The Canadian Society of Plant Biologists
La Société Canadienne de Biologie Végétale

The Canadian Society of Plant Biologists

Eastern Regional Meeting

December 2-3, 2022

UNIVERSITY OF
TORONTO
SCARBOROUGH

Welcome!

The Canadian Society of Plant Biologists (CSPB-SCBV) Eastern Regional Meeting 2022 will be held at the University of Toronto Scarborough. This annual event brings together plant biology researchers from academic, government, and industry labs in Eastern Canada for a day of talks, posters, and networking on all aspects of plant science. Following in the tradition of the society, this year's event will include three plenary lectures by leaders in their field together with oral and poster presentations given mostly by post-doctoral fellows and graduate students covering all aspects of plant science and technology. We look forward to hosting the meeting at the University of Toronto Scarborough.

See you soon!

The Organizing Committee

Sonia Gazzarrini (Chair), Department of Biological Sciences, University of Toronto Scarborough
Eliana Gonzales-Vigil, Department of Biological Sciences, University of Toronto Scarborough
Adam Mott, Department of Biological Sciences, University of Toronto Scarborough

Acknowledgments

The 2022 CSPB/SCBV ERM organizing committee would like to acknowledge Cindy Insley and Jennifer Green (Retail and Conference Services, UTSC); Tony Rupnaraine (Financial/Business Officer, UTSC); Sophia Stone (Dalhousie University) for organizing poster and oral presentation judges; all poster and oral presentation judges; and our graduate and undergraduate volunteers for their assistance in organizing this meeting, including Myles Matundan, Zachary Kileeg, Jessica Hu, Christine Nguyen, Jasmin Patel, Shi Jia (Daphne) Chen, Pionie Kwok and Bianca Ortiz. A big thank you to all our sponsors!

Statement of Acknowledgement of Traditional Land

We wish to acknowledge this land on which the University of Toronto operates. For thousands of years it has been the traditional land of the Huron-Wendat, the Seneca, and the Mississaugas of the Credit. Today, this meeting place is still the home to many Indigenous people from across Turtle Island and we are grateful to have the opportunity to work on this land.

Covid and masking

U of T Scarborough, consistent with provincial and municipal guidance, has been strongly recommending masks in indoor settings where social distancing cannot easily be maintained since the start of classes in September. That recommendation has taken on particular importance in recent weeks with the increase of respiratory viruses, including COVID, and the resultant pressures on our health care system. We urge you to wear a mask if you are able and to help communicate this recommendation to your students as well to support the well-being of our whole community. Masks continue to be available at entrances to all buildings at U of T Scarborough and in classrooms.

Sponsors

Financial support for the 2022 CSPB/SCBV ERM was generously provided by the following sponsors:

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Program-at-a-Glance

Friday evening mixer will take place in the **Environmental Science and Chemistry (EV) Building**, University of Toronto Scarborough, 1065 Military Trail, Toronto, ON M1C 1A4.

Saturday conference sessions will take place in the **Instructional Center (IC) Building**, University of Toronto Scarborough, 1095 Military Trail, Toronto, ON M1C 5J9. See UTSC campus map on the next pages.

Friday, December 2nd
(EV Building)

5:00 - 7:00 pm	Registration & Mixer (**EV Atrium, main floor**)

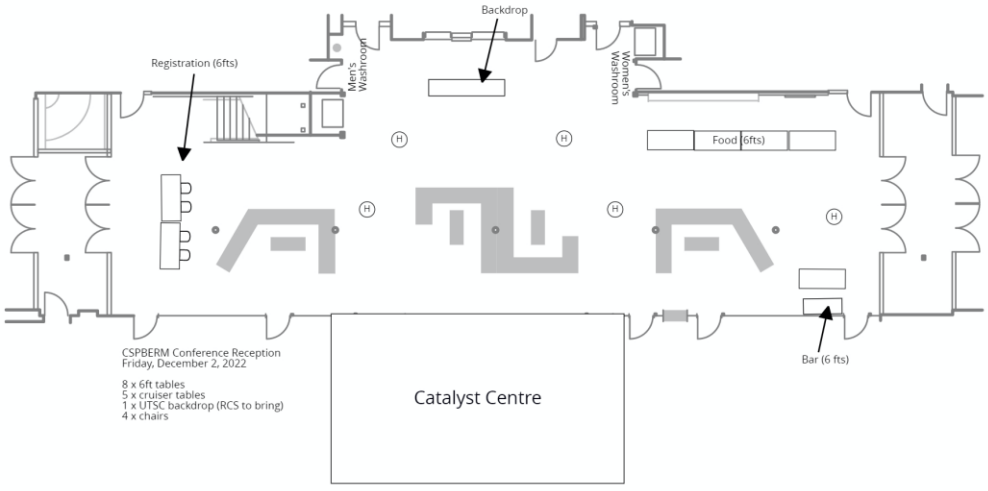
Saturday, December 3rd
(IC Building)

08:00 - 09:10 am	Registration (**IC Atrium, main floor**)
08:00 - 08:50 am	Breakfast and Poster Setup (**IC Atrium**)
08:55 - 09:05 am	Conference Welcome (**room IC130**)
09:05 - 09:40 am	Plenary Lecture I (Isabel Molina, Algoma University)
09:40 - 10:15 am	Plenary Lecture II (Marina Cvetkovska, University of Ottawa)
10:15 - 10:30 am	Morning Refreshment Break (**IC Atrium**)
10:30 - 12:20 pm	**Concurrent Session 1**: Abiotic Interactions (**room IC130**) **Concurrent Session 2**: Cell Biology and Development (**room IC220**) **Concurrent Session 3**: Biochemistry/Post-translational Modifications (**room IC230**)
12:20 - 2:40 pm	Lunch (**EV Atrium**) Poster session (**IC Atrium**) Presenters of odd # posters should be by their poster by 12:40. Presenters of even # posters should be by their poster by 1:30.
2:40 - 4:15 pm	**Concurrent Session 4**: Abiotic and Biotic Interactions (**room IC130**) **Concurrent Session 5**: Systems Biology and Technological Innovations (**room IC220**) **Concurrent Session 6**: Metabolism (**room IC230**)
4:15 - 4:55 pm	Afternoon Refreshment Break (**IC Atrium**) Deliberations for Best Poster (**room IC220**) and Best Oral presentations (**room IC230**]
4:55 - 5:30 pm	Plenary Lecture III (Heather McFarlane, University of Toronto) (**room IC130**)
5:30 pm	Presentation of Student Awards & Conference Closing

Venues

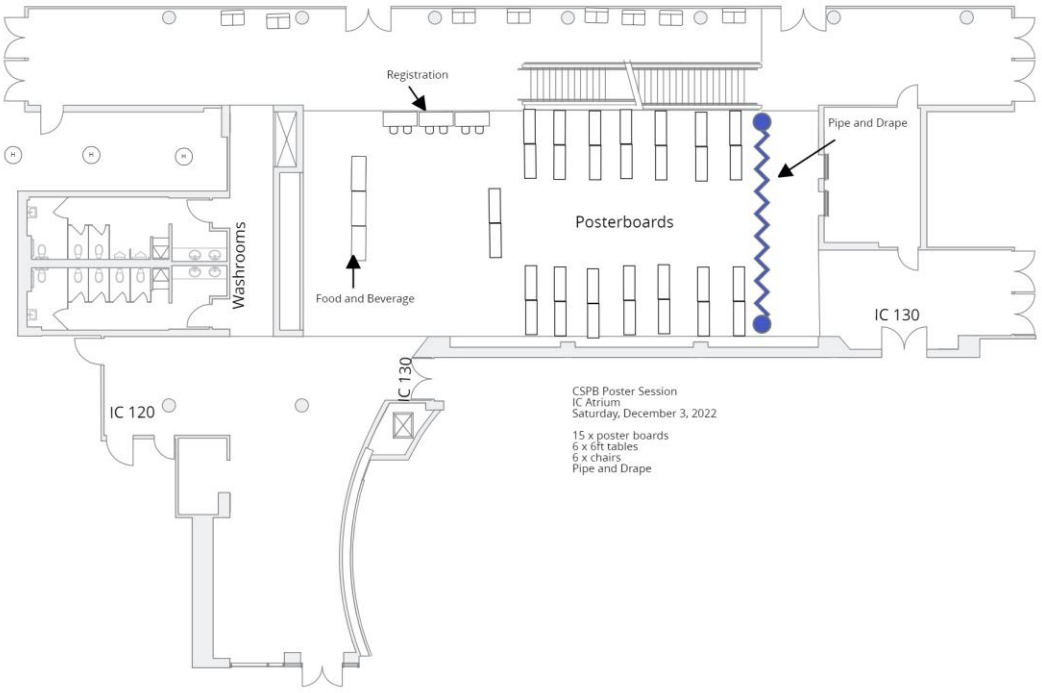
MIXER

Friday, December 2nd
Environmental Science and Chemistry (EV), Atrium
University of Toronto Scarborough, 1065 Military Trail, Toronto, ON M1C 1A4



CONFERENCE

Saturday, December 3rd
Instructional Center (IC)
University of Toronto Scarborough, 1095 Military Trail, Toronto, ON M1C 5J9



UTSC Campus Map

Parking is available in lot G and H. Accessible parking is available in lot L (on the west side of the EV building). You can enter the parking lots from Military Trail.

2022 CSPB/SCBV ERM Program

Friday evening mixer will take place in the **Environmental Science and Chemistry (EV) Building**, University of Toronto Scarborough, 1065 Military Trail, Toronto, ON M1C 1A4.

Saturday conference sessions will take place in the **Instructional Center (IC) Building**, University of Toronto Scarborough, 1095 Military Trail, Toronto, ON M1C 5J9.

Friday, December 2nd (Environmental Science and Chemistry, EV)

5:00 - 7:00 pm	Registration & Mixer (**EV Atrium, main floor**) 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, December 3rd (Instructional Center, IC)

8:00 - 9:10 am	Registration (**IC Atrium**)
8:00 - 8:50 am	Breakfast and Poster Setup (**IC Atrium**)
8:55 - 9:05 am	**Conference Welcome:** CSPB President Robin Cameron, and CSPB-ERM 2022 Chair Sonia Gazzarrini (**room IC130**)
9:05 - 9:10 am	**Chair: Sonia Gazzarrini, University of Toronto Scarborough**
9:10 - 9:40 am	**Plenary lecture I**: Genetic and biochemical mechanisms controlling the water barrier function of adult maize leaf cutin. Isabel Molina, Algoma University
9:40 - 9:45 am	**Chair: Adam Mott, CSPB-ERM 2022 Co-Chair, University of Toronto Scarborough**
9:45 - 10:15 am	**Plenary lecture II**. Life on the Edge: Extremophilic Green Algae and their Responses to a Changing Environment. Marina Cvetkovska, University of Ottawa
10:15 - 10:30 am	**Morning Break** (coffee, tea, and cookies) (**IC Atrium**)

Concurrent Session 1: Abiotic Interactions (room IC130)

	10:30 - 10:35 am	**Chair: Christian Danve Castroverde, Wilfrid Laurier University**
1.1	10:35 - 10:50 am	Summer water stress leads to earlier downregulation of photosynthesis at the end of the growing season in temperate forest tree species. Siyu Wang, Bridget K. Murphy, Noelle Perkins, Ivory Craige P. Rivera, Kyle Do, Monika Peterkova, Ingo Ensminger

1.2 10:50 - 11:05 am The impact of autumn warming on the development of sustained non-photochemical quenching (NPQ) in white spruce. Anchalya Balasubramaniam, Noelle Perkins, Ingo Ensminger

