiently store excess photosynthates, which are degraded at night and support respiration. Starch is composed of two glucan polymers: branched amylopectin and linear amylose. The relative quantity and structure of amylopectin is the main determinant of granule crystallinity and biochemical properties. Amylopectin is produced through the coordinated activities of multiple starch biosynthetic enzymes. One key enzyme, starch synthase 2 (SS2), forms the core of a heteromeric protein complex involved in amylopectin formation; mutations affecting its glucan-binding domain not only reduce SS2 catalytic activity, but also prevent the trafficking of all associated enzymes into the granule, causing an altered granule phenotype. Previous studies also indicate that SS2 is phosphorylated when present within the starch granule, but the site and effects of this modification on catalytic activity and protein complex assembly remain unknown. The objectives of this project are to identify and characterize post-translational modifications of SS2 in Arabidopsis thaliana in vitro, using recombinant proteins, and in vivo, by complementing SS2-null mutants using Agrobacterium-mediated transformation. Given that SS2 is necessary for amylopectin synthesis and multi-protein complex trafficking, it is important to understand the mechanisms underpinning protein complex formation, and the role protein phosphorylation plays in mediating this process.

6.7. A systematic characterization of the isoflavonoid-specific prenyltransferase gene family in Soybean

Arjun Sukumaran[1], Sangeeta Dhaubhadel[2]
[1]*Western University, Canada*, [2]*Agriculture and Agri-Food Canada, Canada*

Soybeans are one of the most predominantly grown legumes worldwide because of the versatility of its uses. However, one deterrent to maximizing its yield is the pathogen, Phytophthora sojae, which causes stem and root rot disease. Many strategies have been implemented throughout the years to combat this pathogen, such as the use of pesticides and certain agricultural practices. However, these have been largely ineffective in completely preventing P. sojae infection. An alternative strategy would be to improve the innate resistance of soybeans by promoting increased glyceollin production. Glyceollins are soybean-specific antimicrobial agents which are derived from the isoflavonoid branch of the general phenylpropanoid pathway. Soybeans produce 3 forms of glyceollin: glyceollin I, glyceollin II, and glyceollin III. The forms are a result of differential prenylation of either the C2 or C4 carbon of glycinol. Currently, only the C4-prenyltransferase (PT) referred to as glycinol 4-dimethylallyltransferase (G4DT), which results in glyceollin I formation, has been identified. The prenyltransferase responsible for the branching towards glyceollin II and glyceollin III, G2DT, has yet to be identified. Eight putative GmPTs have been identified in this project, and will be characterized by their subcellular localization, their expression under pathogen stress, and then through verification of their enzymatic activity. Increased knowledge of components of the isoflavonoid pathway will allow for more precise manipulation of glyceollin production, thus in effect, increasing soybean resistance to P. sojae.

Session 7. Abiotic and Biotic Interactions III

7.1. Identification of gene sets with roles in foliar browsing and heartwood rot defenses in western redcedar by comparative RNA-seq analysis

Jim Mattsson[1]
[1]*Simon Fraser University, Canada*

Western redcedar (Thuja plicata) trees can live for more than 1000 years, indicating exceptionally strong biotic resistance. An example of that is the naturally rot resistant heartwood, popular for various outdoor applications. Nevertheless, reforestation is inefficient and expensive due to extensive deer and elk browsing, and second-growth trees are frequently afflicted by extensive heart rot, jeopardizing the reputation of durable cedar lumber. We used RNA-seq to identify candidate genes and chemical biosynthesis pathways contributing to biotic resistance in both foliage and heartwood-forming tissues. First, a comparison of transcriptomes from wildtype foliage and a natural variant lacking resin glands yielded +500 genes, including complete biosynthetic pathways of foliar terpenoids, compounds that are linked to browsing resistance. Functional assays identified sabinene synthase and hydroxylases, key enzymes in the biosynthesis of the deer repellant [?] -thujone. Secondly, a comparison of transcriptomes from the sapwood to heartwood transition zone with other wood fractions provided candidates for complete biosynthetic pathways for tropolones and lignans, compounds that are linked to heartwood rot resistance, and surprisingly also pathways for flavonoid biosynthesis, compounds that hitherto have not been linked to rot resistance in this species. Finally, the bark-forming region also expressed genes in lignan, terpenoid and flavonoid biosynthesis, suggesting that the bark and heartwood have similar biotic defenses, but also expression indicating production of bark-specific toxins such as phenazines. Identified genes are now being explored for use in multi-trait Genomic Selection in this economically important species.

7.2. Towards guard cell-specific transcriptomics during drought - development of a guard cell-INTACT in Arabidopsis thaliana

Anna van Weringh[1], Nicholas Provart[1]
[1]*University of Toronto, Canada*

The INTACT (Isolation of Nuclei Tagged in Cell-Types) system expresses a GFP tagged, nuclear membrane protein that is biotinylated for affinity-mediated nuclear purification. When expressed under a cell-type specific promoter, nuclei from single cell types along with the enclosed RNA may be isolated. We have recently built a guard cell (GC)- specific INTACT system in Arabidopsis, which will be used to profile gene expression changes in GCs during drought stress. Confocal microscopy demonstrated GC-specific expression in the leaves of seedlings and of mature, 5 week old plants. GCs are signaled to close stomatal pores during drought, playing a key role in the reduction of water loss and survival under water limiting conditions. Hormones within the plant relay the severity of the drought, yet the signaling thresholds that decide the degree of stomatal closure are defined by the genes expressed at a given time within GCs. The use of cell-type specific analyses is necessary to detect such changes within GCs, as they represent only a small fraction of leaves. Future work will profile GC-transcriptomes in Arabidopsis during a progressive drought experiment to identify genes with altered expression during mild, moderate and severe drought stress, as well as following rehydration.

7.3. The relationship between genome, epigenome, and physiology in poplar in response to drought

Katharina Brautigam[1], Yunchen Gong[2], Stefan Schreiber[3], Pauline Wang[2], Barb Thomas[3], Uwe Hacke[3], Malcolm Campbell[4]
[1]*University of Toronto Mississauga, Canada*, [2]*University of Toronto, Canada*, [3]*University of Alberta, Canada*, [4]*University of Guelph, Canada*

Long-lived organisms such as forest trees are likely to encounter environmental change throughout their life cycle. Efficient responses to fluctuating environmental conditions are of paramount importance for tree growth and survival, and in light of recent and predicted climate change. Here, responses to drought, one of the key factors determining tree growth and survival in Populus, were comprehensively investigated by combining physiological measures with the analyses of underlying molecular patterns. Genome responses through remodeling of the transcriptome were analyzed by next generation sequencing (RNA-seq), and plant growth and performance were studied by assessing biomass parameters and water relations. Economically important poplar genotypes were propagated vegetatively, grown in a common environment, and studied under non-stress and stress conditions. Biomass partitioning between above and below ground organs and physiological responses uncovered specific strategies that were employed by different genotypes under stress conditions. These morphological and physiological characteristics were underpinned by distinct genotype-, tissue-, and stress-specific transcriptome patterns. Epigenetic phenomena, mediated by e.g. DNA methylation or histone modifications can mediate lasting changes in the state of gene expression and thus influence phenotypic outcomes in the absence of genetic diversity. Genome-wide analyses of DNA methylation patterns (bisuflite sequencing) of our vegetatively propagated plants uncovered widespread epigenetic variability that paralleled differences in transcriptome responses. The work indicates a possible mechanism for adding an additional layer of plasticity in long-lived organisms and contributes to our understanding of plant-environment interactions, with applications related to genotype selection, plasticity of responses, and impacts on future clone performance in plantations.

7.4. Regulation of Quinate Metabolism in Listeria monocytogenes

Stephanie Prezioso[1], Dinesh Christendat[1]
[1]*University of Toronto, Canada*

The shikimate pathway is an essential metabolic pathway in bacteria, plants, and fungi, which leads to the synthesis of the three aromatic amino acids among other important aromatic compounds. Quinate is an abundant plant derived compound that can be utilized by some bacteria. Quinate metabolism converges at a branch point of the shikimate pathway for either the biosynthesis of aromatic compounds or diversion through a catabolic pathway to generate energy. The utilization of quinate by microbes is highly regulated, however this process is still highly speculative. The genome of Listeria monocytogenes contains two operons containing quinate utilization enzymes (shikimate/quinate dehydrogenase and dehydroquinate dehydratase) in various combinations. We identified a LysR type transcriptional regulator (LTTR) that is divergently transcribed from each of these quinate operons, named QuiR1 and QuiR2. We are studying the transcriptional control mechanisms by QuiR1 and QuiR2 as well as enzyme kinetic properties of the two quinate dehydrogenases, to understand their roles in regulation of quinate biosynthesis and utilization in L. monocytogenes. This would establish the ability of bacteria to synthesize what was previously thought to be solely a plant derived compound.

7.5. Characterization of a Putative Gene Operon Implicated in Pseudomonas Putida Rhizosphere Interactions

Sophie Qin[1], Stephanie Prezioso[1], Dinesh Christendat[1]
[1]*University of Toronto, Canada*

Strains of the gram-negative soil bacteria Pseudomonas putida is well known for their efficiency in rhizosphere colonization and protective and growth promoting effects on agricultural and research plant species. In Arabidopsis thaliana P. putida has been shown to elicit changes in gene expression upon colonization in addition to altering root exudate composition and inducing systemic resistance. Similar findings have also been shown in agricultural plants like bean and maize, where the specific chemotactic recruitment of P. putida was also determined to depend on aromatic constituents of root exudate such as 2,4-dihydroxy-7methoxy-2H-benzoxaxin-3(4H)-one (DIMBOA). Induced systemic resistance in these plants has also been shown to be dependent upon rhizhosphere levels of aromatic compounds like phenylalanine, salicylic acid and jasmonic acid that P. putida metabolizes for energy while secreting systemic resistance elicitor compounds in return. As bacterial aromatic synthesis is dependent upon the shikimate pathway, our team investigated a putative operon containing an RifI homolog of the shikimate dehydrogenase enzyme (among many other proteins of unknown function) to test for possible involvements in rhizosphere interactions. We found that the regulator of this putative operon, an IcLR type protein, thermostabilizes in the presence of the A. thaliana root exudate. Using a high-throughput ligand screen utilizing a LacZ reporting system, we aim to identify the ligand of this reporter and through it, determine how this putative operon is involved in rhizosphere intereactions.

7.6. Differential Temperature Response of Wetland Monocots with Contrasting Root Strategies

Jacob Porter[1], Peter Ryser[1]
[1]*Laurentian University, Canada*

Among Northern Ontario wetland monocot species, most species maintain roots throughout the winter, but in some species roots go through an annual senescence in the autumn. This is analogous to the leaves of deciduous and evergreen trees. Advantages of the annual senescence aren't fully known. We hypothesized that the autumn senescing (annual) roots could be adapted to warmer soil temperatures, and would realize a greater advantage from higher temperatures compared to species with the longer lived (perennial) roots. To test that hypothesis, we investigated the growth responses of the two contrasting root strategies to different soil temperatures. Six species arranged in three phylogenetic pairs, were investigated. Two annual perennial pairs: Sparganium americanum and Typha latifolia (Typhaceae); Rhynchospra alba and Trichophorum caespitosum (Cyperaceae); and one pair with both species with annual roots but they different flowering phenology: Eriophorum vaginatum and E. virginicum (Cyperaceae). The plants were grown outdoors for a period of 10 weeks, from early May to Mid July, in pools where a 2-5 degree difference in daytime soil temperature was maintained throughout daylight hours with the use of pumped groundwater. At harvest shoot and root mass and root length were measured. Preliminary results indicate that above-ground dry mass and root length responded positively to warmer soil temperatures in general, species with an annual root strategy gaining significantly more benefit from the warmer soils than the species with perennial roots. These results indicate that the annual root strategy is poorly adapted to cool temperatures.

7.7. The Effect of High Temperature on Pollen Maturation and Germination in Diverse Taxa

Haley A. Branch[1], Reginald McDonald[1], Tammy L. Sage[1], Rowan F. Sage[1]
[1]*Ecology and Evolutionary Biology, University of Toronto, Canada*

Chronic high temperature stress effects pollen development in a range of crop species, leading to decreased pollen viability and reduced crop yield. This has become a leading concern for crop production as temperatures rise. Previous studies have shown that crop species exhibit a reproductive thermal sterility threshold near 32-36degC; however, pollen temperature tolerance of natural populations has not been widely studied. We examined the effect of heat stress (36degC) on pollen maturation in ten species from diverse taxa; seven agricultural species and three wild species. Eight species, including one desert species, showed significant pollen abortion ([?]70%) at the uninucleate stage of development, providing further evidence for the thermal sterility threshold. In contrast, two different species from hot desert environments, Trianthema portulacastrum and Tribulus cistoides, had significantly lower pollen abortion (<18%) at 36degC. T. portulacastrum and T. cistoides were studied further by examining esterase activity and pollen germination at day/night temperatures of 38/24degC and 40/24degC. T. cistoides halted flower production above 36degC, suggesting that high temperature affected the floral meristem, and thus pollen could not be examined further. However, in T. portulacastrum, esterase activity and pollen germination rate significantly declined at both 38/24degC and 40/24degC in comparison to the control (30/24degC). This data shows that pollen from T. cistoides and T. portulacastrum potentially has higher heat tolerance than many agricultural species, and could indicate novel ways to improve heat tolerance in crops.

Session 8. Cell and Developmental Biology III

8.1. Lipid droplet-associated proteins (LDAPs) are required for the dynamic regulation of lipid droplets in vegetative plant cells

Michal Pyc[1], Satinder Gidda[1], Kent Chapman[2], John Dyer[3], Robert Mullen[1]
[1]*University of Guelph, Canada*, [2]*University of North Texas, United States of America*, [3]*U.S. Department of Agriculture, United States of America*

Lipid droplets (LDs) are small, evolutionarily conserved organelles found in a wide range of unicellular and multicellular organisms. Uniquely delineated by a single phospholipid monolayer and coated with a diverse set of proteins, LDs function primarily in the storage of energy-rich neutral lipids, such as triacylglycerols (TAGs). In plants, LDs have been mostly studied in seeds, although all plant cell types have the machinery required for accumulating and storing lipids in cytosolic LDs. We recently identified a class of proteins in Arabidopsis called LD-associated proteins (LDAPs) that are abundant components of LDs in non-seed cell types. Here we show that all three LDAPs (LDAP1-3) are expressed in a variety of vegetative organs/tissues and target to LDs with high specificity. Targeting of LDAP3, for instance, requires the entire protein sequence, but based on binding experiments with biomimetic liposomes, targeting fidelity is not determined by the phospholipid composition of the LD membrane alone. Investigations of LD dynamics in leaves revealed that LD abundance is modulated during the diurnal cycle and abiotic stress response, and that LDAPs participate in both processes. Furthermore, over-expression of LDAPs resulted in an increase not only in LD abundance, but also TAG content in leaves, indicating that LDAPs function in neutral lipid homeostasis. Taken together, these studies shed new light on the roles of LDAPs in the proper maintenance and regulation of LDs in plant cells. Recent efforts aimed at identifying other novel LD proteins using the three LDAPs as 'bait' in yeast two-hybrid screens are also discussed.

8.2. Possible role of a grape DREB2 transcription factors in stomatal development

Chevonne E. Carlow[1], Christine Lee[1], M. Atikur Rahman[1], Annette Nassuth[1]
[1]*University of Guelph, Canada*

Stomatal development in Arabidopsis has been shown to consist of three steps. First the division of pavement cells to form meristomoids, second the conversion of these meristomoids into guard mother cells (GMCs), and third the ultimate formation of a mature stoma with two guard cells. These steps are regulated by the bHLH transcription factor dimers ICE.SPCH, ICE.MUTE and ICE.FAMA respectively. Results from our lab suggest a similar situation in grapes, however two different forms of FAMA could be present. As part of our continuing investigations into this process, we analyzed the possible involvement of DREB2 transcription factors. Transient overexpression in developing tobacco leaves of DREB2-5 but not DREB2-3 affected stomatal development in that it caused a significantly higher density of pavement cells, and a lower GMC and stomatal index. Overexpression of SPCH and to a lesser extent FAMA(E) gave similar results. The DREB2-5 promoter contains four copies of the stomatal-specific element TAAG and nine MYC elements (recognized by bHLH factors) within 2 kb upstream of the translational start site. An in vivo transactivation assay showed that this DREB2-5 promoter could be activated by Vitis SPCH, MUTE, FAMA(E) and FAMA(L), as well as Vitis ICE1, ICE2, ICE3 and ICE4. A different type of transcription factor, Vitis CBF8, did not. A combination of ICE with FAMA(E) increased the transactivation values significantly. Taken together, these results indicate a role for Vitis DREB2-5 in the first step of stomatal development.

8.3. HSP90C is a chloroplast chaperone that assists in the stromal targeting of PsbO1 in Arabidopsis thaliana

Tim Jiang[1], Lingjie Zhang[1], Rongmin Zhao[1]
[1]*University of Toronto, Canada*

PsbO1 plays an important role in the oxygen-evolving complex by stabilizing the catalytic manganese cluster required for splitting of water into oxygen and proton. Having an intrinsically disordered nature, the protein must be transported across the thylakoid membrane in an unfolded state using the SEC translocon. The SEC machinery is inherited through the chloroplast's bacterial lineage and as such, the mechanism of the SEC pathway is comparable in both organisms. However, the bacterial SEC translocation requires the SecB chaperone to bind and recognize substrates while in plants, the SecB homolog does not exist. In order to study the stromal targeting of PsbO1, we fused a well folded GFP to the C-terminus of PsbO1 to delay translocation through the SEC complex. Fusion to GFP was shown to hinder thylakoid transport resulting in three distinct observable processing forms, the cytosolic pre-protein, the stromal intermediate and the lumen mature form. We then elucidated the stromal and thylakoid targeting mechanism of PsbO1 by identification and characterization of a key stromal chaperone, HSP90C. HSP90C directly interacts with PsbO1 and by overexpressing the chaperone in the form of HSP90CFLAG, we detected a distinct shift in the amount of intermediate to mature form ratio. In addition, we also observed similar results when using PsbO1T200A, a mutant which interacted more strongly with HSP90C in vitro. Taken together, we propose that HSP90C may act as a replacement for SecB in plants and aid in the protein translocation of PsbO1 through the SEC complex.

8.4. Mitochondrial pleomorphy is mediated by contiguous ER dynamics

Ashley Jaipargas[1], Kiah Barton[1], Neeta Mathur[1], Jaideep Mathur[1]
[1]*University of Guelph, Canada*

Mitochondria exhibit rapid changes in appearance in response to intra- and extracellular signals. Whereas mitochondria in animal cells appear elongated and tubular under normal conditions, in plants they are primarily spherical to ovate in shape. In hyperglycemic animal cells however, mitochondria undergo fragmentation taking on a small, spherical morphology. As cytosolic sugar levels increase through the light-driven process of photosynthesis, we investigated whether sugar and light have this effect in plants as well. Confocal laser scanning based live imaging of mitochondria in Arabidopsis expressing a mitochondrial targeting signal sequence of the beta-ATPase subunit fused to GFP or to photo-convertible mEosFP was used for analyzing mitochondrial dynamics in response to sugar and light stimuli. Plants grown without sugar or in the dark had a lower soluble sugar content and more elongated mitochondria (>0.85mm in length) than plants grown with sugar or in the light. Furthermore, tubular mitochondria fragmented when plants were exposed to light. These observations suggested that light and the increases in cytosolic sugar are major contributors to the small, round mitochondria typical of plant cells. We also observed that low oxygen conditions induced the formation of giant, plate-like mitochondria. Simultaneous live imaging of mitochondria and the endoplasmic reticulum (ER) further illustrated that the pleomorphic nature of mitochondria is mediated by the ER. Here, we identified conditions to induce transitions in mitochondrial morphology and applied them to illuminate that the ER plays a more imperative role in mitochondrial dynamics in plant cells than previously thought.

8.5. 'Piggy-back' transport of AROGENATE DEHYDRATASE 5 into the nucleus in Arabidopsis thaliana using IMPORTIN a isoform 6

Sara Abolhassani Rad[1], Susanne Kohalmi[1]
[1]*University of Western Ontario, Canada*

Arogenate dehydratases (ADTs) are a family of enzymes that catalyze the last step of phenylalanine biosynthesis in plants, the dehydration/decarboxylation of arogenate into phenylalanine. Since biosynthesis of phenylalanine in plants happens in chloroplasts all ADTs have a transit peptide sequence for targeting. Through localization studies in Arabidopsis thaliana we found that all ADTs localize to extensions of the chloroplast stroma called stromules. Surprisingly, we found that one member of this family, ADT5, is not only targeted to stromules, but also found in the nucleus. We predict that ADT5 is a moonlighting protein that has a non-enzymatic role, possibly as transcription factor, in the nucleus beside its enzymatic role in the chloroplast. Since ADT5 is too big for diffusion into the nucleus we hypothesize that it is imported by interacting with importin proteins. Using in silico research we found that ADT5 is proposed to interact with IMPORTIN a isoform 6 (IMPA6). We performed two different protein-protein interaction assays: the in vivo Yeast-2-Hybrid (Y2H) assay and the in planta Bi-molecular Fluorescence Complementation (BiFC) assay. The results from both assays are consistent with ADT5 being transferred into the nucleus via a piggy-back mechanism. However, to our surprise this ability was not restricted to ADT5 but a general property of all Arabidopsis ADTs. Consequently, the 'piggy-back' mechanism can explain how ADT5 appears in the nucleus, however it does not clarify the unique localization pattern of ADT5. We will discuss our current approaches to understand the unique nuclear properties of ADT.

8.6. Epidermal pavement cells of Arabidopsis have chloroplasts

Kiah Barton[1], Martin Schattat[2], Torsten Jakob[3], Gerd House[2], Christian Wilhelms[3], Jaideep Mathur[1]
[1]*University of Guelph, Canada*, [2]*Martin-Luther-Universitat, Germany*, [3]*University of Leipzig, Germany*

It is textbook knowledge that with a few notable exceptions, the epidermis of higher plants contains chloroplasts only in the guard cells. Pavement cell plastids are considered to be non-green leucoplasts. However there are conflicting reports in the literature concerning the identity of pavement cell plastids in Arabidopsis, and this can have major effects on the interpretation of the source-sink relationship between plastids. Here we evaluate the pigmentation, ultrastructure and photosynthetic ability of Arabidopsis epidermal plastids to determine their identity. Chlorophyll autofluorescence was detected in both pavement and guard cell plastids in leaves and cotyledons under a variety of growth conditions and at several stages of development, but was not seen in trichome plastids. Two mutants were used to investigate this further: immutans, a variegated mutant with sectors showing either leucoplasts and chloroplasts in the leaves, and glabra2, in which trichome differentation begins but is followed by a reversion to the pavement cell fate. At an ultrastructural level, pavement cell plastids possess structured internal membranes that are less developed than mesophyll chloroplast thylakoids, but similar to the thylakoids in guard cell chloroplasts. The presence of an active photosynthetic electron transport chain in these membranes was confirmed using the Pulse-Amplitude Modulated (PAM) fluorescence technique, which showed fluorescence patterns indicative of electron flow through Photosystem II. Taken together, these results demonstrate that under normal growth conditions the plastids in Arabidopsis pavement cells are true chloroplasts.