1.3 11:05 - 11:20 am Greater respiratory losses impact growth under summer warming in two genotypes of field-grown white spruce. Bridget K. Murphy, Noelle Perkins, Siyu Wang, Anchalya Balasubramaniam, Maia Dall'Acqua, Tyler Muchos, Ingo Ensminger

1.4 11:20 - 11:35 am Effects of separate or combined soil compaction and/or drought stresses on growth and yield of maize (Zea mays L.) single-cross hybrids. Maciej Grzesiak, Tomasz Hura, Anna Maksymowicz, Kinga Dziurka, Grzegorz Rut, Stanisław Grzesiak

1.5 11:35 - 11:50 am bHLH059 may not be involved in warm temperature-modulated immunity in Arabidopsis thaliana. Christina Rossi, Christian Danve M. Castroverde

1.6 11:50 - 12:05 pm Differences in the timing of cold acclimation and deacclimation of pine and spruce species. Noelle Perkins, Anchalya A Balasubramaniam, Siyu Wang, Ingo Ensminger

1.7 12:05 - 12:20 pm Comparative transcriptome profiling shows differential responses between two Eutrema salsugineum ecotypes exposed to low phosphate and elevated salt. Haoran Jia, Solmaz Irani, Guannan Wang, Maheshi Dassanayake, Elizabeth Weretilnyk

Concurrent Session 2: Cell Biology and Development (room IC220)

10:30 - 10:35 am **Chair: Xue Pan, University of Toronto Scarborough**

2.1 10:35 - 10:50 am Plant Plasma Membrane Nano-organization and Cell Signaling. Xue Pan

2.2 10:50 - 11:05 am Natural variation analysis reveals two major SMAX1 variants that fine-tune the Karrikin dependent germination pathway. Bruno Aquino, James Bradley, Shelley Lumba

2.3 11:05 - 11:20 am RING E3 Ligase XERICO and ABA guide stomatal density and patterning through the SPCH-EPF signaling pathway. Deka Mohamed, Eliana Vonapartis, Dennedy Corcega, Sonia Gazzarrini

2.4 11:20 - 11:35 am Sugar code interactors in N-glycan quality control. Jenny Jiahui Huang, Rowan K. Brookman, Heather E. McFarlane

2.5 11:35 - 11:50 am The role of epigenetics in plant development: from profiles to phenotype. Katharina Braeutigam

2.6 11:50 - 12:05 pm Arabidopsis CML13 and CML1, Interact with IQD, CAMTA, and Myosin Protein Families to Support Cytoskeletal Activity and Gene Expression. Kyle Symonds, Howard Teresinski, Bryan Hau, Takeshi Haraguchi, Kohji Ito, Vikas Dwivedi, Eduard Belausov, Sefi Bar-Sinai, Einat Sadot, David Chaisson, Wayne Snedden

2.7 12:05 - 12:20 pm Transcriptional regulation of FUSCA3 during seed coat development in Arabidopsis thaliana. Jasmin Patel, Lin Wu, Maryam Said, Sonia Gazzarrini

Concurrent Session 3: Biochemistry/Post-translational modifications (room IC230)

10:30 - 10:35 am **Chair: Rongmin Zhao, University of Toronto Scarborough**

3.1 10:35 - 10:50 am Investigating the role of CDPK-mediated phosphorylation on Arabidopsis E3 ubiquitin ligase AtATL6 in stress signaling. Faranak Soleimani, Devang Mehta, Tiffany Yip Delormel, Marie Boudsocq, R. Glen Uhrig, William Plaxton, Jacqueline Monaghan

3.2 10:50 - 11:05 am The root-specific glutamate decarboxylase-1 (AtGAD1) is essential for efficient acclimation of Arabidopsis thaliana to nutritional phosphorus deprivation. Kirsten Benidickson, Lee-Marie Raytek, Gordon Hoover, Barry Shelp, Wayne Snedden, William Plaxton

3.3 11:05 - 11:20 am Investigating the biochemical function and biological role of a unique family of multi-functional enzymes containing both a protein kinase and an E3 ligase domain in Arabidopsis thaliana. Thakshila Dharmasena, Natasha Kelkar, Nick Smith, Jacqueline Monaghan

3.4 11:20 - 11:35 am A post translational phosphorylation at Ser600 is responsible for regulating Arabidopsis lipoxygenase, a key enzyme in jasmonate biosynthesis. Diljot Kaur, Sonia Dorion, Souleimen Jmii, Laurent Cappadocia, Jacqueline Bede, Jean Rivoal

3.5 11:35 - 11:50 am The influence of ferritins on protein carbonylation in Arabidopsis thaliana plants. Adesola Tola, Tagnon Missihoun

3.6 11:50 - 12:05 pm Phosphorylation philosophicals: The regulation of AROGENATE DEHYDRTASE5 in Arabidopsis thaliana. Eileen Barac, Emily A. Hornung, Sara Abolhassani Rad, Susanne E. Kohalmi

3.7 12:05 - 12:20 pm JAZ1 proteins bind NAC42 transcription factors to suppress the biosynthesis of diverse phytoalexins in plants. Jie Lin, Asraful Jahan, Ivan Monsalvo, Melissa Ly, Nik Kovinich

12:20 - 2:40 pm **Lunch** (Boxed lunches & refreshments; **EV Atrium**) and **Poster Session (IC Atrium)**

Presenters of posters with **odd #** should be by their poster by **12:40**
Presenters of posters with **even #** should be by their poster by **1:30**

Concurrent Session 4: Biotic/Abiotic Interactions (room IC130)

2:40 - 2:45 pm **Chair: Jacqueline Monaghan, Queen's University**

4.1 2:45 - 3:00 pm Identifying E3s involved in maintaining nutrient homeostasis. Erin MacKinnon, Sophia Stone

4.2 3:00 - 3:15 pm Legume hosts drive the evolutionary decline in symbiotic benefits under soil nitrogen addition. Rebecca Batstone, Katy Heath, Jennifer Lau

4.3 3:15 - 3:30 pm Identifying the Strigolactone Receptor in Fungi. Michael Bunsick, James Bradley, Bruno Aquino, Flora Wang, Nina Marsh, Omar As'sadiq, Shelley Lumba

4.4 3:30 - 3:45 pm Bringing us together: Connections between Age-Related Resistance and Systemic Acquired Resistance. Garrett Nunn, Natalie Belu, Robin K. Cameron

4.5 3:45 - 4:00 pm Breaking the cell wall: Role of SRF receptors in the DAMP pathway. Aparna Haldar, Ishal Dave, Adam Mott

4.6 4:00 - 4:15 pm Strigolactones trigger phosphate starvation in fungi by inhibiting phosphate uptake. James Bradley, Michael Bunsick, Bruno Aquino, Dario Bonetta, Peter McCourt, Shelley Lumba

Concurrent Session 5: Systems Biology and Technological Innovations (room IC220)

2:40 - 2:45 pm **Chair: Katharina Braeutigam, University of Toronto Mississauga**

5.1 2:45 - 3:00 pm Sightless but not blind - plants expand receptor kinase families related to stress adaptation. Zachary Kileeg, Adam Mott

5.2 3:00 - 3:15 pm High-throughput Phenotyping of Growth in White Spruce Genotypes using Drone based Time-series LiDAR Remote Sensing Data. Aravind Harikumar, Gregory Millar, Siyu Wang, Malaika Gomes, Ingo Ensminger

5.3 3:15 - 3:30 pm Comparing Phenotypic Selection with Machine Learning-Based Genomic Selection for Developing New Varieties of Common Bean (Phaseolus vulgaris): A Validation Study. Robert McGee, Isabella Chiaravalotti, Valerio Hoyos-Villegas

5.4 3:30 - 3:45 pm Towards virtual structure-function analyses of protein-ligand interactions using computational docking and in silico mutagenesis. Carlo Perolo, Heather McFarlane, Nicholas Provart

5.5 3:45 - 4:00 pm Leveraging phosphoproteomics to uncover mechanisms of cell wall integrity signaling. Eduardo A Ramirez Rodriguez, Rylan Vincent, Leo Tullo, Heather Mcfarlane

5.6 4:00 - 4:15 pm Characterizing drought response phenotypes of guard cell-specific genes in Arabidopsis thaliana. Paul Gamueda, Anna van Weringh, Abdeljalil El Habti, Nicholas Provart

Concurrent Session 6: Metabolism (room IC230)

2:40 - 2:45 pm **Chair: Yang Xu, University of Guelph**

6.1 2:45 - 3:00 pm Engineering Chlamydomonas reinhardtii for heterologous synthesis of cannabinoids. Serge Basile Nouemssi, Natacha Mérindol, Fatma Meddeb-Mouelhi, Hugo Germain, Isabel Desgagné-Penix

6.2 3:00 - 3:15 pm Untargeted isotopolog metabolomics reveals the antagonism of methyl jasmonate and salicylic acid in central carbon metabolism of Arabidopsis. Matthew Bergman, Sonia Evans, Michael Phillips

6.3 3:15 - 3:30 pm RUBISCO β-elimination supplies pyruvate to the chloroplast 2C-methyl-D-erythritol-4-phosphate pathway. Sonia Evans, Michael Phillips

6.4 3:30 - 3:45 pm A lesion-mimic mutant of Catharanthus roseus accumulates the opioid agonist, akuammicine. Fanfan Li, Steven Bordeleau, Kyung-Hee Kim, Jonathan Turcotte, Benjamin Davis, Lan Liu, Stéphane Bayen, Vincenzo De Luca, Mehran Dastmalchi

6.5 3:45 - 4:00 pm A multi-product farnesyl diphosphate synthase-like protein supplies geranyl diphosphate for cytosolic monoterpene biosynthesis in Pelargonium graveolens. Anya Franks, Matthew Bergman, Michael Phillips

6.6 4:00 - 4:15 pm Strategy for High-Yield Expression of Recombinant Proteins in the marine diatom Phaeodactylum tricornutum. Gabriela Carolina Gajón Robles, Elisa Fantino, Karen Cristine Gonçalves Dos Santos, Fatma Meddeb-Mouelhi, Isabel Desgagné-Penix

4:15 - 4.55 pm **Afternoon Break** (coffee/tea & cookies)

Deliberations for Best Poster (**room IC 220**) and Best Oral Presentations (**room IC 230**)

4:55 - 5:00 pm	**Chair: Eliana Gonzales-Vigil, CSPB-ERM 2022 Co-Chair, University of Toronto Scarborough (room IC130)**
5:00 - 5:30 pm	**Plenary lecture III**: Sending the right signals for plant cell wall synthesis and remodeling. Heather McFarlane, University of Toronto **(room IC130)**
5:30 PM	Presentation of Student Awards & Conference Closing **(room IC130)**

List of Posters

ABIOTIC STRESS

P1 Daily changes in the hydration of leaves or roots and hydraulic conductivity in maize grown under simultaneous exposure to the compact soil and to the soil drought. Stanisław Grzesiak, Maciej Grzesiak

P2 The relationship between flavonoid metabolism and the drought response of Phaseolus vulgaris. Luis E. Peña Barrena, Lili Mats, Hugh Earl, Gale Bozzo

P3 Which is better? Exploring cuticular wax and trichome responses to heat and drought in bread wheat. Aswini Kuruparan, Eliana Gonzales-Vigil, Raju Soolanayakanahally