8.7. The localization of carbon-phosphate translocators on the inner plastid envelope membrane exhibits a stochastic relation to stromules

Michael Wozny[1], Kate Delfosse[1], Neeta Mathur[1], Jaideep Mathur[1]
[1]*University of Guelph, Canada*

Carbon metabolism in plants begins with the synthesis of 3-phosphoglycerate and triose phosphate within photosynthetic chloroplasts. These three carbon molecules are then exported to the cytosol via TRIOSE PHOSPHATE TRANSLOCATORs (TPT) for the synthesis of sucrose and subsequent energy mobilization throughout the plant. In heterotrophic tissues, such as the roots, plastids import fixed carbon in the form of phosphorylated glucose for storage as starch via, primarily, GLUCOSE 6-PHOSPHATE TRANSLOCATORs (GPT). Both TPT and GPT are known to be embedded within the inner membrane of the plastid envelope which, along with the outer membrane, can be drawn away from the plastid body in the form of thin, stroma filled tubules known as stromules. Stromule formation is diurnal in nature, increases during the day/light period, and can be stimulated with exogenous glucose and sucrose application. As such stromules have been proposed as a means to increase the surface area of the plastid for the exchange of carbon metabolites between the plastid and the cytosol. Using fluorescent protein fusions with TPT and GPT we investigated whether they preferentially localize to stromules to facilitate metabolite exchange. Our observations strongly suggest that although both translocators are found as 'patches' on the plastid envelope, their occurrence on stromules is stochastic.

Session 9. Photosynthesis and Metabolism III

9.1. Combining EMS mutagenesis and enhanced screening identifies novel mutants in monoterpenoid indole alkaloid (MIA) biosynthesis in Catharanthus roseus (Madagascar periwinkle).

Trevor Kidd[1], Michael Easson[1], Paulo Cazares[1], Kyung-Hee Kim[1], Yang Qu[1], Vincenzo De Luca[1]
[1]*Brock University, Canada*

The Madagascar periwinkle is the only source of powerful anticancer dimeric MIAs, vinblastine and vincristine. Over 40 years of extensive research to elucidate the biochemistry and molecular biology of MIA biosynthesis has recently led to functional expression of the 13 gene geraniol to strictosidine (PNAS (2015) 112, 3205-10] and the 7 gene tabersonine to vindoline [PNAS (2015) 112: 6224-9] pathways in yeast. However much remains to be discovered about the steps involved in the conversion of strictosidine to tabersonine or catharanthine required to complete the assembly anticancer dimer MIAs. Ethyl methane sulfonate (EMS) mutagenesis produces random point mutations through nucleotide substitution. Specifically it converts guanine to 6-O-ethylguanine, which in turn combines with thymine instead of cytosine. Subsequent DNA repair results in A/T replacing the original G/C, leading to random mutations within the organism. Initial screening of 8000 EMS mutants for altered MIA production was carried by qualitative thin layer chromatography after extraction of MIAs found on the surface of Catharanthus leaves with chloroform. This simple screen was carried out over 4 months by 2 students to yield 5 mutants exhibiting altered MIA phenotypes. This presentation will describe the biological properties of a low/no MIA mutant which was highly susceptible to infection by Phythopthora parasitica. While the mutant could only be cultivated under sterile in vitro conditions, this permitted several new research approaches for understanding MIA biosynthesis that will be described in this presentation.

9.2. Biochemistry-enabled genomics: the Ca2+-dependent protein kinase RcCDPK1 phosphorylates bacterial-type PEP carboxylase at serine-451 in developing castor oil seeds

Sheng Ying[1], Allyson Hill[1], Michal Pyc[2], Erin Anderson[2], Wayne Snedden[1], Robert Mullen[2], Yimin She[3], William Plaxton[1]
[1]*queen's university, Canada*, [2]*university of guelph, Canada*, [3]*Chinese Academy of Sciences, Canada*

Although Ca2+-dependent protein kinases (CDPKs) have crucial functions in plant development and stress response signaling, a major problem has been pinpointing their in vivo substrates. The aim of the current study was to identify and further characterize the putative CDPK (Hill et al. 2014 Biochem J) that catalyzes in vivo inhibitory phosphorylation of bacterial-type PEP carboxylase (BTPC) subunits of an unusual PEP carboxylase hetero-octameric complex in developing castor oil seeds (COS). A novel immunological assay was developed to monitor BTPC kinase activity during its purification from developing COS. This assay is based upon the use of anti-(phosphorylation site-specific) antibodies and immunoblotting to monitor P incorporation from unlabeled ATP into the conserved serine-451 phosphosite of heterologously-expressed BTPC. BTPC kinase was purified from developing COS via FPLC and identified as RcCDPK1 (GI: XM_002526769) by nanoHPLC MS/MS using an Orbitrap Fusion Tribrid mass spectrometer. Maximal RcCDPK1 expression occurred during COS development. Heterologously expressed RcCDPK1 catalyzed Ca2+-dependent (Kd [?] 5 uM), inhibitory BTPC phosphorylation at Ser-451. Pull-down experiments established the Ca2+-dependent interaction of GST-RcCDPK1 with BTPC. RcCDPK1-mCherry localized to the cytosol and nucleus of tobacco BY-2 cells, but co-localized with mitochondrial-surface associated BTPC-EYFP when both fusion proteins were co-expressed. Our collective results indicate a potential link between cytosolic Ca2+-signaling and the control of respiratory CO2 recycling and anaplerotic carbon flux through a central metabolic hub of developing seeds.

9.3. K+ Dependent and K+ Independent Asparaginase from Common Bean (Phaseolus vulgaris): Mechanism of Activation by K+

Ebenezer Ajewole[1], Frederic Marsolais[2], Mariusz Jakolski[3], Agnieszka Pajak[4]
[1]*Department of Biology, University of Western Ontario, London, ON, N6A 5B7, Canada, Canada*, [2]*Genomics and Biotechnology, Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, ON, N5V 4T3, Canada and Department of Biology, University of Western Ontario, London, ON, N6A 5B7, Canada, Canada*, [3]*Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland and Department of Crystallography, Faculty of Chemistry, A. Mickiewicz University, Poznan, Poland, Poland*, [4]*Genomics and Biotechnology, Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, ON, N5V 4T3, Canada, Canada*

Asparagine is broadly known to be the major nitrogen (N) transport and storage form in most plants, especially legumes. Catabolism of L-asparagine provides N for protein biosynthesis in developing plant tissues. The enzyme, L-asparaginase, is responsible for the catalytic deamidation of L-asparagine into L-aspartate and ammonia, which is necessary for further transformation into other amino acids. Previous studies showed that there are two isoforms of the hydrolytic enzyme (potassium dependent - PvASPG1 and potassium independent asparaginase - PvASPG-T2) as a result of their K+ requirements for activation. The crystal structure of the potassium dependent asparaginase (PvASPG1) suggested that Ser118 in the activation loop of PvASPG1 plays a critical role in coordinating potassium for activation; however, this amino acid residue is replaced by an isoleucine in PvASPG-T2. Reciprocal mutants of the enzymes were engineered, the effect of the amino acid residue substitution on the kinetic parameters, optimum pH and K+ dependence of the wild type and mutant enzymes were studied, both in the presence and absence of potassium. There appears to be a catalytic switch on enzyme's dependence on K+ for activation, while activity was optimum at pH 7.5 for both enzymes. The extent of conformational changes, stability and binding affinities of the enzyme as a result of the point mutation is also being investigated in the presence and absence of K+. This study seeks to elucidate the mechanism of catalytic activation of the plant-type asparaginase by K+.

9.4. Arabidopsis thaliana BGLU15 is required for flavonol catabolism in plants

Jonathon Roepke[1], Gale Bozzo[1]
[1]*University of Guelph, Canada*

Flavonols are natural products with known human health benefits. The flavonol bisglycosides kaempferol and quercetin 3-O-b-glucoside-7-O-a-rhamnoside (K3G7R and Q3G7R, respectively) accumulate in Arabidopsis leaves under simultaneous Nitrogen Deficiency and Low Temperature (NDLT) but disappear with recovery from NDLT. In vitro biochemistry demonstrates an Arabidopsis, b-glucosidase, BGLU15, hydrolyses the b-O-linked glucoside bond of flavonol 3-O-b-glucoside-7-O-a-rhamnosides and flavonol 3-O-b-glucosides. Here we tested the hypothesis that BGLU15 is physiologically relevant for the catabolism of these flavonols. Analysis of BGLU15 T-DNA knockout lines (bglu15-1 and bglu15-2) revealed negligible levels of transcripts whereas wild type (WT) leaves had a transient 280-330% increase in BGLU15 transcript levels within 1 d of recovery from NDLT. In

addition, leaves of both bglu15 mutants contained negligible Q3G7R hydrolase activity, whereas this activity increased by 223% within 2 d of NDLT recovery in WT leaves. UHPLC-DAD-MSn revealed that levels of Q3G7R, K3G7R and quercetin 3-O-b-glucoside remained elevated in leaves of bglu15 mutants during recovery from NDLT, whereas losses occurred in WT leaves. Moreover, degradation of other flavonol bisglycosides was evident after NDLT recovery, regardless of whether BGLU15 was present. A transient increase in kaempferol 7-O-a-rhamnoside occurred with stress recovery, regardless of germplasm. The accumulation of this metabolite in the BGLU15 mutants suggest a contribution from hydrolysis of kaempferol 3-O-b-rutinoside-7-O-a-rhamnoside and/or kaempferol 3-O-a-rhamnoside-7-O-a-rhamnoside by hitherto unknown mechanisms. Thus, BGLU15 is essential for catabolism of flavonol 3-O-b-glucoside-7-O-a-rhamnosides and flavonol 3-O-b-glucosides in Arabidopsis. On-going research is focused on the impact of altered flavonol bisglycoside catabolism on auxin-mediated growth phenomena.

9.5. The role of quinate biosynthesis in plants

Artyom Gritsunov[1], James Peek[1], Dinesh Christendat[1]
[1]*UofT, Canada*

Biosynthesis of aromatic amino acids in plants and microbes begins with the shikimate pathway. In plants, the bifunctional 3-dehydroquinate dehydratase/ shikimate dehydrogenase enzyme, DHQ-SDH, catalyzes the formation of shikimate from dehydroquinate. Although plants are the predominant source of quinate in the environment, the enzyme quinate dehydrogenase has only been isolated from a single plant species, poplar. This is rather intriguing with the advent of powerful bioinformatics tools and modern plant sequencing platforms. Quinate is important for the biosynthesis of chlorogenic acid, phenylpropanoid-derivatives and other central metabolites that serve as feeding deterrents. Classical biochemical analysis of plant metabolite extracts has established that some plants synthesize and store quinate derivatives. We are working towards establishing an approach to identify plants quinate dehydrogenases. This will allow us to evaluate why certain plants have evolved the quinate biosynthetic pathway and the biological advantage it confers to these plants in adapting to their environment. In this presentation, I will discuss results from our biochemical and structural biology studies in identifying plant quinate dehydrogenases.

9.6. Molecular cloning and characterization of a phenylpropanoid methyltransferase with unusual para-methylating activity.