P4 Palmelloid formation alters the organization of the photosystems which enhances photoprotection in the Antarctic psychrophile, Chlamydomonas priscuii. Beth Szyszka-Mroz, Victoria Kata, Alexander Ivanov, Charles Trick, Norman Huner

P5 Feeding our Future: Effects of Elevated CO2 and Temperature on Wheat. Andrew Cook, Danielle Way, Raju Soolanayakanahally

P6 Identification of drought-responsive genes by differential splicing analysis of guard cell transcriptomes. Hasna Khan, Anna van Weringh, Nicholas J. Provart

P7 Differential phosphorylation correlates with activation of the root-specific glutamate decarboxylase-1 (AtGAD1) in phosphate-starved Arabidopsis. Lee Marie Raytek, Brittany Menard, Nathan Doner, Maria Rodriguez, Barry Shelp, Glen Uhrig, Rob Mullen, Wayne Snedden, William Plaxton

P8 Activation of a singlet oxygen signaling pathway by competition cues in Arabidopsis thaliana. Nicole Berardi, Sasan Amirsadeghi, Clarence Swanton

P9 Responses of Balsam Poplar to Extreme Weather Events Simulating Late 21st Century Climate. Laura Jones, Andrew Yu, Oscar Nunez, Katharina Braeutigam

P10 Investigating the role of EPF signaling peptide in regulating drought stress response in Arabidopsis thaliana. Kritika Bharti

P11 Chlorophyll fluorescence as a tool for tracking photosynthetic phenology in spruce genotypes. Qi Liu, Aravind Harikumar, Siyu Wang, Malaika Gomes, Ingo Ensminger

P12 Characterizing Real-Time Sensors of Cell Wall Stress in Arabidopsis thaliana. Vicky Zhu, Raegan Larson, Lella Erceg, Heather McFarlane

P13 lncRNA mediated LRR-RLK regulation in Arabidopsis thaliana. Robert Yaremko, Hemal Bhasin, Hasna Khan, Adam Mott.

BIOTIC STRESS

P14 Caterpillar detoxification of plant specialized metabolites: Battle of the sexes! Yinting Chen, Ryan J. Smith, Jacqueline C. Bede, Hongliang Su

P15 Investigation of Flavonoid Synthesis in M. truncatula during S. meliloti Inoculation Based on Co-Evolutionary History. Mithusha Peragerasingam, Rebecca Batstone

P16 To eat or not to eat? Surveying resistance to insect herbivores in the wild tomato Solanum habrochaites. Andreea Bosorogan, Osmond Hui, Eliana Gonzales-Vigil,

P17 Tomato specialized metabolites can modulate the insect-gut microbiome. Andreea Bosorogan, Eliana Gonzales-Vigil

P18 Tomato defence hormone responses to the foodborne pathogen Salmonella under elevated temperatures. Karen Liu, Eric Marchetta, Sam Snider, Robin Slawson, Joel Weadge, Christian Danve M. Castroverde

P19 Diverse interactions between Solanum lycopersicum and beneficial Canadian soil-borne bacteria regulate increased immunity. Mack Loranger, Miya Tseng-West, Winfield Yim, Wolfgang Moeder, Nadia Morales-Lizcano, Arivin Nickzad, Eric Déziel, Keiko Yoshioka

P20 Involvement of two cyclic nucleotide-gated ion channel subunits in jasmonic acid-mediated immune signaling. Hyunsuh Lee, Angelica Miraples, Robin Goh, Shingo Maruyama, Andreea Bosorogan, Eliana Gonzales-Vigil, Wolfgang Moeder, Hanae Kaku, Keiko Yoshioka

P21 Resistance to bacterial brown spot in adzuki bean. Ujomonigho Omoregie, K. Peter Pauls

P22 Investigation of 13 Immunity-Priming Bacterial Strains from the Canadian Soilborne Bacteria Library with Respect to their Plant Growth Promoting Effects. Matthew Toffoli, Wolfgang Moeder, Eric Déziel, Thomas Berleth, Keiko Yoshioka

P23 Characterization of Effector Binding Elements present on genes from Phaseolus vulgaris L. targets of the Transcription Activator- like Effectors from Xanthomonas species. Mylene Corzo-Lopez, Gregory Perry, Weilong Xie, K. Peter Pauls

P24 Identifying proteins that interact with DIR1 during Systemic Acquired Resistance (SAR) using an estrogen-inducible SAR system and LC-MS/MS-based detection methods. Natalie Belu, Phillip Carella, Garrett Nunn, Rowan Brookman, Robin Cameron

P25 Genetic Expression of the Tomato CBP60g Gene Family in Response to Bacterial Infection and Elevated Temperature. Vanessa Shivnauth, Eric Marchetta, Danve Castroverde

P26 Production of Cannabis (Cannabis sativa L.) Synthetic Seeds for In vitro Clonal Mass propagation and Germplasm Conservation. Benjamin Davis, Elham Tavakouli Dinani, Biruk Feyissa, Adel Zarei

CELL BIOLOGY AND DEVELOPMENT

P27 Elucidating the role of RKF1 interactors in post-pollination responses in Arabidopsis thaliana. Stephen Bordeleau, Daphne Goring

P28 Investigating downstream regulators in the self-incompatibility pathway of transgenic SI A. thaliana. Paula Beronilla, Daphne R. Goring

P29 Investigating the role of LRR-VIII-2 Receptor-Like Kinase genes in intra- and inter-species pollinations in Arabidopsis thaliana. Laura Canales Sanchez, Daphne Goring

P30 Investigating the role of NAC transcription factors in Arabidopsis thaliana seed coat development. Myles Matundan, Sonia Gazzarrini

P31 Investigating the role of ubiquitination in cell wall signal transduction. Yu Zhu, Una McNally, Heather McFarlane

P32 Characterizing immunity and physiological phenotypes in temperature-sensitive and -resilient accessions of Arabidopsis thaliana. Dhrashti Patel, Christina Rossi, Christian Danve. M Castroverde

P33 Functional analysis of the N-terminal intrinsically disordered region of an immune kinase. Anamika Rawat, Ruoqi Duo, Katherine Dunning, Melissa Bredow, Kyle Bender, Lauren Grubb, Danielle Ciren, Wayne Snedden, Jacqueline Monaghan

P34 A cell-based system to study gene expression and protein function: Arabidopsis mesophyll protoplasts. Carmen Mei, Oscar Nunez, Stefan Heinen, Katharina Braeutigam

P35 Investigating the role of subgroup IV calcium-dependent protein kinases (CDPKs) across the plant lineage. Ruoqi Dou, Karima El Mahboubi, Melissa Bredow, Cailun Tanney, Pierre-Marc Delaux, Jacqueline Monaghan

P36 Investigating the mechanistic role of Arabidopsis HSP90.7 in auxin-mediated plant development. Jenan Noureddine, Wai Lam Mok, Rongmin Zhao

P37 Functional studies of Arabidopsis MAPK phosphatases reveal novel regulators of chloroplast biogenesis. Pooja Kaushik, Lahouari Zakaria Brahim, Jianlei Sun, Jin Suk Lee

GENOMICS, SYSTEMS BIOLOGY AND TECHNOLOGICAL INNOVATIONS

P38 miR156/SPL12 modulates nodulation, nitrogen fixation and root regeneration in Medicago sativa by Silencing AGAMOUS-LIKE 6. Vida Nasrollahi, Abdelali Hannoufa, Susanne Kohalmi

P39 Comparative Analysis of Different Phenotypic and Genotypic Selection Strategies to Increase the Yield Genetic Gain using Nested Association Mapping Population in Dry Bean. Maryam Vazin, K. Peter Pauls

P40 Diversity, function, and regulation of the immunity-associated CALMODULIN-BINDING PROTEIN 60 (CBP60) family in Solanum lycopersium (tomato) under biotic and abiotic stresses. Christian Danve Castroverde, Vanessa Shivnauth, Sonya Pretheepkumar, Keaun Amani

P41 A Genetic Suppressor Screen to Identify New Alleles and Downstream Targets of SMAX1. Jenna Hountalas

P42 Importance of the development of a co-dominant marker in the introgression of novel traits through marker-assisted backcrossing. Sajida Noor, Peter K. Pauls

P43 Facilitating High Throughput Screening of Common Beans (Phaseolus vulgaris L.) for Anthracnose Resistance Genes. Marysia Zaleski-Cox

BIOCHEMISTRY AND METABOLISM

P44 Heterologous expression of non-proteolyzed glutamate decarboxylase-1 (AtGAD1) from Arabidopsis thaliana. Brittany S. Menard, Lee-Marie Raytek, Barry J. Shelp, Wayne A. Snedden, William C. Plaxton

P45 Investigating the function of chloroplast chaperone HSP90C C-terminal extension in abiotic stress resistance. Bona Mu, Tim Jiang, Wei-tse Tseng, Rongmin Zhao

P46 What is the role of phosphorylation of the cytosolic glucose-6-phosphate dehydrogenase isozyme AtG6PD6 in response to phosphate nutrition of the model plant Arabidopsis thaliana? Millie Smith, William Plaxton

P47 Highlighting the in vitro impact of chalcone isomerase-like on legume-specific chalcone biosynthesis. Brandon Saltzman, Mehran Dastmalchi

P48 A waxy coat makes poplars popular in Northern Hemisphere. Mahbobeh Zamani Babgohari, Raju Soolanayakanahally, Eliana Gonzales-Vigil

P49 A combinatorial bioengineering approach aimed at enhancing the accumulation of α-eleostearic acid-containing neutral storage lipids in Nicotiana benthamiana leaves. Alyssa Clews, Yang Xu, Nathan M. Doner, Lingling Zhang, Shiyou Lü, Damien Seay, Jay M. Shockey, John M. Dyer, Robert T. Mullen

P50 Mapping the local proteome of the endoplasmic reticulum and chloroplasts membrane contact sites using biotin proximity labeling. Monika Jesionowska, Alyssa C. Clews, Robert T. Mullen, Yang Xu

P51 Investigation of the metabolism and biological activity of (+)-tetralone ABA, an ABA analog, in Arabidopsis. Christine Nguyen, Dawei Yan, Naveen Diddi, Leon Lai, Suzanne Abrams, Eiji Nambara

P52 The role of phosphorylation in regulating AROGENATE DEHYDRATASE 2 function in Arabidopsis thaliana. Erin N Brownscombe, Emily A Hornung, Emily J Clayton, Sangeeta Dhaubhadel, Susanne E Kohalmi

P53 Probing interacting regions of lipid biosynthetic enzymes from flax. Katelyn Hockemeyer, Monika Jesionowska, Yang Xu

P54 Subcellular localization of enzymes in the isoflavonoid pathway. Audrey Cote, Mehran Dastmalchi

P55 Interaction analysis between chloroplast molecular chaperone HSP90C and subunits of the thylakoid SEC translocase. Adheip Nair, Bona Mu, Rongmin Zhao

P56 Characterizing the targeting pathway of chloroplast outer membrane protein OEP18. Ceaira Hiemstra, Tianlun Zhou, Simon Chuong, Matthew Smith

P57 TOC159 Receptors are Targeted to the Chloroplast Outer Membrane Using a Bipartite Targeting Signal at the C-Terminus. Michael Fish, Simon Chuong, Masoud Jelokhani-Niaraki and Matthew Smith

ORAL SESSIONS ABSTRACTS

PLENARY LECTURE I

Isabel Molina

Genetic and biochemical mechanisms controlling the water barrier function of adult maize leaf cuticles

Richard Bourgault[1], Meng Lin[2], Susanne Matschi[3], Pengfei Qiao[2], Miguel Vasquez[3], Marc Mohammadi[1], Annika Sonntag[1], Isabel Molina[1], Michael Scanlon[2], Michael A. Gore[2], Laurie Smith[3]

[1]Algoma University, [2]Cornell University, [3]University of California San Diego

The plant cuticle, a hydrophobic layer of cutin and waxes synthesized by epidermal cells, establishes a vital interaction interface with the environment. This lipid barrier controls the movement of water, gases and solutes. Although biochemical, genetic and genomic studies continue to advance our understanding of cuticle biogenesis and function in model systems, these advances have yet to be translated into improvements in agronomically important grasses such as maize. Transpiration across the cuticle (cuticular conductance, g_c) is the major source of water loss at night and in water-limiting conditions when stomata are closed. g_c is therefore a target trait for efforts to improve maize drought tolerance. We have characterized the composition and barrier properties of the adult leaf cuticle at multiple developmental stages and linked our findings to epidermal gene expression. This study identified phytochrome-mediated light signaling as a key regulator of cuticle development. Taking advantage of the extreme genetic diversity of domesticated maize, we have conducted genome wide (GWAS) and transcriptome wide association studies (TWAS) to identify genomic regions and candidate genes associated with water loss thorough the cuticle. This approach highlighted 21 candidate genes for cuticle biosynthesis and regulation of cuticle development. Furthermore, natural variation in adult leaf g_c values across genotypes is associated with the abundance of wax esters, which emerged as key contributors to the water barrier function of adult maize leaf cuticles. Taken together, these findings provide insights into the potential for cuticle modification to improve agriculturally important traits related to cuticle function in maize.