Brent Wiens[1], Razvan Simionescu[1], Vincenzo De Luca[1]
[1]*Brock University, Canada*

The monoterpene indole alkaloids (MIAs) reserpine and rescinnamine found in the roots of Rauwolfia serpentina are known as antihypertensive and as potential anticancer agents. However little is known about their biosynthesis in plantae. Both molecules contain an acyl side chain featuring a 3,4,5-trimethoxyphenolic moiety that requires a para-methyltransferase activity for its assembly. Using crude enzyme preparations from the roots of R. serpentina and [14C]-labeled S-adenosylmethionine, we detected the activity of a methyltransferase able to methylate sinapic (3,5-dimethoxy-4-hydroxycinnamic) acid at the para- position to form 3,4,5-trimethoxycinnamic acid. We next employed a correlative analysis of transcriptomic and metabolomic datasets to target candidate genes; and were able to identify, clone, and heterologously express a methyltransferase that exhibited this same sinapic acid methyltransferase activity, however the activity towards this substrate was weak in comparison to its activity with caffeic (3,4-dihydroxycinnamic) acid. Surprisingly, UPLC-MS analyses of in vitro assays using caffeic acid as substrate revealed the formation of two products in equal amounts, which were determined to be ferulic (3-methoxy-4-hydroxycinnamic) acid and para-isoferulic (3-hydroxy-4-methoxycinnamic) acid, thus displaying an unusual para!-methylating activity towards caffeic acid. The recombinant enzyme also accepted gallic acid and a range of flavonoids, preferring those with a vicinal dihydroxy- system. These data suggest potential biosynthetic routes to 3,4,5-trimethoxycinnamic acid in the phenylpropanoid pathway.

9.7. Altered MTA metabolism affects lipid homeostasis and vascular development

Benjamin Tremblay[1], Ishari Waduwara-Jayabahu[1], Hitoshi Sakakibara[2], Maye Saechao[1], Barbara Moffatt[1]
[1]*University of Waterloo, Canada*, [2]*RIKEN Yokohama Institute, Japan*

5'-Methylthioadenosine nucleosidase (MTN) catalyzes the recycling of 5'-methylthioadenosine (MTA), a by-product of multiple biosynthetic pathways including those producing polyamines (PAs). One PA, spermidine (Spd), is required for hypusination of eukaryotic initiation factor 5A (eIF5A), and in conjunction with the PA thermospermine (Tspm) is essential for proper vascular development and general plant growth. Several Arabidopsis MTN-deficient mutant lines are the focus of this research: mtn1-2mtn2-2 completely lacks MTN activity and is embryo lethal; mtn1-1mtn2-1 has a very severe phenotype including altered PA homeostasis, disturbed vasculature and infertility, and mtn1-1mtn2-5 has a more moderate phenotype and is fertile. A microarray analysis of the severe mutant revealed an increased transcript abundance of stress tolerance-related genes and a decreased mRNA abundance of lipid metabolism-associated genes. Metabolite measurements of the same mutant documented its disturbed hormone homeostasis. We have used a chemical biology approach and the lipid stain Nile red to explore lipid and hormone metabolism in the moderate mutant in order to identify the primary effects of MTN-deficiency. We are currently using the reactive oxygen species (ROS) stain DCF-DA to look for evidence of increased ROS indicative of the increased transcription of stress tolerance-related genes, as well testing for tolerance to drought and salt stresses. Ongoing work is aimed at identifying possible roles for eIF5A and Spd in regulating lipid metabolism as well as possible links between increased PA catabolism and stress tolerance in MTN-deficient plants.

Poster Abstracts

Abiotic and Biotic Interactions:

P1. The negative effect of high temperature stress on reproduction in Arabidopsis thaliana

Vanessa Lundsgaard-Nielsen[1], Dinesh Christendat[1], Tammy L Sage[1]
[1]*University of Toronto, Canada*

The negative effect of high temperature (HT) stress on pollen development is a primary cause of reduced seed set in arable ecosystems. We have identified a gene, HTT (High Temperature Tolerance) in Arabidopsis that encodes a plastid-targeted protein functioning in the removal of reactive carbonyl species (RCS). RCS are formed following lipid peroxidation and damage cells by oxidizing proteins. In the HT tolerant Arabidopsis Ler, HTT is expressed in the anther, pollen remains viable and plants produce seed. In contrast, Cvi has no seed set at HT, pollen is inviable, and HTT has significantly lower expression. The loss of pollen viability in Cvi and htt1-1 at HT is associated with abnormal plastid development and lipid accumulation. We assessed protein carbonylation and used mass spectrometry to identify carbonylated proteins to test the hypothesis that HTT functions to maintain plastid homeostasis and pollen viability during HT stress. Pollen plastids in Arabidopsis are essential for pollen maturation due to their critical role in fatty acid and carbohydrate metabolism. Consistent with our hypothesis, carbonylated protein levels were higher in Cvi and htt1-1 than Ler at HT. Proteomic analysis indicated that Cvi and htt1-1 accumulated carbonylated proteins localized to the plastid that function in carbohydrate metabolism, fatty acid transport and lipid biosynthesis. Other carbonylated proteins produced at HT in Cvi and htt1-1 included those involved in protein folding, amino acid metabolism, ROS detoxification, and the heat-induced signal transduction pathway. Our results provide a novel role for HTT in removing RCS to maintain pollen viability.

P2. A long non-coding RNA associated with sulfur nutrition in Eutrema salsugineum: An example of local adaptation?

Amanda Garvin[1], Caitlin Simopoulos[1], Wilson Sung[2], Brian Golding[1], Elizabeth Weretilnyk[1]
[1]*McMaster University, Canada*, [2]*The Centre for Applied Genomics, The Hospital for Sick Children, Canada*

Eutrema salsugineum is an extremophile plant with a naturally high tolerance to abiotic stresses. E. salsugineum grown in controlled environment cabinets have a different phenotype from field plants. To discern genes contributing to these differences we compared transcriptomes generated from field and cabinet plants using principal component analysis. Thirteen genes that are associated with the cabinet phenotype are related to sulfur nutrition with seven previously shown to be upregulated during sulfur deficiency. Also upregulated in cabinet plants was a long non-coding RNA (lncRNA) of 786 bp. With no known homologs in close relatives, the lncRNA's function remains uncertain, however the existence of 15 regions of > 70% similarity within the E. salsugineum genome suggests this transcript is important in this species. Using RT-qPCR we found that expression of the lncRNA decreased in leaf tissue of plants grown on soil supplement with sulfur as did the expression of two sulfur deficiency marker genes. We hypothesized that the lncRNA may be associated with the local adaptation of E. salsugineum to an environment naturally high in sulfur. Using bioinformatics, two small open reading frames were identified in the lncRNA and a putative binding partner was identified with an expression pattern negatively correlated to that of the lncRNA. We are now empirically testing the biological role of the lncRNA using virus induced gene silencing (VIGS).

P3. Differentiating viable/non-viable and mature/immature Plasmodiophora brassicae resting spores using propidium monoazide-assisted qPCR

Fadi Al-Daoud[1], Bruce Gossen[2], Mary Ruth McDonald[1]
[1]*University of Guelph, Canada*, [2]*Agriculture and Agri-Food Canada, Canada*

Soil-borne resting spores (RS) of Plasmodiophora brassicae Woronin, the causal agent of clubroot on canola (Brassica napus L.) and other Brassicas, can remain viable for years. Soil spore load can be quantified using quantitative polymerase chain reaction (qPCR); however, qPCR amplifies DNA from both viable and non-viable RS. Propidium monoazide (PMA) has been used in conjunction with qPCR (PMA-PCR) to prevent amplification of DNA from non-viable microorganisms. The objective of this study was to assess the potential of PMA-PCR to differentiate RS based on viability and maturity. Relatively immature and mature RS were isolated from younger and older clubs, respectively, and heat-treated at 80 degC to produce a mixture of viable and non-viable spores. The spores were then treated with PMA (40-120 uM) followed by qPCR analysis. PMA-PCR analysis indicated that almost all of the non-heat-treated mature RS were viable whereas only 26% of non-heat-treated immature RS were viable, when compared to qPCR without PMA. After exposing mature and immature RS to heat treatment, PMA-PCR detected a decrease of up to 98% in viable RS, when compared to qPCR without PMA. Bioassays using a susceptible canola cultivar confirmed that non-heat-treated and mature RS produced more clubroot than most of the heat-treated and immature RS, respectively. Collectively, these data indicate that PMA-PCR can be used to differentiate viable and non-viable as well as mature and immature RS. Current research is assessing various protocols for use with PMA-PCR on soil samples.

P4. Do plant growth-promoting rhizobacteria increase plant growth and reduce the production of ethylene in Arabidopsis under cadmium and copper stress?

Joshua Frank[1], Sheila Macfie[1]
[1]*Western University, Canada*

Stressed plants produce ethylene, which leads to stunted growth and senescence. Pseudomonas putida, a plant growth-promoting rhizobacterium promotes plant growth under stress conditions. The proposed mechanisms for this involves (1) metabolizing the precursor to ethylene, 1-amino-cyclopropane-1carboxylate (ACC) by the bacterial enzyme ACC deaminase (AcdS) and (2) synthesising the plant growth hormone indole-3-acetic acid (IAA). Pseudomonas putida UW4/AcdS+ (UW4) and a mutant, P. putida UW4/AcdS- (ACC-) that lacks AcdS, were used to test the hypothesis that, under cadmium and copper stress, Arabidopsis thaliana (Col-0) inoculated with UW4 will have reduced ethylene and larger size than both uninoculated control plants and those inoculated with ACC-. Confocal microscopy combined with BacLight tm Live/Dead(r) staining confirmed that both bacterial strains grew on the plant roots. Furthermore, the expression of AsdS in UW4 and lack of AcdS expression in ACC- was confirmed by PCR. When grown in the presence of cadmium or copper, plants inoculated with UW4 produced less ethylene compared to plants inoculated with ACC- and uninoculated plants however, UW4 did not increase plant growth under metal stress conditions. Lastly, tryptophan in plant exudates and IAA produced by both bacterial strains were measured by HPLC-MS. It is unclear why uninoculated plants maintained high growth under both metal stress conditions. Therefore, further work is needed to determine the factors that contribute to the apparent pathogenicity of the bacteria.

P5. Evaluating the roles of seed coat constituents in protecting embryos from chromium toxicity in Arabidopsis thaliana

Nayana De Silva[1], Celine Boutin[2], Anna Lukina[2], Isabel Molina[3], Owen Rowland[1]
[1]*Department of Biology and Institute of Biochemistry, Carleton University, Ottawa, Canada*, [2]*Science and Technology Branch, 1125 Colonel by Drive, Carleton University Campus, Ottawa, Canada*, [3]*Department of Biology, Algoma University, Sault Ste. Marie, Ontario, Canada, Canada*

Soil contamination with chromium is one of the main environmental issues in areas where chromium mining occurs. This may impact negatively on the survival of some plant species depending on the ability of their seeds to germinate in chromium-contaminated soils with subsequent effects on plant community and other trophic levels. Seed coat covers embryonic tissues and may play a role in preventing chromium from penetrating into the seed. During development, the seed coat undergoes cell differentiation and produces a variety of protective chemical compounds. In Arabidopsis, the outer cell layer accumulates mucilage, which is a pectin-rich polysaccharide that is released upon hydration. Other cell wall-associated compounds present in seed coat are the lipid polyesters suberin and cutin, and the oligomeric flavonoids proanthocyanidins. These various polymeric constituents provide protection from adverse environmental conditions, including toxins, UV radiation, and pathogens, while allowing for germination under favourable conditions.
In the present study, Arabidopsis mutants that are defective in seed coat development or specific chemical constituents were tested for altered viability under a set of chromium (CrCl3*6H2O) concentrations that could be expected in areas where mining occurs. Reduced speed of germination and percent germination were found in seeds defective in suberin deposition. These mutants also had higher seed coat permeability as measured by uptake of tetrazolium salts. Further tests will be conducted with mutants defective in cutin, mucilage, and proanthocyanidins. Our investigation will provide evidence on the constituents of seed coat that specifically confer protection to embryos against chromium contamination.

P6. Protein content and protease activity in senescing roots and leaves of wetland monocot species with contrasting root turnover strategies.

Mona Alsahame[1], Peter Ryser[1]
[1]*Laurentian University, Canada*

Perennial herbaceous monocots in Northern Ontario wetlands can be classified in two distinct types of root turnover strategies: species with a low fine root mortality in the fall and winter, and species which a complete fine root mortality in the fall. Leaves of all species die for the winter, and the species with senescing roots overwinter as rhizomes, bulbs, bulbils, corms or tubers. To understand the adaptive value of the two strategy types, information is needed about the effect of root mortality on plant nutrient balance. Existing data on root nutrient remobilization is based on potential changes in root element content in dying roots, which does not differentiate between remobilization and leaching out. In the present project we investigated three species with fall-senescing root systems (Rhynchospora alba, Sagittaria latifolia, Sparganium americanum) and three species with overwintering root systems (Carex oligosperma, Iris versicolor, Scirpus microcarpus). For these species protein content and protease activity was assessed in senescing roots and leaves from September to November. The aim was to confirm and quantify active remobilization processes in the roots. We hypothesize that this process is more pronounced in the fall-senescing roots. Preliminary results support the hypothesized stronger decline of root protein content in the fall for species with fall senescing roots, compared to species overwintering roots.

P7. Comparative transcriptomics of allopolyploid Glycine dolichocarpa and its diploid progenitors in response to rhizobial inoculation

Adrian Powell[1], Jeff Doyle[1]
[1]*Cornell University, United States of America*

Polyploidy, or whole genome duplication, is a genetic phenomenon known to affect biotic interactions in plants. Rhizobial nodulation symbiosis, by which plant hosts can gain access to fixed nitrogen from bacterial symbionts, is a key biotic interaction for many legumes. Recently, there has been speculation that polyploidy events may have played a role in the evolution of nodulation, but the impacts of more recent allopolyploidy events have yet to be directly, comprehensively assessed. Glycine subgenus Glycine (the wild perennial relatives of soybean) contains a complex of recently formed allopolyploids that we have used to study these effects on rhizobial interactions. Experiments have found varying parental and transgressive responses to rhizobial inoculation in the allopolyploid species G. dolichocarpa. Here, we present evidence of differential gene expression responses to rhizobial inoculation in this allopolyploid relative to its diploid progenitors. These responses can be observed at the whole transcriptome level, as well as in expression of particular nodulation-related genes including NIN, NFR1, and NFR5.

P8. Adaptation of two-spotted spider mite, Tetranychus urticae, to Arabidopsis indole glucosinolates

Golnaz Salehipour-Shirazi[1], Kristie Bruinsma[1], Huzefa Ratlamwala[1], Vojislava Grbic[1]
[1]*University of Western Ontario, Canada*

The generalist herbivore, the two-spotted spider mite (Tetranychus urticae), has the capacity to develop resistance to a wide variety of pesticides and defense compounds of host plants. Rapid development of resistance to several classes of pesticides in T. urticae necessitates introduction of alternative management strategies to control it. Indole glucosinolates (IGs), plant secondary metabolites found in the plants of the family Brassicaceae are shown to negatively affect performance of spider mites. In this study, it is examined whether the spider mites can overcome the effects of IGs and what the mechanism of adaptation would be. Results show that, compared to bean-reared spider mites, those that were reared on IGs-containing Arabidopsis for several generations performed better, and induced more damage on Arabidopsis. This adaptation to Arabidopsis could have been due to suppression of plant defense or detoxification of IGs by spider mites. Results imply that suppression of plant defense was not the mechanism of spider mite adaptation to Arabidopsis, since mite adaptation did not change expression of genes required for IGs biosynthesis in Arabidopsis. However, higher expression of detoxification genes in spider mites and decreased performance of adapted mites treated with detoxification enzyme inhibitors on Arabidopsis indicate involvement of spider mite detoxification enzymes in adaptation of spider mites to Arabidopsis IGs. This shows that use of IGs against T. urticae as a control strategy is valid but spider mites ability to detoxify IGs must be considered to avoid development of resistance in pest management strategies.

P9. Investigation of the importance of various VrICE2 domains and the effect of cold treatment on their posttranslational modifications effect activity as a transcription factor

Rob Brandt[1], Scott Hughes[2], Annette Nassuth[2]
[1]*Unviersity of Guelph, Canada*, [2]*University of Guelph, Canada*

The Vitis species is an economically important crop that provides 6.8 billion dollars annually to the Canadian economy. Wild Vitis riparia (Vr) is able to survive in harsh Canadian winters (-40 degC) while the Mediterranean adapted wine grape Vitis vinifera (Vv) is only able to withstand milder temperatures (-25degC). The differences in survival come from the species abilities to acclimate to cold. Cold-induced Inducer of CBF expression (ICE) proteins confer some of this acclimation through the control of downstream regulators. Post translational modifications such as ubiquitination, sumoylation, and phosphorylation are proposed to activate/deactivate the ICE proteins under cold conditions. Using site directed mutagenesis of the VrICE2 gene and subsequent transient trans-activation, the role of these posttranslational modifications was elucidated. The mutations of the PEST and Phosphorylation site all resulted in similar activation to the WT while the SUMO mutation and Phosphomimic resulted in increased activation under normal conditions. Interestingly, under cold conditions, the phosphorylation site mutation resulted in increased activation while all other mutants resulted in lower activation. Through further investigation of these modifications and their domains within the protein, a better understanding of part of the cold acclimation process is achieved. This knowledge can then be further applied with Qualitative Trait Loci to attempt to breed a more cold hardy variety of wine producing grape.

P10. New insights into the spider mite mouthparts, their mode of action and their effect on plant tissues associated with feeding.

Nicolas Bensoussan[1], Vladimir Zhurov[1], Maria Estrella Santamaria[2], Vojislava Grbic[1]
[1]*University of Western Ontario, Canada*, [2]*Centro de Biotecnologia y Genomica de Plantas (UPM-INIA), Spain*

Tetranychus urticae is one of the most polyphagous arthropods and is a major agricultural pest worldwide. The spider mite has a rapid life cycle and feeds on over 1000 plant species belonging to more than 150 different plant families, including many agriculturally important crops such as tomatoes, peppers, strawberries, grapes and citrus. Therefore, understanding the molecular mechanism underlying plant's abilities to recognize and respond to spider mites is essential for addressing this agricultural problem. Presently, the primary effect of spider mite feeding on plant tissue is largely unknown. The objective of this study is to further characterize the feeding behavior of T. urticae and the direct effect on plant tissues. Comparative analysis of internal and external anatomical structure of T. urticae mouthpart reveals complex organs associated with feeding. Analyses of histological leaf cross section from Arabidopsis and beans infested with spider mites were performed to identify the feeding site and to reconstitute the stylet penetration pathway through the mesophyll cells. Microscopic analysis of these sections reveals for the first time that stylets insert between epidermal cells and follow a straight route through the tissue. Dead cells were stained with a selective dye to determine the pattern of tissue damage. According to preliminary results, it seems that spider mite feeding causes the death of individual/small number of mesophyll cells. Understanding the immediate effects of T. urticae feeding will help us better understand Plant-Arthropod interactions at the cellular level and will ultimately lead to novel crop protection strategies.

P11. An in silico analysis of fatty acid QTL and related genes in soybean

Yarmilla Reinprecht[1], K. Peter Pauls[1]
[1]*University of Guelph, Department of Plant Agriculture, Canada*

Soybean oil is composed of five major fatty acids: palmitic (16:0), stearic (18:0) oleic (18:1), linoleic (18:2) and linolenic acid (18:3)]. De novo fatty acid biosynthesis occurs in the chloroplast stroma of leaves and proplastids of seeds through a repeated series of condensation, reduction and dehydration reactions that add two carbon units derived from malonyl ACP to the elongating fatty acid chain. Fatty acid desaturation involves the creation of a double bond (at the delta position) by the enzymatic removal of hydrogen from a methylene group in an acyl chain. Previously, we identified QTL for all five major fatty acids in the RG10 x OX948 mapping population and identified omega-3 fatty acid desaturase genes responsible for the low level of linolenic acid in RG10. The focus of the current research was to identify genes underlying QTL for all five major fatty acids using an in silico approach. Literature and databases were searched for fatty acid QTL and genes associated with fatty acid biosynthesis. Over 150 fatty acid QTL were deposited in SoyBase. They were mapped on 19 chromosomes in soybean Composite_2003 genetic map. More than 30 genes involved in the fatty acid biosynthesis were identified on 15 chromosomes. Some of the fatty acid biosynthetic genes were mapped in silico in regions of fatty acid QTL in soybean sequence map. The co-localization of fatty acid biosynthetic genes and QTL can provide better understanding of molecular mechanisms of oil accumulation in soybean seed and potential for genetic modifications.

P12. Attenuation of tomato induced transcriptional response upon herbivory by adapted two-spotted spider mites

Nicky Wybouw[1], Vladimir Zhurov[2], Catherine Martel[2], Kristie Bruinsma[2], Frederik Hendrickx[3], Vojislava Grbic[2], Thomas Van Leeuwen[4]
[1]*Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Netherlands*, [2]*Department of Biology, University of Western Ontario, Canada*, [3]*Terrestrial Ecology Unit, Biology Department, Ghent University, Belgium*, [4]*Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Belgium*

Co-evolution of plants and herbivores has led plants to develop defences against herbivores due to the selection pressure exerted by herbivores on plants. These defenses are eventually overcome by herbivores, forcing plants to develop new ones. Therefore, in the context of agriculture, it is herbivore adaptation that makes resistant varieties of crops ineffective in pest management strategies. Studying adaptation mechanisms is needed to overcome this issue. The two-spotted spider mite, Tetranychus urticae, is a generalist herbivore, feeding on over1100 plant species, including over 100 important crops. The ability of T. urticae to feed on such a wide range of hosts is, in large part, attributed to its' detoxification abilities. The adaptive potential of T. urticae has been well documented, but the molecular and genetic mechanisms of adaptation is not fully understood. Recent work studying mechanisms of adaptation of experimentally derived adapted populations of mites suggest there are two predominant mechanisms. One is detoxification of plant defence metabolites and the other is suppression of plant defences. Our research of tomato whole genome transcriptional response to tomato adapted and non-adapted spider mites revealed an attenuated response induced by adapted mites relative to the non-adapted reference strain, indicating that suppression of plant defences may play a role in the adaptation process to tomato. However, further analysis revealed this suppression to be ineffective in providing a benefit to mites, therefore it is likely this adapted population is relying on detoxification of tomato toxic defence compounds as

its' predominate mechanism of adaptation.

P13. Investigating the role of plant mitochondria in NO synthesis and signalling

Nicole Alber[1], Greg Vanlerberghe[1]
[1]*University of Toronto, Canada*

Plant mitochondria are proposed to act as signaling organelles orchestrating acclimation responses to abiotic and biotic stress. While the primary signals generated by mitochondria are largely unknown, reactive oxygen and nitrogen species are considered strong candidates. Using fluorescence confocal microscopy, we have shown that AOX knockdown plants have increased concentrations of leaf mitochondrial-localized superoxide and leaf nitric oxide (NO) in comparison to wild-type. Given these findings, we sought evidence that the electron transport chain can act as a site of NO synthesis under normoxic conditions. AOX knockdowns showed higher amounts of NO than wild-type when treated with inhibitors of Complex III or ATP synthase, but not following treatment with an inhibitor of the TCA cycle enzyme aconitase, suggesting NO production is influenced by AOX under specific mitochondrial dysfunction scenarios. Interestingly, treatment with antimycin A, an inhibitor of the Qi-site of Complex III, generated much higher NO amounts than treatment with myxothiazol, an inhibitor of the Qo-site, even though both inhibitors were equally effective at inhibiting oxygen consumption. Plants grown with ammonium instead of nitrate showed no increase in NO when treated with either complex III inhibitors. When these plants were injected with nitrite in addition to the inhibitors treatment, the levels of NO mimicked those of nitrate grown plants. The results suggest that the Q-cycle of Complex III can be a source of NO generation from nitrite, even under normoxic conditions, and that this NO production can be regulated by AOX.