PLENARY LECTURE II

Marina Cvetkovska

Life on the Edge: Extremophilic Green Algae and their Responses to a Changing Environment

Marina Cvetkovska[1]

[1]University of Ottawa

Green algae from the order Chlamydomonadales, including the flagship species *Chlamydomonas reinhardtii*, have fascinated biologist for decades. Important developments in major life science areas trace their origin in Chlamydomonas research, spanning diverse fields from plant and algal biology (e.g., photosynthesis), evolution (e.g., multicellularity) and medical research (e.g., optogenetics). In the Cvetkovska lab we work with obligate psychrophilic Chlamydomonas species from Antarctic and Arctic ice-covered aquatic habitats. Polar ecosystems contribute more than 30% of global carbon sequestering through photosynthesis but we know very little of how this process operates at extreme conditions. Psychrophilic Chlamydomonas are some of the best studied polar primary producers and are a largely untapped resource for identifying cold adaptation traits, novel metabolites, and cold-active enzymes. Psychrophiles are exceptionally adapted to thrive at a plethora of extreme conditions but are sensitive to environmental change and typically cannot survive even moderate temperature increases. Today, with rapid climate change upon us, studying psychrophiles is more important than ever. We are broadly interested in two questions: (1) How do polar algae adapt to an extreme environment characterized by permanently low temperatures, high salinity, and low light? (2) How do psychrophilic algae respond to climate-induced environmental stress that threatens the sensitive polar ecosystems? I will discuss our recent advances in these fields, and some of the future opportunities and challenges that still await us.

PLENARY LECTURE III

Heather McFarlane

Sending the right signals for plant cell wall synthesis and remodeling

Heather McFarlane[1]

[1]University of Toronto

The plant cell wall is a polysaccharide-based extracellular matrix that surrounds and protects all plant cells. Since plants are constantly growing and developing within the confines of their cell walls, plant cells must be in constant communication with their cell walls. Furthermore, cell walls are a critical line of defence between plant cells and their environment; changes to the cell wall are often early warning signs of pathogen attack or abiotic stress, and plants fortify their cell walls in response to these stresses. This ongoing communication between the plant cells and their cell walls is collectively called “cell wall signaling”. The McFarlane Lab at The University of Toronto studies the molecular mechanisms of cell wall signaling and responses, including cell wall secretion and remodeling. We have previously implicated a G-protein coupled receptor-like module in cell wall signaling and demonstrated that this module is required to fortify the cell wall during stress conditions. We are continuing to uncover the mechanisms of cell wall signaling by studying post-translational modifications in the context of cell wall signal transduction.

CONCURRENT SESSION 1: Abiotic Interactions

1.1 - Summer water stress leads to earlier downregulation of photosynthesis at the end of the growing season in temperate forest tree species

Siyu Wang[1, 2], Bridget K. Murphy[1, 3], Noelle Perkins[1, 3], Ivory Craige P. Rivera[1], Kyle Do[1], Monika Peterkova[1], Ingo Ensminger[1, 2, 3]

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Climate warming has increased the intensity and frequency of drought events. Understanding how photosynthetic traits and phenology of temperate forests respond to water stress can help understand changes in spatial and temporal patterns of terrestrial ecosystem carbon uptake under climate change. The goal of our project is to understand the response of autumn phenology of two deciduous (White oak, Red maple) and one evergreen (White pine) forest species to drought. We evaluated phenology in response to drought in seedlings of the three tree species in a factorial experiment, using rainout structures to reduce 50% of the precipitation in treatment plots compared to control plots. Physiological and phenology responses to drought were captured by measuring growth, gas exchange, chlorophyll fluorescence, leaf and canopy spectral reflectance at weekly to bi-weekly intervals during the growing season. Soil moisture and pre-dawn leaf water potential were monitored as proxies of water stress. Our results show significant effects of the drought treatment on growth, photosynthetic CO_2 uptake, stomatal conductance, photochemical energy partitioning and photosynthetic reflectance during summer. The intrinsic water use efficiency of the white pine was more sensitive to water stress compared to white oak and red maple. Most importantly, the timing of autumn phenology of the deciduous trees subjected to drought stress showed an earlier downregulation of photosynthesis by one to two weeks compared to control seedlings. Our findings demonstrate that summer water stress leads to a shorter growing season in temperate trees with implications for productivity in a future climate.

1.2 - The impact of autumn warming on the development of sustained non-photochemical quenching (NPQ) in white spruce

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Global environmental change leads to warmer autumn temperatures whilst the autumn decrease in photoperiod remains unaffected. Asynchronous timing of the decrease of temperature and photoperiod is likely to affect the development of low temperature acclimation during autumn in evergreen conifers and impact the timing of the downregulation of photosynthesis. In conifers, low temperature acclimation and the downregulation of photosynthesis involves dynamic changes in two components of photoprotective non-photochemical (NPQ) quenching. The dynamic NPQ component is increasingly lost as photosynthetic activity decreases towards the end of the growing season, whereas the sustained component of NPQ increases. The goal of our project is to identify how autumn warming affects the development of sustained NPQ in white spruce (*Picea glauca*). We used a temperature free-air enhancement (T-Face) experimental setup at the Koffler Scientific Reserve, where we can raise leaf temperature by 5 ˚C above ambient in heated plots using infrared heaters compared to unheated control plots. We tracked seasonal variation in autumn photosynthetic phenology by measuring chlorophyll fluorescence and leaf spectral reflectance from early autumn to early winter. We observed differences in physiology between heated and control plots where warming resulted in a delayed downregulation of photosystem II efficiency and the downregulation of dynamic NPQ. Warming also delayed the induction of sustained NPQ. Overall, our findings indicate that autumn warming, despite the decrease in photoperiod, is able to extend the length of photosynthetic activity towards the end of the growing season.

1.3 - Greater respiratory losses impact growth under summer warming in two genotypes of field-grown white spruce

Bridget K. Murphy[1, 2], Noelle Perkins[1, 2], Siyu Wang[1, 3], Anchalya Balasubramaniam[1, 2], Maia Dall'Acqua[4], Tyler Muchos[1], Ingo Ensminger[1, 2, 3]

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Global temperatures are projected to increase by 5.7˚C by 2100 with the most severe warming in northern latitudes. Local adaption to environment may impact the ability of different trees to cope with future climate that will be warmer and drier. Our main objective is to determine how intraspecific variation affects photosynthesis, growth, and survival of two latitudinally distinct white spruce (*Picea glauca)* genotypes when exposed to environmental stresses. The experimental design consisted of four treatments in replicate field plots: (1) control, (2) drought, (3) warming and (4) warming combined with drought. To track stress responses of the two genotypes, physiological and morphological parameters were quantified across four measurement campaigns from June to September. Both genotypes appeared to be resilient to air temperatures as high as 34.5˚C and soil moisture as low as 16.3% as mortality was low and photosynthesis remained unaffected. However, towards the end of the growing season, seedlings exposed to and measured under warming of +5˚C showed reductions in stomatal conductance and increases in respiration rates compared to seedlings grown under ambient temperatures. Warming also decreased water potential and seedling height. Warming appeared to impact the biomass accumulation of the southern, fast-growing genotype negatively compared to the northern, slow-growing genotype. Increased respiration rates and decreased stomatal conductance likely contributed to reduced growth under warming. Overall, respiratory losses caused through warming affected growth in both genotypes, with warming having a greater impact on the southern genotype, highlighting the importance of identifying genotypes better adapted for future climates in reforestation.

1.4 - Effects of separate or combined soil compaction and/or drought stresses on growth and yield of maize (Zea mays L.) single-cross hybrids

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In this study, we examined responses of maize hybrids differing in the susceptibility to soil compaction (LI or HI) and drought stresses (D). We ran field and greenhouse experiments and determined effects of both stress factors on the grain yield, biomass, weight of 1000 grains, shoot and roots dry matter, shoot to roots ratio, harvest index, plant height, emergence index, leaf area and greening, and root number and length. Individual and combined effects of both stresses were studied in the field and greenhouse. Compared with plants growing in loose soil and optimal irrigation (LI), the resistant hybrids in treatments HI, LD and HD showed smaller reduction in studied traits. In both groups, stress influence on harvest index, weight of 1000 grains, leaf area and greening was smaller and insignificant. Comparing with LI treatment, the roots of LD, HI and HD increased their dry matter, number and length in the upper layer of the soil profile and the number of roots growing at the angle of 0-30 and 30-60 degrees in relation to the root main axis. Analysis of those traits revealed a pattern of defence responses. Our study shows that soil compaction and soil drought, which usually occur simultaneously, caused significant changes in components of plant yield and revealed the plants plasticity in responses to environmental factors under natural conditions.

1.5 - bHLH059 is not involved in warm temperature-modulated immunity in Arabidopsis thaliana

Christina Rossi[1], Christian Danve M. Castroverde[1]

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Plant diseases compromise plant health and lead to worldwide crop losses, which are exacerbated by our increasing global temperatures. Previous studies in the model plant *Arabidopsis thaliana* demonstrated suppression of the central plant defense hormone salicylic acid (SA) at elevated temperatures (28˚C) via downregulation of the master immune regulatory genes *CBP60g* and *SARD1*. However, the molecular mechanisms underlying temperature-sensitive immune system modulation remain largely underexplored. At normal, non-stress temperatures (16˚C and 22˚C), the basic helix-loop-helix transcription factor bHLH059 was previously discovered to act as a thermoresponsive SA-immunity regulator. In our study, we aimed to establish if bHLH059 is also involved in the regulation of SA at elevated temperatures. In planta bacterial disease assays showed that *bHLH059* mutants exhibit temperature-sensitive disease resistance, similar to the wildtype control accession Col-0, which is known to exhibit temperature-sensitive SA production. Preliminary gene expression analyses demonstrated that *CBP60g* and *SARD1* genes (encoding master immune transcription factors) are downregulated at higher temperatures in the *bhlh059-11 and bhlh059-14* mutants. Consistent with these results, pangenomic polymorphisms in *bHLH059* in natural *Arabidopsis* accessions did not strictly correlate with temperature-sensitive or -resilient immunity. SA quantification in these two *bHLH059* mutants is currently underway. Overall, our results suggest that bHLH059 may not be involved in the regulation of SA and plant immunity at elevated temperatures above the optimal growth range. This opens the horizon for potentially novel regulators governing the temperature-vulnerability of CBP60g/SARD1-mediated plant disease resistance.

1.6 - Differences in the timing of cold acclimation and deacclimation of pine and spruce species

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Conifers in boreal forests undergo an active growing season followed by a period of dormancy during the winter season. Evergreen conifers are dominant in northern temperate and boreal forests as they can withstand the extreme winter conditions because they are cold hardy. Cold hardiness involves physiological and biochemical processes that protect these conifers from freezing and low temperatures. We were interested in determining if there are species specific differences in the timing of cold acclimation and deacclimation in pine and spruce. We performed an experiment to determine species differences in the physiological response to cold acclimation and deacclimation in white spruce (*Picea glauca)*, Norway spruce (*Picea abies*) and Eastern white pine (*Pinus strobus*). For this purpose, we monitored photosynthetic traits using chlorophyll fluorescence from October to May. We found that all species showed a similar pattern with a decline in the maximum quantum efficiency of photosystem II (F_v/F_m), quantum yield of PSII photochemistry (Φ_{PSII}), and dynamic non-photochemical quenching (Φ_{NPQ}); and an increase in sustained non-photochemical quenching ($\Phi_{f,D}$). In the spring we saw an increase in F_v/F_m, Φ_{PSII}, and Φ_{NPQ}; and a decrease in $\Phi_{f,D}$. Interestingly, there were consistent differences in the timing between pine and spruce species in the dynamic and sustained components of NPQ during the autumn and spring. This indicates that there are species specific differences in the regulation of dynamic and sustained NPQ during the autumn and spring.

1.7 - Comparative transcriptome profiling shows differential responses between two Eutrema salsugineum ecotypes exposed to low phosphate and elevated salt

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[1]McMaster University, [2]Thompson Rivers University, [3]Louisiana State University

Eutrema salsugineum is a highly stress tolerant and halophytic relative of *Arabidopsis thaliana* and the oilseed crop, canola. *E. salsugineum* found in the Yukon, Canada, tolerates low inorganic phosphate (low-Pi) with seedlings displaying similar root architecture when grown on agar with low-Pi (0.05 mM) or Pi-supplemented (0.5 mM) conditions. In contrast, plants of an *E. salsugineum* ecotype from Shandong, China, had shortened primary roots and increased lateral root density when grown on media with 0.05 mM Pi compared to plants on 0.5 mM Pi agar. Using RNA-Seq, we found that the Pi treatment conditions led to 118 and 13 differentially expressed genes (DEGs) between Yukon and Shandong plants, respectively, including nine shared DEGs. The low number of Pi-responsive DEGs suggests transcriptomic reprogramming under low-Pi involves a small fraction of the full genome coding capacity. Gene Ontology analysis of the low-Pi responsive DEGs suggests that 144 stimulus-responsive processes and 13 metabolic processes were down-regulated in Yukon plants with only 24 processes responsive to stimuli down-regulated in Shandong plants. Only five and eight ion-homeostasis processes were up-regulated in Yukon and Shandong plants, respectively. The addition of 150 mM salt to low-Pi agar increased DEGs in Shandong plants to 5070 whereas under the same treatment no DEGs were detected for Yukon plants. This outcome suggests that salinity can differentially impact low-Pi transcriptomic reprogramming, even for closely related halophytes. Understanding how extremophile plants cope with low-Pi offers insights into the significant challenges of managing crops on global soils experiencing declining fertility and increasing salinization.

CONCURRENT SESSION 2: Cell Biology and Development

2.1 - Plant Plasma Membrane Nano-organization and Cell Signaling

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The molecular organization and dynamics of the plasma membrane (PM) play a crucial role in the regulation of diverse cell signaling events implicated in plant growth, develop and stress responses, yet our understanding of the mechanisms underlying its organization and dynamics in plants is still rudimentary. By studying multi-polar, puzzle piece-shaped leaf pavement cells in *Arabidopsis thaliana*, we discovered the first signaling protein/sterol nanoclustering-based mechanism for auxin-mediated ROP6 activation at the PM. In addition, we illustrated that the plant hormone auxin induces membrane lipid ordering and formation of ordered lipid nanodomains to coordinate the re-organization and activation of polarity signaling molecules for patterned cell morphogenesis. In my talk, I will also discuss how we combined data-based modeling with model-inspired experiments to study the contribution of membrane protein/lipid nanoclustering to cell polarity establishment. It is anticipated that a better understanding of membrane biology and its impact on signaling holds great promise for developing novel engineering strategies to modulate membrane nano-organization to improve plant growth.

2.2 - Natural variation analysis reveals two major SMAX1 variants that fine-tune the Karrikin dependent germination pathway.

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Karrikin (KAR) is a class of butenolide compounds that controls several aspects of plant development. In Arabidopsis, this molecule is perceived by an α/β hydrolase (KAI2), which interacts with an F-BOX protein (MAX2) to target the SMAX1-like family of transcriptional repressors for degradation.
One phenotype activated by SMAX1 degradation is germination. Although this phenotype is controlled by many different pathways, experiments using natural accessions often fail to fully explain the high variation in germination.
To assess if the KAR pathway can contribute towards this phenotypic variation, we first analyzed the genetic diversity across a global Arabidopsis population for the core signaling components of this pathway. We identified a region of very high genetic diversity in just one component of the pathway, SMAX1. This genetic diversity could be explained by two major variants of SMAX1 (SMAX1^{Cvi} and SMAX1^{Col-0}) that have been maintained in the Arabidopsis population, suggesting a history of balancing selection. Moreover, we found an association of those variants with environmental conditions such as temperature and light. *In silico* structure analysis of the two variants showed different conformations, suggesting a potential change in the interaction with other proteins. Pull-down assays indicated that SMAX1^{Col-0} interacted with KAI2 better than SMAX1^{Cvi} even in the presence of the ligand, possibly indicating that accessions carrying the SMAX1^{Col-0} variant are more likely to germinate than SMAX1^{Cvi}. Together these data suggest that SMAX1 might play a role as a key regulatory point to control germination in response to appropriate environmental conditions.

2.3 - RING E3 Ligase XERICO and ABA guide stomatal density and patterning through the SPCH-EPF signaling pathway

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Long-term adaptation to dehydration stress requires plants to adjust stomatal response and development by coordinating a diverse range of environmental and hormonal signals that restrict stomatal proliferation and modulate patterning. Abscisic acid (ABA) acts to restrict stomatal lineage to lower stomatal number. In Arabidopsis, XERICO (XER) is a stress-induced RING E3 ligase that promotes ABA accumulation and downstream responses, including regulating stomatal movements and density. Analysis of *Arabidopsis xer* null mutants revealed that *XER* inhibits stomatal development and ensures adequate stomata spacing, contributing to ABA-mediated suppression of stomatal development. *XER* is required for correct stomatal asymmetric cell division, due to enhanced rate of stomatal division errors that produce stomatal clusters in *xer-1*. Reporter analysis and expression kinetics revealed that stomatal clusters arise in ABA-deficient mutants *xer-1* and *aba2-2* due to reduced expression of *EPF1* and *EPF2*. Application of ABA partially rescued *epf1* and *epf2* stomatal defects, suggesting ABA can compensate for loss of stomatal signaling ligands. We show *XER* and ABA are required for adequate expression of *EPF1* and *EPF2* positional cues to fine-tune stomatal patterning. Genetic analysis demonstrated that *XER* converges on the *SPCH-EPF2* pathway to further suppress stomatal proliferation. My talk will detail how a XER-mediated regulation of stomatal density and patterning likely represents another branch by which the environment modulates stomatal development.

2.4 - Sugar code interactors in N-glycan quality control

Jenny Jiahui Huang[1, 2], Rowan K. Brookman[3, 4], Heather E. McFarlane[1, 2]

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During protein synthesis, covalent modifications affect protein conformation, function, and recognition by other molecules. N-glycosylation is the major co- and post-translational modification pathway that takes place across the endoplasmic reticulum (ER) and Golgi apparatus and is largely conserved across yeast, mammals, and plants. In the ER, N-glycosylation quality control is regulated by the N-glycan-binding proteins (lectins) calnexin and calreticulin. There is presumably also quality control present in the Golgi but the mechanism is unknown. Therefore, we first used bioinformatics to identify candidate lectin proteins in *Arabidopsis thaliana* that are predicted to be capable of reversibly binding glycans and localized to the Golgi. We then screened 92 mutants corresponding to 44 lectins for phenotypes that suggest that protein N-glycosylation is affected. We have identified several lectin candidates with phenotypes and are exploring their effects on glycoprotein trafficking and maturation. We are also developing fluorescent protein tools to understand how N-glycosylation impacts glycoprotein maturation and trafficking in live cells.

2.5 - The role of epigenetics in plant development: from profiles to phenotype

Katharina Braeutigam[1]

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In addition to the genetic information, epigenetic factors play central roles in shaping cell function and ultimately phenotype. Such factors can modify chromatin structure, modulate gene expression, and guide genome interpretation. Here, we investigate the role of epigenetic marks in reproductive development in *Populus*, i.e. trees with a wide natural distribution and economic importance in Canada.
Sexual reproduction is one of the most important processes in life: it ensures genetically diverse offspring and therefore the ability to survive in an ever-changing environment. Despite this, little is known about sex determination in several crop and forest species. Just recently, the mechanism of sex determination has been uncovered in poplars. Similar to humans, poplars separate male and female function onto different individuals (dioecy).
Here, we utilize high-resolution epigenome maps to study sex determination in the genus *Populus*. Using unbiased machine learning approaches on complex epigenome profiles allowed us to identify the central regulator of sex, a response regulator specific to female development. In males this regulator is silenced via RNA-directed DNA methylation. In-depth analyses of full male and female developmental programs from reproductive meristems to mature flowers further indicated successive waves of chromatin alteration to coordinate gene expression and to ensure correct spatial and temporal development. The discovery of this central regulator now raises interesting questions related to developmental pathways, its relevance in a broader taxonomic context, or the application of this knowledge in silviculture. Approaches can be expanded to advance our understanding of other developmental paths in forest trees and beyond.