P14. Translational regulation via codon usage bias during cold acclimation in Vitis

Trent Faultless[1], Sanjeena Dang[1], Annette Nassuth[1]
[1]*University of Guelph, Canada*

Regulation of gene products in plants has traditionally been ascribed primarily to transcriptional regulation, with gene expression being the primary determinant of protein production. Gene products are also regulated at the translational level via biases in their use of synonymous codons. Highly-expressed genes, such as ribosomal proteins, exhibit distinct codon biases to ensure efficient translation. Genes expressed under stress conditions exhibit codon biases distinct from genes expressed constitutively under all conditions. Rather than simply reflecting their status as 'non-optimal', the codon use biases present in stress-induced genes have functional relevance, prioritizing their expression under stress conditions and restricting translation of these products from any aberrant mRNA when the cell is not experiencing stress. This is achieved through alteration of the available tRNA species within the cell. tRNA complement is highly dynamic, varying between cells and tissue type, and changing through cell development and under stress via modification of extant tRNA isoacceptor species. While stress-related codon use has been demonstrated and specific examples have been demonstrated to have an in vivo effect on translational efficiency, it is not known whether different stress responses are distinct from one another in their codon use. Genes differentially regulated under cold stress and during fungal infection were compared and found to differ in their codon use, supporting the idea that stress-specific codon use exists in plants and together with tRNA dynamics, regulates translation during stress responses.

P15. The experimental evolution of Pseudomonas syringae pv. phaseolicola 1448A within the non-host Arabidopsis thaliana

Andrew Jamnik[1], David Guttman[1]
[1]*University of Toronto, Canada*

The plant pathogen Pseudomonas syringae has a multitude of pathogen variants (pathovars) with the ability to infect a large range of hosts. Plant species can inhibit infection to a broad spectrum of non-adapted plant pathogens through an immune response known generally as non-host resistance. In order for a pathogen to successfully infect a plant host, it must first suppress and evade this broad-spectrum immunity. Little is currently known about how pathogens evolve to overcome this broad-spectrum immunity within non-hosts, and a further understanding of this could help in uncovering how newly emerging diseases arise. P. syringae pv. phaseolicola (Pph) 1448A is a strain that specifically infects a broad range of cultivars within Phaseolus vulgaris (common bean). Interestingly, Pph 1448A is also able to achieve low levels of growth within the non-host Arabidopsis thaliana, but not to the same degree as adapted strains. In this study we are performing an experimental evolution experiment in which multiple independent lines of a hyper-mutating strain of Pph 1448A are serially passaged through the non-host A. thaliana. The main goal of this passaging will be to evolve lines of Pph 1448A that can achieve greater pathogenicity within A. thaliana, and to whole genome sequence these lines to uncover the genomic changes associated with this phenotype. This project has the potential to uncover the pathways in which pathogens evolve to overcome non-host resistance and to reveal the most prevalent mechanisms of A. thaliana non-host resistance against Pph 1448A.

Cell and Developmental Biology:

P16. High throughput chemical screening using an Arabidopsis ShHTL7 system to identify strigolactone signaling agonists

Asrinus Subha[1], Shigeo Toh[1], Duncan Holbrook-Smith[1], Peter McCourt[1]
[1]*University of Toronto, Canada*

Strigolactones (SLs) are a newly discovered collection of terpenoid-derived small molecule hormones that control germination and shoot architecture in Arabidopsis. Unfortunately, SLs have been co-opted as a germination cue enabling parasitic plants of the genus Striga to coordinate their life cycles with their host plants. For example, Striga hermonthica has evolved strong seed dormancy, which is broken only when they sense SLs extruded from host roots. Upon germination, Striga attaches to the host root and depletes it of nutrients. In sub-Saharan Africa alone, Striga has infested up to two-thirds of the arable land affecting over 100 million people in 25 countries. Chemical control has mostly followed the idea of making SL mimics that germinate Striga in the absence of a host resulting in the death of these obligate parasites. High throughput chemical screening (HTS) could identify potent and inexpensive SL analogs that germinate Striga seed. However, screening directly for compounds that germinate Striga seeds is problematic because Striga seeds vary greatly in their SL responsiveness, and therefore are not amenable to consistent high throughput screening methodologies. The identification of ShHTL7 as a picomolar sensitive SL receptor in Arabidopsis allows the design of such a system. I have developed a germination assay using Arabidopsis ShHTL7 seeds in a 96 well format for HTS of SL agonists and have screened approximately 4000 compounds and identified 5 leads that germinate this line.

P17. The effect of APK1A and APK1B knockout in reinstated self-incompatibility of Arabidopsis thaliana

Hyun Kyung Lee[1], Rajiv Rampersaud[1], Daphne Goring[1]
[1]*University of Toronto, Canada*

The self-incompatibility (SI) pathway in the Brassicaceae family is a conserved trait which reduces inbreeding depression. The pathway is initiated when S-locus Cysteine Rich (SCR) and S-locus Protein 11(SP11) binds to S receptor kinase (SRK) to confirm matching S-haplotype. In Brassica species, the M-locus protein kinase (MLPK), a receptor-like cytoplasmic Ser/Thr kinase, binds to SRK and act as a positive downstream regulator. MLPK and SRK are proposed to promote the activation of Armadillo Repeat Containing 1(ARC1) which then polyubiquitinate Exo70A1, targeting it for degradation. This process causes self-pollen rejection. While Arabidopsis thaliana no longer has a functional self-incompatibility system, this trait can be reinstating by transforming the Arabidopsis lyrata SCRb, SRKb and ARC1 genes into A. thaliana. APK1A and APK1B are orthologues of MLPK in A. thaliana and their function is largely unknown. Here, we conducted an assay to observe the functionality of APK1A and APK1B by testing T-DNA knockout lines of both genes in transgenic SCRb-SRKb-ARC1 A. thaliana. Self-pollen rejection was examined by aniline blue staining of pollen tubes and seed counts were measured. The homozygous double knockout transgenic plants showed trends of higher seed counts and pollen tube penetration. This however was not conclusive due to the possibility of transgene silencing and more lines need to be tested to confirm this trends. Nevertheless, by studying their possible functionality, we can further characterize the evolutionary origin of SI pathway.

P18. Characterizing an alternative targeting signal to the chloroplast outer membrane in Arabidopsis

Nicholas Grimberg[1], Matthew Smith[1], Simon Chuong[2]
[1]*Wilfrid Laurier University, Canada*, [2]*University of Waterloo, Canada*

Chloroplast biogenesis and differentiation relies on the coordinated activities of the nucleus and organelle. Most nuclear-encoded proteins are targeted to the interior of chloroplasts by signals in the N-terminal presequences known as transit peptides. However, the nature of sequence information of transit peptides is not fully understood due to their high divergence in length and composition. A translocon complex at the outer envelope of chloroplasts (TOC) is responsible for the import of nuclear-encoded preproteins from the cytosol. Over the last 8 years, the number of proteins identified in the outer envelope membrane of Arabidopsis chloroplasts has tripled to 117. Although the functions for many of these outer envelope proteins (OEPs) have been characterized, the precise mechanism of their targeting to the chloroplast outer membrane is not fully understood. The majority of OEPs do not contain a cleavable N-terminal presequence and are targeted to the outer membrane either via a transmembrane a-helix or via multiple transmembrane b-strands. Recently, ChloroP analysis and protoplast transient expression assay were used to identify a novel chloroplast targeting signal in the C-terminus of Toc159, an OEP lacking a canonical transmembrane domain. To determine whether other OEPs use this novel targeting pathway, ChloroP analysis identified eight potential candidates possessing the putative C-terminal targeting signal. Transient expression assays of one candidate,OEP17, are underway to examine its subcellular localization and identify sequences required for targeting. Overall, these results suggest that some OEPs other than Toc159 may use this novel targeting pathway.

P19. The Arabidopsis HOTHEAD gene is involved in regulating seed size

Pearl Pei-Chun Chang[1], Susan J Lolle[1]
[1]*University of Waterloo, Canada*

Mutations in the HOTHEAD gene lead to higher cuticle permeability, fused flowers, and impaired fertility. In this study, we show that on average, hth-9 and three T-DNA insertion lines (hth-13, -14 and -15) produced seeds that are 2-2.5 times larger than their wildtype counterpart. Moreover, using the tetrazolium assay we show that hth mutant seed coats were more permeable then wildtype seeds. When the hth-9 plant was transformed with native promoter-driven fluorescent protein-tagged constructs (HTHpro:HTH-FP), it produced wildtype-looking flowers and seeds, and the cuticle and seed coat permeability was restored to normal levels. Analysis of these transgenic lines using fluorescence microscopy revealed that the HTH protein is localized in the adaxial (inner) epidermis of the outer integument (oi-ad). The oi-ad integument is separated from the inner integument by an electron-dense cell wall layer ('wall 3') that is rich in cutin-like material. The vast majority of the wall material deposited in wall 3 is produced by the oi-ad layer. The localization of the HTH protein to oi-ad suggests that the increased seed size and seed coat permeability of hth mutants could be due to altered composition of wall 3, possibly reflecting abnormal cutin deposition in the wall 3 matrix.

P20. Stability of VNI2 protein is regulated by an interacting protein

Masatoshi Yamaguchi[1], Junko Kitagawa[1], Hirofumi Uchimiya[1], Maki Kawai-Yamada[1], Taku Demura[2]
[1]*Saitama University, Japan*, [2]*NAIST, Japan*

A NAC domain transcription factor VNI2 has been isolated as an interacting factor with another NAC domain transcription factor VND7, a master regulator of xylem vessel formation. VNI2 negatively regulates the xylem vessel differentiation by inhibiting VND7 activity. Here, to understand how VNI2 is controlled during the differentiation, we screened interacting factors with VNI2 by using yeast two-hybrid system. When full length VNI2 was used as a bait, a cDNA encoding a protein containing a BTB domain was isolated. The BTB protein family is known to act as an ubiquitin E3 ligase by forming complex with other proteins. Previously we showed that VNI2 has a PEST motif at its C-terminus, and VNI2 stability is regulated by the PEST motif. The BTB protein is unable to interact with VNI2 lacking the PEST motif. We generated a mutant line, which seems to reduce in the ubiquitin-E3 ligase activity. VNI2 protein is more stable in the mutant than that in wild type plants. Furthermore, discontinuous xylem vessels are observed in cotyledon of the mutant. These results suggest that the BTB protein regulates stability of the VNI2 protein, and it is necessary for xylem vessel formation to control the VNI2 protein stability precisely.

P21. Stigma-specific kinase knockdown lines show reduced compatible pollen acceptance in Arabidopsis thaliana

Nethangi Udugama[1], Jennifer Doucet[1], Daphne Goring[1]
[1]*University of Toronto, Canada*

The mustard (Brassicaceae) family have dry stigmas, meaning pollen acceptance is tightly regulated through a family-wide compatible pollen recognition system. Once a compatible pollen is recognized by the female stigma, water and other necessary resources are delivered to the pollen grain through vesicle trafficking to promote pollen germination and subsequent fertilization. Despite a thorough understanding of the factors involved in regulating downstream post-pollination stages, we have limited knowledge on the signalling events that govern initial pollen recognition and acceptance. In this study, we explored two potential stigma-specific compatibility factors, Pollen Acceptor

Stigma Kinase 1 and 2 (PASK1 and PASK2). PASK1 and PASK2 were identified by screening for genes with similar expression patterns as S-locus-related1 (SLR1), a known stigma-specific gene. Two RNAi lines expressing double knockdown of PASK1/2 were generated to characterize the role of PASK1 and PASK2 in compatible pollen acceptance. The phenotypic response to reduced PASK1/2 expression was observed by measuring pollen adhesion, pollen tube penetration, silique size, and seed set. The results demonstrated that RNAinterference-mediated suppression of PASK1 and PASK2 cause a significant reduction in compatible pollen acceptance responses in Arabidopsis thaliana. This suggests that stigma-specific expression of PASK1 and PASK2 is required for pollen acceptance. Given the significance of Brassicaceae species, such as Canola, in the agricultural field, these findings are of high value as they will steer us closer to regulating fertilization more efficiently.

P22. Characterizing the roles of Arabidopsis calmodulin-like protein, CML39, in hormonal regulation of early seedling development and fruit formation.

Ubaid Midhat[1], Wayne Snedden[1]
[1]*Queen's University, Canada*

Calcium (Ca2+) is considered among the most ubiquitous and versatile second messengers in eukaryotes. Cytosolic Ca2+-oscillations are evoked by environmental stimuli such as biotic or abiotic stresses and developmental cues. In turn, these Ca2+ signals are detected by Ca2+-binding proteins, termed Ca2+ sensors, that help coordinate physiological responses by binding to and regulating the activities of various proteins. Calmodulin (CaM) is an evolutionarily-conserved eukaryotic Ca2+ sensor involved in many signal transduction pathways. Interestingly, plants possess large families of unique Ca2+ sensors related to CaM and known as calmodulin-like (CML) proteins. Several CMLs have been implicated in developmental and stress-response signalling but the roles of most CMLs remain unknown. We recently reported the importance of Arabidopsis CML39 in early seedling establishment (Bender et al 2013, Plant J: 76:634). CML39 knock-out (KO) mutants display developmental arrest in the absence of exogenous sucrose. Our ongoing phenotypic analysis of cml39 knock-out plants has identified several additional developmental abnormalities in these mutants. In comparison to wild-type plants, cml39 mutants display perturbations in response to exogenous hormones, altered fruit morphologies, and unusual germination properties. Qualitative and quantitative studies describing the phenotypic characteristics of cml39 vs wild-type plants are presented. In addition, a putative interacting partner (CML39IP) of CML39 was identified through yeast-two-hybrid screens. Here, we present preliminary data for the delineation of the interaction domain along with the phenotypic analysis of KO mutants of CML39IP.

P23. Arabidopsis At3g11620 encodes an evolutionarily conserved protein involved in the intracellular positioning of cytosolic lipid droplets

Samantha C. Watt[1], Satinder K. Gidda[1], John M. Dyer[2], Joe Hull[2], Robert T. Mullen[1]
[1]*University of Guelph, Canada*, [2]*USDA-ARS, United States of America*

Lipid droplets (LDs) are organelles found in the cytosol of all eukaryotic cells, where they serve primarily as storage sites for neutral lipids. While the enzymatic machinery involved in neutral lipid synthesis has been well studied, the molecular mechanisms underlying the biogenesis of LDs remains unclear. This paucity of information is particularly relevant for plant LD biogenesis, where only a few LD proteins have been identified to date. There is also considerable interest in bioengineering strategies aimed at enhancing the accumulation of these energy-rich organelles in vegetative biomass for biofuel production. Toward this end, we have taken advantage of the generally conserved nature of LD biogenesis across evolutionarily diverse species to identify a potential Arabidopsis homolog of a Drosophila protein known to be important for neutral lipid storage and proper positioning of LDs within cells. We show that the Arabidopsis gene product, AT3G11620, which is annotated as a member of the a/b hydrolase superfamily and has homologs in all plants, is expressed throughout Arabidopsis growth and development, suggesting it plays a fundamental role(s) during the plant lifecycle. We show also that AT3G11620, similar to its Drosophila counterpart, localizes to both the endoplasmic reticulum and LDs, and its overexpression in plant (and insect) cells results in the pronounced coalescence of LDs. Together, these results and those obtained from preliminary experiments assessing potential AT3G11620 protein-protein homotypic interactions and LD targeting signal motifs are providing new mechanistic insights to the regulation of LD biogenesis and positioning in plant cells.

P24. Identifying the genetic requirements for recognition of a Pseudomonas syringae HopF effector in Arabidopsis

Derek Seto[1], Noushin Koulena[1], Timothy Lo[1], David Guttman[1], Darrell Desveaux[1]
[1]*University of Toronto, Canada*

Pseudomonas syringae is a Gram-negative bacterial pathogen capable of infecting a wide range of plant species. P. syringae possesses a needle-like type III secretion system, which is required for injecting effector proteins into plant cells. These effectors can disrupt the signaling components of the plant immune system, thereby aiding in the virulence of the pathogen. However, plants have also evolved resistance genes to recognize effectors and activate effector-triggered immunity (ETI) against invading bacteria. In response, P. syringae has diversified its collection of effectors to maintain its ability to suppress ETI in a wide range of plant species. The HopF family of effector proteins is one of over 30 different families and is very diverse. However, research has focused primarily on only two members of this family: HopF2 from P. syringae pv. tomato DC3000 and HopF1 from P. syringae pv. phaseolicola 1449B. Another HopF allele has been found to trigger a resistance response in Arabidopsis. However, recognition of this HopF allele appears to be independent of any known resistance signalling components. Through a reverse genetic screen using the Arabidopsis R gene T-DNA Insertion Collection (ARTIC), the genetic requirements for recognition of this HopF allele will be identified. Further studies will be carried out to identify other major components of this novel resistance pathway. The role of effectors in both bacterial virulence and resistance in plants make them essential for further understanding these mechanisms.

P25. Determination of cellulose synthase complex composition using BioID

Sara Behnami[1], Dario Bonetta[1]
[1]*University of Ontario Institute of Technology, Canada*

Cellulose is an aggregate of unbranched polymers of b-1,4-linked glucose residues which constitutes a major component of plant cell walls. Cellulose biosynthesis occurs at the plasma membrane and involves multimeric complexes (CSCs) composed of cellulose synthase (CESA) proteins as well as other structural proteins. The exact stoichiometry of CESA proteins within CSCs or interactions among themselves and structural proteins are not well understood. To gain insight into the nature of the protein composition of CSCs, we are employing a strategy aimed at identifying proximate and vicinal proteins in vivo associated with a CESA in Physcomitrella patens. Expression analysis of PpCESA3 indicates that it is exclusively expressed in gametophore and is not essential for plant survival. The approach we are is based on

the use of a promiscuous prokaryotic biotin protein ligase, called BioID, which has been shown to detect proteins that are proximate to and/or interact with the target protein to which it is fused. To achieve this in moss we are generating a BioID-CESA3 translational fusion by homologous recombination. Once created, the BioID knock-in line will be used to identify biotinylated CESA3 proximate proteins which can be isolated by affinity chromatography and identified by mass spectrometry.

P26. A genetic screen for a suppressor of the irrepressible variant of AUXIN RESPONSE FACTOR 5/MONOPTEROS

Adriana Caragea[1], Thomas Berleth[1]
[1]*University of Toronto, Canada*

Multiple interactions between the 23 AUXIN RESPONSE FACTORs (ARFs) and 29 Aux/IAA proteins play a central role in the regulation of the auxin response in Arabidopsis. Our approach was to reduce this complexity by asking how specific known components of auxin-mediated gene regulation can affect specific patterning decisions in vascular tissues. Specifically, one Auxin Response Factor, ARF5/MONOPTEROS(MP), with an established role in vascular development, disconnected from auxin-input through Aux/IAA co-regulators (MP[?]), but controllable in its function through steroid-dependent post-translational regulation (MP[?]:GR), was used to identify effectors important in executing regulatory input from MP[?] (and therefore MP). As a result, a suppressor of MP[?] was identified and designated P24.

P27. Clade I TGA bZIP factors are essential for BLADE-ON-PETIOLE-dependent regulation of flowering and inflorescence architecture in Arabidopsis thaliana

Ying Wang[1], Brenda C. Salasini[1], Madiha Khan[1], Bhaswati Devi[1], Mike Bush[1], Shelley R. Hepworth[1]
[1]*Department of Biology, Carleton University, Canada*

The transition from vegetative to reproduction development is an important decision in the life cycle of a plant. Internal and external signals acting on the shoot apical meristem cause its restructuring to form an inflorescence meristem. This results in changed patterns of aerial development resulting in the production of flowers separated by internodes. Two related BELL1-like homeodomain factors, PENNYWISE (PNY) and POUND-FOOLISH (PNF) enable this transition by preserving a boundary at the meristem periphery. In pny pnf mutants, misexpression of boundary genes including BLADE-ON-PETIOLE1/2 (BOP1/2) and its downstream effectors, KNOTTED-LIKE FROM ARABIDOPSIS THALIANA6 (KNAT6) and ARABIDOPSIS THALIANA HOMEOBOX GENE1 (ATH1) blocks meristem responsiveness to floral inductive signals. Inactivation of genes in this module fully restores flowering in pny pnf mutants. Here, we show that clade I bZIP transcription factors TGA1 and TGA4 previously associated with plant defense are essential components of this module. First, inactivation of TGA1/4 fully rescues pny pnf defects in flowering. Second, TGA1 and TGA4 expression is enriched at lateral organ boundaries. Third, TGA1/4 are required by BOP1/2 to exert changes in flowering and inflorescence architecture. BOP1/2 are BTB-ankryin domain-containing transcriptional co-activators lacking a DNA binding domain. We are currently investigating the model that clade I TGA factors are essential in recruiting BOP1/2 to the promoter of target genes. Our data provide intriguing new evidence of dual function for Clade I TGA factors in defense and development.

Photosynthesis and Metabolism:

P28. Can we develop an assay to determine arogenate dehydratase activity in vivo?

Emily Clayton[1], Susanne Kohalmi[2]
[1]*University of Western Ontario, Canada*, [2]*Unversity of Western Ontario, Canada*

Phenylalanine (Phe) can be synthesized via two pathways: the arogenate or the prephenate pathway. The arogenate pathway requires the transamination of prephenate to arogenate by a prephenate aminotransferase (PAT), followed by a decarboxylation/dehydration of arogenate to Phe by an arogenate dehydratase (ADT). The prephenate pathway consists of the same two steps, but in reverse: a decarboxylation/dehydration of prephenate to phenylpyruvate by a prephenate dehydratase (PDT), then a transamination of phenylpyruvate to phenylalanine by a phenylpyruvate aminotransferase (PPAT). PDT and ADT sequences are very conserved and currently cannot be used to distinguish between the two enzymatic functions. We can quickly determine PDT activity using an in vivo yeast complementation assay, however time consuming biochemical assays are required to detect ADT activity. It is therefore desirable to create a simple and cheap assay to test for ADT activity in vivo. We have developed a strategy based on a Saccharomyces cerevisiae PPAT knockout strain. Two PPATs were identified in S. cerevisiae: ARO8 and ARO9. We predict that a [?]aro8aro9 homozygous strain will be unable to synthesize Phe and will not grow without Phe supplementation. This [?]aro8aro9 homozygote strain can be stably transformed with an Arabidopsis PAT which should direct Phe synthesis to the arogenate pathway. We propose that the yeast will only grow on media lacking Phe in the presence of a transformed enzyme with ADT activity. This assay will be a cheap and simple alternative, and its results will be directly comparable to the existing yeast PDT assay.