2.6 - Arabidopsis CML13 and CML14, Interact with IQD, CAMTA, and Myosin Protein Families to Support Cytoskeletal Activity and Gene Expression

Kyle Symonds[1], Howard Teresinski[1], Bryan Hau[1], Takeshi Haraguchi[2], Kohji Ito[2], Vikas Dwivedi[3], Eduard Belausov[3], Sefi Bar-Sinai[3], Einat Sadot[3], David Chaisson[4], Wayne Snedden[1]

[1]Queen's University, [2]Chiba University, [3]The Volcani Center, Israel , [4]St. Mary's University

Calcium ions (Ca^{2+}) are ubiquitous secondary messengers in eukaryotes. Ca^{2+}-binding proteins, termed sensors, interpret Ca^{2+} signals and regulate downstream responses. In addition to the evolutionarily-conserved calmodulin (CaM), plants possess a unique family of CaM-like proteins (CMLs) that act as Ca^{2+} sensors. CMLs possess no functional domain besides Ca^{2+}-binding EF-hands and are thought to act as sensor-relays by undergoing Ca^{2+}-induced conformational changes and interacting with target proteins. Arabidopsis has 50 CMLs, of which the closely related paralogs AtCML13,14 stand out as biochemically unique. To understand CML13,14 function, we screened a yeast two-hybrid library and identified 3 unrelated families of putative targets: IQ67 domain proteins (microtubule scaffolds), CAMTAs (transcription factors), and myosins (motor proteins). These proteins share a structural feature, tandem IQ-motifs which are a special type of CaM-binding domain. Using *in vitro* and *in vivo* protein-interaction approaches we show that among the CML family, CML13, CML14, and CaM are likely the only interactors of these targets via their IQ domains. Using myosins as representative CML13,14 targets, a combination of confocal microscopy, *in vitro* kinetic assays, and *in vitro* binding tests, we provide data to show that these CMLs represent novel myosin light chains. To further explore their function, we used an inducible RNAi system to silence either CML13 or CML14 in Arabidopsis and observed very strong, pleiotropic phenotypes. Collectively, our data suggest these CMLs play important roles in development by regulating cytoskeletal activity via myosins and IQ67 domain proteins and may also participate in gene expression via CAMTAs.

2.7 - Transcriptional regulation of FUSCA3 during seed coat development in Arabidopsis thaliana

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Seeds assure the spread and survival of angiosperms and other higher plants. The Arabidopsis seed comprises three major compartments: the embryo, the endosperm, and the seed coat. The FUSCA3 (FUS3) transcription factor plays an important role in seed development by coordinating embryo and endosperm growth. Recently, we found that two NAC transcription factors, preferentially expressed in the seed coat, bind to the *FUS3* genomic region. *fus3* and *nac* loss-of-function mutant seeds showed altered seed coat morphology and mucilage release from the seed coat. Interestingly, in *35S:FUS3* and *35S:NAC* lines the seed coat is detached from the embryo, and "naked embryos" without a seed coat are found within the siliques. These seeds also show increased permeability and altered germination. We hypothesize that the FUS3-NAC module regulates cell wall related processes, which are required for seed coat development and seed permeability. Taken together, these results illustrate a role for the NAC-FUS3 module in shaping seed coat development, including mucilage deposition, seed coat permeability and adhesion.

CONCURRENT SESSION 3: Biochemistry/Post-translational modifications

3.1 - Investigating the role of CDPK-mediated phosphorylation on Arabidopsis E3 ubiquitin ligase AtATL6 in stress signaling

Faranak Soleimani[1], Devang Mehta[2], Tiffany Yip Delormel[3], Marie Boudsocq[3], R. Glen Uhrig[2, 4], William Plaxton[1], Jacqueline Monaghan[1]

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Protein phosphorylation and ubiquitination are important post-translational modifications that play pivotal roles in controlling the activation and duration of plant stress signaling. Calcium dependent protein kinases (CDPKs) exhibit both Ca^{2+} sensing and enzymatic activities in a single polypeptide that can directly translate Ca^{2+} signals into protein phosphorylation events. Amongst the 34 CDPK isozymes of the model plant *Arabidopsis thaliana*, AtCPK4 and AtCPK11 share high sequence identity (>90%) and function in several signal transduction and metabolic pathways, including abscisic acid signaling, immune gene expression, reactive oxygen species production, and programmed cell death. However, there are many gaps in our understanding of these CDPK paralogs, including the identification and characterization of additional AtCPK4/11 substrates. Here, we focus on the interaction of AtCPK4 with the ubiquitin E3 ligase *Toxicos en Levadura* 6 (AtATL6). AtATL6 functions as a positive regulator of AtBIK1-mediated immunity by targeting AtCPK28 for degradation via polyubiquitination. Previous work has shown that AtCPK5 can trans-phosphorylate AtATL6 and its close paralog AtATL31. Here, we will present evidence that AtCPK4 can also trans-phosphorylate AtATL6 *in vitro* on multiple residues. We will lay out our research plan to assess the impact of AtCPK4-mediated phosphorylation on the E3 ligase activity of AtATL6 and biological function using phosphoablative and phosphomimetic AtATL6 transgenic lines. Understanding how plants perceive stress signals and acclimate to adverse conditions can be translated to applications in biotechnology to improve crop resistance to different stresses.

Keywords: *Arabidopsis*, AtCPK4 and AtCPK11, E3 ubiquitin ligase, immune signaling, AtATL6, protein phosphorylation.

3.2 - The root-specific glutamate decarboxylase-1 (AtGAD1) is essential for efficient acclimation of Arabidopsis thaliana to nutritional phosphorus deprivation

Kirsten Benidickson[1], Lee-Marie Raytek[1], Gordon Hoover[2], Barry Shelp[2], Wayne Snedden[1], William Plaxton[1]

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Inorganic phosphate (**Pi**) is an environmentally limiting macronutrient critical for plant growth. Pi deprived (**-Pi**) plants elicit a Pi starvation response (**PSR**) that alters gene expression and metabolism to improve their efficiency of Pi acquisition and use. Our phosphoproteomics study discovered that the glutamate decarboxylase (**GAD**) isozyme AtGAD1 became hyperphosphorylated at multiple serine residues 48 h following Pi-resupply to -Pi *Arabidopsis* cell cultures (Mehta *et al*. 2021 Plant J). GAD is a Ca^{2+}/calmodulin-activated, cytosolic enzyme that catalyzes the first committed step of the γ-aminobutyrate (**GABA**) shunt, which bypasses two reactions of the tricarboxylic acid cycle. This research asks: do AtGAD1 and the GABA shunt play an adaptive role during *Arabidopsis* acclimation to Pi starvation? This was investigated by assessing the impact of Pi nutrition on the phenotype of wild-type (**WT**) plants versus two T-DNA insertional mutant *atgad1* lines. Biomass accumulation by -Pi, but not Pi fertilized *atgad1* plants was significantly reduced relative to that of WT; this was correlated with elevated leaf anthocyanin and reduced Pi levels in the -Pi mutants. Pi deprivation also triggered a marked, 71% increase in root GABA concentration of WT, but not *atgad1* plants. AtGAD1 and GABA are believed to play an adaptive role during various abiotic stresses. However, this is the first genetic evidence of their involvement in the plant PSR. Examining the interactions between GAD, GABA, and Pi nutrition may facilitate engineering 'Pi-efficient' crops, urgently needed to reduce inputs of unsustainable and non-renewable Pi fertilizers for long-term food security and ecosystem preservation.

3.3 - Investigating the biochemical function and biological roles of a unique family of multi-functional enzymes containing both a protein kinase and an E3 ligase domain in Arabidopsis thaliana

Thakshila Dharmasena[1], Natasha Kelkar[1], Nick Smith[1], Jacqueline Monaghan[1]

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Phosphorylation and ubiquitination are two major post-translational modifications (PTMs) that co-exist in nature, respectively catalyzed by protein kinases and ubiquitin ligases. While protein kinases and E3 ligases are usually encoded by distinct genes, there is a conserved family of proteins that contain both domains, which we call 'E3Ks'. Although these proteins are found across the plant lineage and have a long evolutionary history, their molecular and biological functions are completely unknown. Here, I will present my current work to unravel the biochemical mechanism and biological function of the most highly expressed E3Ks in the model plant Arabidopsis thaliana.

3.4 - A post translational phosphorylation at Ser600 is responsible for regulating arabidopsis lipoxygenase2, a key enzyme in jasmonate biosynthesis

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Plant defense against chewing herbivores or necrotrophic pathogens is regulated by a group of oxylipin phytohormones called jasmonates. *Arabidopsis thaliana* lipoxygenase2 (AtLOX2), an early enzyme in jasmonate biosynthesis, was previously reported to be post-translationally phosphorylated at Ser^{600}. AtLOX2 is constitutively phosphorylated and dephosphorylated in damaged leaves. The current study characterizes the kinetics of recombinant wildtype LOX2 ($AtLOX2^{WT}$). The enzyme shows positive cooperativity with a-linolenic acid (a-LeA), linoleic acid (LA) and arachidonic acid (AA) as its substrates. Higher enzyme velocity is observed with the substrates a-LeA and LA as pH increases. As well, the substrate affinity for a-LeA is lower in this range. Ser^{600} phosphovariants showed that pseudophosphorylation inhibits enzymatic activity. The phosphomimics $Atlox2^{S600D}$ and $Atlox2^{S600M}$ has negligible activity with a-LeA as its substrate. In contrast, the phosphonull variant $Atlox2^{S600A}$ has activity comparable to wildtype with all three substrates. AtLOX2 AlphaFold model reveals that Ser^{600} lies at the enzyme catalytic site, indicating its crucial position in the AtLOX2 structure. Overall, results indicated that AtLOX2 phosphorylation delivers a fundamental mechanism in the modulation of its activity and, consequently, the possible regulation of the jasmonate biosynthetic pathway.

3.5 - The influence of ferritins on protein carbonylation in Arabidopsis thaliana plants

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Protein carbonylation is an irreversible and non-enzymatic post-translational modification triggered by reactive oxygen species (ROS). The carbonylated proteins are often used as stress markers and degraded by the 20S proteasome system. But in recent studies, significant amounts of carbonylated proteins were found in unstressed Arabidopsis plants. How these proteins become carbonylated and impact the cellular processes is unknown. Since ROS-mediated protein carbonylation requires H_2O_2 and a transition metal like iron (Fe), we hypothesized that iron homeostasis could influence the occurrence of protein carbonylation *in vivo*. To investigate this, we compared the profile and the contents of the carbonylated proteins in the *Arabidopsis thaliana* wild type and mutant deficient in three ferritin genes. Additionally, we examined the proteins specifically carbonylated in the wild type seedlings exposed to iron-deficiency conditions by mass spectrometry. Our results revealed that certain proteins were differentially carbonylated between the wild type and the triple ferritin mutant *Fer1-3-4* in the leaves, stem, and flowers in either control or heat stress conditions. A change in the iron availability in the growth medium greatly influenced the carbonylation of certain proteins involved in the intracellular signal transduction, translation, and iron-deficiency response, in the absence of oxidative stress. Overall, the study revealed that i) the occurrence of protein carbonylation is influenced by iron availability, ii) several enzyme and stress-responsive proteins are carbonylated in the plants under control condition in the absence of stress. The results also suggested that protein carbonylation could be involved in the trigger of the plant response to iron-deficiency.

3.6 - Phosphorylation philosophicals: The regulation of AROGENATE DEHYDRATASE5 in Arabidopsis thaliana

Eileen Barac[1], Emily A. Hornung[1], Sara Abolhassani Rad[1], Susanne E. Kohalmi[1]

[1]Western University

Phenylalanine is an aromatic amino acid that is required by all organisms for protein synthesis. In plants, phenylalanine is particularly important for the synthesis of specialized metabolites. Plants can synthesize phenylalanine *de novo*, primarily using the arogenate pathway, where arogenate is converted into phenylalanine by an arogenate dehydratase (ADT). In *Arabidopsis thaliana*, a family of six *ADT*s has been identified. While all six *At*ADTs perform their enzymatic role in the chloroplast, *At*ADT5 is the only member which has been shown to also localize to the nucleus. Though its role in the nucleus remains unknown, an *in silico* analysis revealed several putative phosphorylation sites unique to *At*ADT5, potentially regulating its localization. To confirm *in vivo* that *At*ADT5 is a phosphoprotein, we are using bimolecular fluorescence complementation to test for interactions between *At*ADT5 and a 14-3-3 protein, since 14-3-3 proteins interact with phosphoproteins. We are also preparing to use mass spectrometry for a comprehensive analysis of post-translational modifications in *At*ADT5. As no ADT5 antibody is available, we will isolate total proteins from a stably transformed ADT5-CFP *Arabidopsis* line, using CFP to pull down ADT5. Furthermore, we are using phosphomimetics (on or off replacements) to determine the functional effects of phosphorylation events. The subcellular localization of the phosphomimetic proteins will be determined using confocal microscopy, and any change from the wildtype *At*ADT5 pattern will suggest that phosphorylation impacts its localization. This exploratory research will be the first to demonstrate the effect of phosphorylation on ADT5 functions.

3.7 - JAZ1 proteins bind NAC42 transcription factors to suppress the biosynthesis of diverse phytoalexins in plants

Jie Lin[1], Asraful Jahan[2], Ivan Monsalvo[1], Melissa Ly[1], Nik Kovinich[1]

[1]York Univeristy, [2]Shahjalal University of Science and Technology

Phytoalexins are defense metabolites produced by plants in response to pathogens and certain abiotic stresses. Many have important pharmaceutical activities, yet they are produced only in minor amounts in plants. Phytoalexins are biosynthesized by diverse metabolic pathways in different plant species, including the indole alkaloid, stilbenoid, and isoflavonoid pathways of Arabidopsis, grapevine, and soybean, respectively. Despite their biosynthetic heterogeneity, recent research has suggested that diverse phytoalexins share common regulatory networks. We recently identified the soybean transcription factors GmNAC42-1 and GmMYB29A2 that are essential and direct activators of glyceollin biosynthesis genes in soybean. Notably, they are homologous to Arabidopsis indole alkaloid and grapevine stilbenoid regulators. Yet neither of these transcription factors could activate phytoalexin biosynthesis when overexpressed in the absence of an elicitor treatment. Here, by searching our RNAseq data for genes that were oppositely regulated compared to glyceollin biosynthesis, we identified *GmJAZ1s* as the most highly represented gene function. RNAi silencing *GmJAZ1s* in elicited soybean hairy roots enhanced glyceollin biosynthesis 2-fold, whereas in the absence of an elicitor it activated biosynthesis to 60% of the elicited control. In contrast, overexpressing *GmJAZ1-9* inhibited the activation of glyceollin biosynthesis. The GmJAZ1-9 protein localized to the nucleus of soybean cells and physically interacted with the C-terminal domain of the GmNAC42-1 protein *in vivo* and *in vitro*, indicating a direct point of negative regulation of phytoalexin biosynthesis. Our results exemplify a strategy that could, in-part, be used bioengineer economical and elicitor-free production of phytoalexins for use in agriculture and the pharmaceutical industry.

CONCURRENT SESSION 4: Biotic/Abiotic Interactions

4.1 - Identifying E3s involved in maintaining nutrient homeostasis

Erin MacKinnon[1], Sophia Stone[1]

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To cope with abiotic stress, plants utilize a variety of molecular mechanisms, including the ubiquitin proteasome system (UPS), to facilitate changes to the proteome that facilitate tolerance. The UPS involves the attachment of ubiquitin, a small, highly conserved eukaryotic protein (ubiquitination) to select substrates followed by the proteasomal degradation. Ubiquitination is achieved through the sequential actions of three enzymes: the E1, which initiates the process, and the E2 that works with the substrate-selecting E3 to attach a chain of ubiquitin molecules to the substrate. The UPS has recently emerged as an important regulator of the uptake and translocation of essential nutrients, through the degradation of transporters, nutrient-responsive transcription factors and other regulatory proteins. This study aims to discover *Arabidopsis thaliana* E3s involved in maintaining nutrient homeostasis. Twelve E3s, which showed differential gene expression under iron (Fe), phosphorus (P) or nitrogen (N) limitation, were selected for analyses. Growth E3 loss-of-function mutants were compared to ecotype Columbia (*Col-0*) wild type seedlings under deficient, sufficient, and excess levels of Fe, P, and N. Preliminary results identified two E3s involved in responses to Fe deprivation and excess conditions, one enzyme facilitates P deficiency tolerance, and another mutation was found to alter responses to both P excess and N deprivation. RT-PCR results confirmed differential gene expression of these four under the stress which the corresponding mutants exhibited differential growth. Understanding the role of the UPS in nutrient uptake is important, as maintaining optimal levels of essential nutrients mitigates the negative effects of other environmental stresses.

4.2 - Legume hosts drive the evolutionary decline in symbiotic benefits under soil nitrogen addition

Rebecca Batstone[1], Katy Heath[2], Jennifer Lau[3]

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Plant resiliency to stressful conditions depends not only on genetic variation present within the plant's own genome, but also within their associated microbiomes. While some abiotic conditions select for microbes that benefit plants under those conditions, such as drought, others, including nutrient addition, can have the opposite effect. For example, in the legume-rhizobium symbiosis, whereby legumes trade carbon for nitrogen (N) fixed by rhizobia, long-term N-addition resulted in an evolutionary decline in the benefits rhizobia provide to their legume hosts. However, the selective agents driving this evolutionary shift remain unclear. Here, we experimentally evolved a population of rhizobia with or without legume hosts under both N-addition and N-free conditions across four plant growing seasons. At the end of the experiment, we created soil slurries from each pot, inoculated them onto host plants in N-free conditions, and measured plant growth. When legumes were present during experimental evolution, our results recapitulated previous findings: hosts benefited less when inoculated with rhizobia that had evolved under high- compared to low-N conditions. However, when legumes were absent during experimental evolution, we found no difference in growth when hosts were inoculated with rhizobia that had evolved in either N condition. Overall, our results suggest that the evolutionary shift towards less beneficial rhizobia under N-addition is mediated by the indirect effects of hosts rather than the direct effects of N itself. Accounting for plant-mediated drivers of microbial adaptation is therefore required to make more accurate predictions of the effects of environmental change on plant resiliency.

4.3 - Identifying the Strigolactone Receptor in Fungi

Michael Bunsick[1], James Bradley[1], Bruno Aquino[1], Flora Wang[1], Nina Marsh[1], Omar As'sadiq[1], Shelley Lumba[1]

[1]University of Toronto

When starving for phosphate, plants produce strigolactone: a phytohormone which facilitates nutrient exchanging symbioses with arbuscular mycorrhizal (AM) fungi. Unraveling strigolactone's precise role in these symbioses remains difficult, however, because AM fungi are challenging experimental systems. Here, we use genome-wide expression profiling of the model fungus *Saccharomyces cerevisiae* to identify the molecular events associated with strigolactone perception. We find that strigolactone strongly upregulates genes associated with the phosphate starvation response. Using a combination of forward and reverse genetics we demonstrate that strigolactone triggers phosphate starvation response by inhibiting the high-affinity phosphate transporter PHO84. Our data suggest that plant-derived strigolactone serves as an inter-kingdom signaling molecule which inhibits PHO84-dependent phosphate uptake in fungi.

4.4 - Bringing us together: Connections between Age-Related Resistance and Systemic Acquired Resistance

Garrett Nunn[1], Natalie Belu[1], Robin K. Cameron[1]

[1]McMaster University

Arabidopsis thaliana plants are susceptible to the bacterial pathogen *Pseudomonas syringae* at young developmental stages but display Age-Related Resistance (ARR) when mature, which includes the accumulation of antimicrobial intercellular salicylic acid (SA). Little is still known about what allows mature plants to respond in a resistant manner to normally virulent pathogens. RNA-sequencing was used to identify genes that contribute to ARR by examining global gene expression in leaves of young and mature plants responding to *Pseudomonas syringae.* Many genes were uniquely upregulated during ARR, including genes involved in Systemic Acquired Resistance (SAR). This data led to the surprising idea that ARR and SAR share signaling components. SAR is a defense response in which infection in one leaf leads to the production of signals that move to distant leaves and enhance defense. Subsequently primed distant leaves can resist a secondary infection. Analysis of the ARR transcriptome revealed the upregulation of biosynthesis genes for N-hydroxypipecolic acid (NHP), a signaling molecule required for the priming of distant leaves during SAR. The ARR response was examined in NHP biosynthesis mutants demonstrating NHP biosynthesis is required for ARR and the accumulation of antimicrobial intercellular SA during ARR. NHP biosynthesis genes and several cell-surface receptors were expressed in mature plants prior to infection, suggesting NHP may be involved in initiating defense priming during ARR. Our study suggests that ARR and SAR share NHP signaling and a priming stage, in which an initial infection (SAR) or reaching maturity (ARR), primes plants to respond effectively to pathogenic infections.

4.5 - Breaking the cell wall: Role of SRF receptors in the DAMP pathway

Aparna Haldar[1], Ishal Dave[1], Adam Mott[1]

[1]University of Toronto Scarborough

Plants cannot see danger, instead they rely on a large network of cell surface receptors to recognize environmental signals and integrate them into adaptive responses. My research focuses on a subset of receptors called leucine-rich repeat receptor kinases (LRR-RKs) of which there are approximately 225 members present in Arabidopsis. These LRR-RKs have been shown to have important roles in both plant growth and immunity, but the functions of many of these receptors remain unknown.
My project combines gene expression data along with protein-protein interaction networks to identify key LRR-RKs that are likely to play a role in biotic stress responses. Based on this analysis, I identified the SRF gene family to play a role in this process. I have shown that the SRF family of receptors is involved in the cell wall damage pathway and monitors the cell wall as a proxy for pathogen presence. Additionally, I have shown that mutation of members of the SRF family results in hyper-responsiveness upon being challenged with cell wall damaging enzymes. Bacterial growth assays were also performed and showed that the *srf* mutants were more susceptible to *Pseudomonas syringae* infection. These findings demonstrate that the SRF receptors play a complex role in the plant's cell wall damage sensing pathway.

4.6 - Strigolactones trigger phosphate starvation in fungi by inhibiting phosphate uptake

James Bradley[1], Michael Bunsick[1], Bruno Aquino[1], Dario Bonetta[1, 2], Peter McCourt[1], Shelley Lumba[1]

[1]The University of Toronto, Cell and Systems Biology, [2]Ontario Tech University, Oshawa

Phosphate is a major resource required for plant growth yet is often inaccessible in the soil. When phosphate is scarce, plants synthesise strigolactones (SLs) and exude these signals into the soil, where they recruit beneficial mycorrhizal fungi to plant roots, which in turn provides plants with additional phosphate for growth. Yet how SLs are perceived by fungi has remained unknown, in part due to the experimental intractability of these organisms. We used genetic, biochemical, and physiological experiments with *Saccharomyces cerevisiae* (Baker's yeast) to understand how SLs are perceived by fungi. We found that SLs trigger a phosphate-starvation response in yeast even when growing in phosphate-replete media. We found this was due to the inhibition of phosphate uptake through the phosphate transporter, *Sc*Pho84. I will present data demonstrating that SLs not only inhibit *Sc*Pho84 in Baker's yeast but are likely to be perceived through a similar mechanism in filamentous fungi that form agriculturally important associations with plants. Finally, I will comment on the implications this may have for understanding the mechanism of nutrient exchange between fungi and plants more generally.