P29. Chilling out: The evolution of psychrophily in algae

Marina Cvetkovska[1], David R. Smith[1], Norman P.A. Huner[1]
[1]*University of Western Ontario, Canada*

The Earth is a cold place, with most of it at or below the freezing point of water. These seemingly inhospitable environments harbour a wide variety of organisms, by definition, can not survive at moderate temperatures. Algae are the dominant photosynthetic eukaryotes in cold environments, forming the base of the food web. Consequently, these organisms are ideal for studying the physiology and biochemistry of photosynthesis under extreme conditions, as well as the evolution of psychrophily in a general, which has implications of the origins of life, exobiology and climate change. In this work, we explore the adaptation and diversification of photosynthetic eukaryotes to permanently cold conditions. We highlight the known diversity of psychrophilic algae and discuss the unique qualities that allow these organisms to thrive in such extreme ecosystems. We focus on the psychrophilic green alga Chlamydomonas sp. UWO241, discussing recent discoveries and directions for future research, and argue that it is among best available models for studying psychrophily.

P30. Characterization of the anatomy, development, and transcriptome of C3 and C4 Atriplex species

Stefanie Sultmanis[1], Matt Stata[1], Udo Gowik[2], Andrea Brautigam[2], Andreas Weber[2], Rowan Sage[1], Tammy Sage[1]
[1]*University of Toronto, Canada*, [2]*Heinrich-Heine University, Germany*

Maintenance of global food security in the presence of climate change and increasing populations requires an enhancement of photosynthetic efficiency of the major C3 cereal and legume crops. Processes leading to enhanced photosynthesis throughout evolution have resulted from the development of carbon

concentrating mechanisms such as C4 photosynthesis. The evolution of C4 photosynthesis was initially enabled by an increase in mitochondria and chloroplast volume within the bundle sheath cells that surround vascular tissue as well as an increase in vascular tissue. The increase in these organelles activated the bundle sheath cells for photosynthesis and engineering of C4 photosynthesis into C3 plants will require similar modifications. The present study examined structural features associated with Kranz anatomy in Atriplex prostrata (C3) and A. rosea (C4), and analyzed the transcriptome in mature leaves and along a leaf developmental gradient. Expression of genes involved in chloroplast and mitochondrial biogenesis and function were assessed as they relate to the development of the carbon concentrating mechanism in the bundle sheath of a C4 leaf. Structural and transcriptome data indicates that mature leaves of A. rosea exhibit typical Kranz anatomy and NAD-ME biochemistry, as well as a higher vein density than A. prostrata. The C4 species displays accelerated development and differential expression of genes known to be involved in organelle and vascular tissue development. These findings facilitate identification of gene candidates required for establishing the carbon concentrating mechanism of the C4 photosynthetic pathway into C3 crop plants.

P31. The Role of SNF1-Related Protein Kinase 1 (SnRK1) in Carbohydrate Metabolism in Arabidopsis thaliana

You Wang[1], Ian Tetlow[1], Michael Emes[1]
[1]*University of Guelph, Canada*

The Sucrose Non-fermenting-1-Related Protein Kinase (SnRK) belongs to a highly conserved protein kinase family which plays an important role in the control of the energy balance in animals, fungi and plants. In higher plants, it has been found that overexpression of SnRK1 protein kinases in transgenic plants can change the levels of starch and soluble sugars. An Arabidopsis mutant lacking a b subunit of SnRK1 has been obtained. Starch content in the SnRK1 b subunit mutant is higher than in WT Arabidopsis; however, there is no significant difference in starch structure. Compared with WT Arabidopsis, the contents of several soluble sugars such as glucose, fructose, sucrose and maltose in the SnRK1 b subunit mutant exhibit changes over a diurnal cycle. Moreover, metabolic profiling of the mutant revealed that levels of organic acids in the tricarboxylic acid (TCA) cycle were lower than in the wild type, which was reflected in a reduced rate of respiration. The cellular localization of a1, b1 and g1 subunits of SnRK1 in vivo has been investigated using transient expression analysis in Tobacco leaves as well as stable gene expression in transformed Arabidopsis lines. Localisation appears to be complex and multi-compartmentailsed for each subunit as will be presented.

P32. Effects of heat waves on photosynthetic performance in Douglas-fir

Andre G. Duarte[1], Nadine Ruehr[2], Genki Katata[3]
[1]*University of Western Ontario, Canada*, [2]*KIT - IMK -IFU, Germany*, [3]*Japanese Atomic Agency, Japan*

The frequency and intensity of climate extremes, such as heat waves, are predicted to increase in the future. Temperature could rise beyond the threshold that allows trees to function optimally, and have severe implications on plant growth and productivity. In this context, the present study aims to assess how photosynthetic traits in Douglas-fir, a tree of worldwide economic and environmental importance, is affected by exposure to three heat waves with temperatures about 10degC above ambient. A combined set of parameters was assessed, including maximum photosynthetic capacity (Amax), maximal rate of carboxylation (Vcmax) and electron transport (Jmax), minimum stomatal conductance (gmin), d13C and needle carbon and nitrogen content. The temporary increase in growth temperature caused Amax, Vcmax, Jmax and gmin to decrease, indicating high-temperature limitations on photochemistry. The observed decline in electron transport rates, carboxylation activity, and stomatal conductance reflected the increase in photorespiration and concomitant decrease in photosynthetic rates. Moreover, even one month after the last heat wave, the photosynthetic apparatus was still impaired. However, Douglas-fir displayed resistance to the elevated temperatures in terms of the d13C signature and the needle C and N content. These results can offer new insights into the physiological response of Douglas-fir to consecutive episodes of increased temperatures, and help further our understanding of the limitations that heat waves could impose on the development and functioning of Douglas-fir in future climatic scenarios.

P33. Biochemical characterization of AtPirin1: a flavonol degrading enzyme

Ashley Crews[1], Jonathon Roepke[1], Jose A. Freixas-Coutin[1], Gale G. Bozzo[1]
[1]*University of Guelph, Canada*

Flavonols are phytochemicals with potential to reduce the risk of chronic illnesses in humans. In Arabidopsis, flavonol bisglycosides accumulate with abiotic stresses, such as nitrogen deficiency and low temperature (NDLT); recovery from this stress leads to rapid loss of these compounds. A simultaneous spike in flavonol 7-O-rhamnosides is evident during the recovery, whereas their aglycones (i.e., quercetin) do not accumulate. Taken together, the biochemical data suggests degradation of flavonol aglycones is possible. In bacteria and humans, quercetin is oxidized by pirins rendering quercetin depside. We tested the hypothesis that pirin activity is pivotal for flavonol catabolism in Arabidopsis plants during the recovery from abiotic stress. Real time quantitative PCR was used to determine whether transcripts for any of the four known Arabidopsis pirin genes are up-regulated during recovery from NDLT. A transient increase in AtPirin1 transcripts was evident by day three of the recovery period; transcript levels for AtPirin2, AtPirin3 and AtPirin4 were unaffected. AtPirin1 was cloned, expressed in Escherichia coli, and purified by Ni2+-affinity chromatography. Biochemical analysis of the C-terminal hexahistidine tagged recombinant AtPirin1 evidenced the loss of quercetin in assays performed at pH 8.0. HPLC-diode array detection is being used to assess whether the product of this reaction is a depside; the potential for AtPirin1 to oxidize flavonol 7-O-rhamnosides will also be tested. It is expected that this research will contribute to our current understanding of flavanol catabolism in plants and assist in designing engineering strategies to enhance their levels in edible crops.

P34. Altered leaf starch synthesis results in increased rates of photosynthesis in A. thaliana, before flowering.

Noel Mano[1], Fushan Liu[1], Malgre Micallef[1], Michael Emes[1], Ian Tetlow[1]
[1]*University of Guelph, Canada*

During the day, plants build up leaf starch reserves from the sugars produced in photosynthesis. These reserves are degraded throughout the night as an energy source for plant growth and metabolism. Starch deficient mutants of the model species Arabidopsis thaliana have previously been shown to possess decreased rates of CO2 assimilation (Sun et al, 1999). In recent studies, the maize starch branching enzymes (SBE) I and IIb were expressed in A. thaliana mutants lacking both isoforms of the endogenous starch branching enzyme (sbe2.1/2.2). These transformations resulted in a doubling of plant biomass and an increase in oilseed production (up to 4-fold) due to an increased number of siliques per plant. Leaf starch content at the end of the light period was also greater compared to that of the wildtype. The

present study sought to investigate the nature of carbon assimilate partitioning in these transgenic lines. In 21 day old plants, the transgenic lines had significantly higher rates of photosynthesis than did the wildtype. However, this difference was no longer observed at 28 days after plants had flowered. The relationship between starch synthesis, photosynthesis and plant development will be discussed.

P35. Branching Enzymes as Agents for Modifying Glucan Structure in Industrial Processing

Lily Nasanovsky[1], Ian Tetlow[1]
[1]*University of Guelph, Canada*

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

P36. Interaction of curculin-like lectin with AtPAP26-CW2, a purple acid phosphatase glycoform upregulated by phosphate-starved Arabidopsis thaliana.

Mina Ghahremani[1], Hue Tran[2], H. Del Vecchio[1], M. Choka[1], Erin Anderson[4], Yi-Min She[3], Robert Mullen[4], William Plaxton[1]
[1]*Queen's University, Canada*, [2]*Oncolytics Biotech Inc., Canada*, [3]*Shanghai Center for Plant Stress Biology, China*, [4]*University of Guelph, Canada*

Among 29 predicted Arabidopsis purple acid phosphatases (PAPs), AtPAP26 (At5g34850) has been demonstrated as the principal vacuolar (V), cell wall (CW), and root secreted PAP upregulated by phosphate-starved (–Pi) plants. AtPAP26 facilitates Pi acquisition by scavenging Pi from extracellular and intracellular P-monoesters and anhydrides. During lectin-affinity chromatography on Concanavalin-A, a pair of differentially glycosylated AtPAP26 'glycoforms' were purified from CW extracts of –Pi Arabidopsis suspension cells. AtPAP26-CW1 failed to bind to Concanavalin-A, whereas AtPAP26-CW2 was bound and eluted following application of a methyl-α-D-mannopyranoside gradient. A 55-kDa protein extensively co-purified with AtPAP26-CW2 and was identified by MALDI-TOF mass spectrometry as an Arabidopsis curculin-like (mannose-binding) lectin (At1g78850). qPCR indicated that curculin transcripts were induced in seedlings and suspension cells of –Pi Arabidopsis. Anti-curculin immunoblots confirmed that this lectin is upregulated by –Pi Arabidopsis. This is the first purification of a curculin-like lectin or indication of its involvement in plant Pi-starvation responses. Far-western immunoblotting confirmed a specific interaction between purified curculin and AtPAP26-CW2, but not AtPAP26-CW1. AtPAP26-CW2's acid phosphatase activity and thermal stability were enhanced when it was preincubated with curculin. Bifluorescence-complementation and CLSM revealed an in vivo interaction of AtPAP26-V with curculin within the vacuole of the –Pi suspension cells. Research is in progress to assess the phenotype of Arabidopsis T-DNA insertional curculin 'knockout' plants. We hypothesize that curculin plays a key role during Pi deprivation through its interaction with mannose-rich oligosaccharide groups of AtPAP26-CW2 and -V, and consequent impact on AtPAP26-CW2 and -V function and stability.