CONCURRENT SESSION 5: Systems Biology and Technological Innovations

5.1 - Sightless but not blind - plants expand receptor kinase families related to stress adaptation

Zachary Kileeg[1], Adam Mott[1]

[1]University of Toronto Scarborough

Plant disease is a major contributor to crop loss around the world. Although many methods are in use to prevent these losses, tuning basal immunity through receptor kinases (RKs) offers a robust method for crop protection. RKs play an important role in plant immune responses, yet identifying stress-related RKs is challenging. In this research, I leverage the natural variation that exists within Arabidopsis thaliana and across plant species to identify stress-related adaptation. To do so, I first identified all receptor kinases across 26 ecotypes of Arabidopsis thaliana, clustered them into orthogroups, and examined their presence/absence variation. I found a subset of receptor kinase families containing rare genes and evidence of tandem duplication – both indicative of adaptive evolution. I then used these natural variants to calculate the rates of positive and negative selection across the leucine-rich repeat receptor-kinase (LRR-RK) family to identify potential selection events. Lastly, a plant-wide phylogenetic analysis found evidence that different plant orders were more likely to retain genes from specific LRR-RK subfamilies. Altogether, this research provides targets for biochemical analysis to increase our understanding of the role played by RKs in plant immunity.

5.2 - High-throughput Phenotyping of Growth in White Spruce Genotypes using Drone based Time-series LiDAR Remote Sensing Data

Aravind Harikumar[1], Gregory Millar[1], Siyu Wang[1], Malaika Gomes[1], Ingo Ensminger[1]

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White spruce is a dominant tree species in the eastern Canadian temperate and boreal forests. Extreme events and pest attacks triggered by climate change are causing large-scale damage and loss in spruce forests. Thus, the ability to monitor the physiological response such as growth (i.e., increase in height, stem diameter, foliage density, and biomass) of different spruce genotypes to biotic and abiotic factors is important to identify trees that are better adapted to future climate. Here we used drone-based Light Detection and Ranging (LiDAR) Remote sensing for fast and cost-effective monitoring and estimation of tree-level physiological parameters in large forests. We used time-series tree-level LiDAR data collected from low-flying (i.e., less than 50 meters above ground) drones to model physiological parameters of spruce genotypes. We performed all the experiments in a juvenile spruce site located near Pintendre, Quebec, Canada, with 6308 trees representing 1811 different spruce genotypes. Drone data were collected four times for the entire experimental field site between June and October 2022, and the reference data were collected on the field for a set of 90 selected trees. In general, the height increments in tree genotypes were found to follow a Gaussian distribution with the minimum, the mean, and the maximum values at 0.05, 0.24, and 0.55 meters, respectively. The low root mean squared error of 0.06 meters associated with the height increments proves the ability of the proposed high-throughput approach to accurately perform growth phenotyping at large-scale.

5.3 - Comparing Phenotypic Selection with Machine Learning-Based Genomic Selection for Developing New Varieties of Common Bean (Phaseolus vulgaris): A Validation Study

Robert McGee[1], Isabella Chiaravalotti[1], Valerio Hoyos-Villegas[1]

[1]McGill University

By 2050 the world population is projected to increase by two billion, and to feed these people at least 50% more food needs to be produced. To address this global challenge, new plant cultivars need to be rapidly developed that are high yielding, and tolerant to diseases, pests, and extreme weather events brought on by climate change. These types of complex plant traits are, however, challenging to breed for due to their low rates of heritability. Genomic selection (GS) is a machine learning-based plant breeding approach, which has been shown to outperform conventional phenotypic selection (PS) when breeding for complex plant traits in terms of selection accuracy, genetic gains, and time. However, most GS studies to date have not been empirically validated. In this project, the main objective is to determine if GS outperforms PS in terms of selection accuracy, genetic gains, and time when developing new cultivars of common bean (*Phaseolus vulgaris*) market classes such as kidney, cranberry, and black beans. GS models, that are currently being trained using phenotypic data from a previous field season and genotyping data from a SNP array, will be used to select the best common bean parents for crossing. Once advanced to the F_5 generation, yield, days to maturity, and disease tolerance will be measured in multiple field to trials to determine which of the two approaches; GS vs PS, performed best. Elite lines will be the basis for releasing superior cultivars to Canadian farmers and increase yield under a changing climate.

5.4 - Towards virtual structure-function analyses of protein-ligand interactions using computational docking and in silico mutagenesis

Carlo Perolo[1], Heather McFarlane[1], Nicholas Provart[1]

[1]University of Toronto

Computational molecular docking can be used to predict bound conformations of small molecules (ligands) to a protein of interest. Molecular docking software can predict protein-ligand interactions, but they require structures of both ligand and protein. Therefore molecular docking is limited by the significant bottleneck of determining a protein structure. However, the recent advances in the field of protein structure prediction could make molecular docking a valuable tool for researchers in every biological field. Mutations conferring single amino acid changes can be used to directly test protein-ligand interactions, but generating these different protein variants is time-intensive. Here we show that *in silico* mutagenesis using Pymol's mutagenesis tool and molecular docking via Autodock Vina can correctly predict the effect that known mutants have on protein-ligand interactions, using the *Arabidopsis thaliana* receptor BRASSINOSTEROID-INSENSITIVE 1 (BRI1) and its natural ligand brassinolide as a model system. Molecular docking simulations of brassinolide via Autodock Vina on known and predicted BRI1^{G644D} structures differ similarly from docking simulations on wild-type BRI1. *In silico* mutagenesis and molecular docking can therefore be used to "screen" for key residues involved in protein-ligand interactions before conducting wet lab experiments. We are developing an intuitive, web-based tool in the Bio-Analytic Resource (BAR), which integrates Pymol *in silico* mutagenesis with molecular docking, allowing users to conduct their own analysis of protein-ligand interactions.

5.5 - Leveraging phosphoproteomics to uncover mechanisms of cell wall integrity signaling

Eduardo A Ramirez Rodriguez[1], Rylan Vincent[1], Leo Tullo[1], Heather Mcfarlane[1]

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Plants cell walls are polysaccharide matrixes that protect and surround plant cells. These dynamic structures are finely tuned to balance rigidity for protection and structure, and flexibility to grow and respond to environmental cues. Bioengineers looking to exploit these properties have targeted the biosynthetic enzymes to increase the production of cell wall components, yet this approach has had limited success. These results suggest that an underlying regulatory mechanism, called 'cell wall integrity' (CWI) signaling, perceives changes to the cell wall, and in turn, remodels the cell wall and/or regulates plant growth. Various of membrane-bound kinases have been implicated in signal perception of CWI; however, the downstream components remain largely uncharacterized. To identify new CWI signaling components, we undertook a phosphoproteomic approach, by treating plants to induce cell wall stress and analyze phosphorylation state of the Arabidopsis proteome using high-resolution mass spectrometry. Data from 3 independent experiments (n=9) identified 242 differentially phosphorylated sites in 231 proteins. Then, we conducted a bioinformatic analysis for all candidates integrating gene ontology terms, protein-protein interaction networks, phosphomotif analysis, and comparative analysis with other stress-induced phosphoproteomes. These results guided selection of 30 candidates for a reverse genetics screen under cell wall stress conditions. Results from this screen implicate a set of three intracellular kinase candidates, now referred as ISOXABEN PHOSPHO-RESPONSIVE or IPR, in CWI. Further characterization of the most responsive mutants is underway.

5.6 - Characterizing drought response phenotypes of guard cell-specific genes in Arabidopsis thaliana

Paul Gamueda[1], Anna van Weringh[1], Abdeljalil El Habti[2], Nicholas Provart[1, 3]

[1]University of Toronto, [2]University of Adelaide, [3]Centre for the Analysis of Genome Evolution and Function

Drought, an abiotic stress factor, is projected to increasingly affect crop growth in arable lands, threatening global food security. It also stimulates stomatal closure, resulting in reduced water loss via transpiration. Since stomata can regulate water content, many studies have investigated the potential of manipulating stomata to regulate transpiration as a countermeasure to the projected increase in drought incidence. To this end, Anna van Weringh, a Ph.D. candidate in the Provart lab, profiled differentially expressed guard cell-specific drought-responsive genes using an adapted nuclear RNA isolation protocol called INTACT. Using a reverse genetics approach, I aim to characterize GC-specific drought-upregulated genes in A. thaliana. To identify phenotypically interesting candidates, multiple filtering processes were used, followed by initial screening using a thermal imager to estimate wild-type (WT) and knockout (KO) plant temperatures; candidate genes were chosen based on a phenotype of cooler temperature. In collaboration with Dr. Abdeljalil El Habti, a postdoctoral fellow at the University of Adelaide, stomatal aperture and density are being quantified using an AI model that recognizes stomata. KO plants are hypothesized to have increased stomatal aperture compared to WT plants, with stomatal density as a confounding variable. The end goal of this project is to characterize select guard cell-specific drought-responsive gene KO phenotypes and characterize the genes within molecular pathways through overexpression studies and Y2H assays, respectively. Studying these GC-specific genes can identify possible mechanisms for plant acclimation to drought conditions and add to the current understanding of drought response mechanisms of genes that influence water use.

CONCURRENT SESSION 6: Metabolism

6.1 - Engineering Chlamydomonas reinhardtii for heterologous synthesis of cannabinoids

Serge Basile Nouemssi[1], Natacha Mérindol[1], Fatma Meddeb-Mouelhi[1], Hugo Germain[1], Isabel Desgagné-Penix[1]

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Cannabinoids are a family of specialized metabolites produced by *Cannabis sativa,* and some members of this family show bioactive properties. Lately, the demand for cannabis products and cannabinoids has rapidly grown due to its legalization in several countries for medical and/or recreational purposes. The current production of pharmaceutically relevant cannabinoids relies primarily on extraction and purification from *Cannabis*, which is challenging due to variability in the quantity and quality of specific cannabinoids in the plant extract. To overcome this, alternative approaches such as heterologous production of specific and unique cannabinoids in microorganisms (*e.g.*, bacteria, yeast, and microalgae) have been proposed. *Chlamydomonas reinhardtii* is a common single-celled photosynthetic microalgae populating fresh water and moist soil. With a complete sequenced genome and efficient molecular tools, *C. reinhardtii* emerges as a suitable production platform for biotechnological purposes. Thus, engineering *C. reinhardtii* for the heterologous synthesis of cannabinoids may provide a cost-efficient and rapid manufacturing platform to acquire high quantities of specific cannabinoids, as well as novel derivatives. In this study, we transformed by electroporation the *C. reinhardtii*'s nuclear genome with genetic constructs that included two endmost genes encoding enzymes of the cannabinoid biosynthetic pathway, specifically the aromatic prenyl transferase (APT) and the cannabidiolic acid synthase (CBDAS). Stable transformants were selected and characterized for the expression of the APT and CBDAS by high throughput colony-polymerase chain reaction (HT-colony-PCR) and real-time quantitative PCR (RT-qPCR). The production of the desired cannabinoid molecule in relation to the transgene expression was analyzed by HPLC-MS and will be presented.

6.2 - Untargeted isotopolog metabolomics reveals the antagonism of methyl jasmonate and salicylic acid in central carbon metabolism of Arabidopsis.

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The untargeted analysis of ^{13}C label incorporation into whole Arabidopsis rosettes labeled with $^{13}CO_2$ revealed insights into the metabolism of over 100 central carbon metabolites. By using ammonia chemical ionization gas chromatography mass spectrometry, we were able to unambiguously verify the labeling patterns of previously difficult to detect metabolites including shikimate, intermediates of the tricarboxylic acid (TCA) cycle, and intermediates from amino acid, lipid, and sugar metabolism. In Arabidopsis control plants, we observed that while most intermediates of the photorespiratory cycle achieved a labeling plateau of ~35% ^{13}C incorporation, glyoxylate, a key intermediate in the pathway, plateaued at only 4% labeling over the same time course and featured a smaller pool size, suggesting that flux through the photorespiratory pathway deviates from the generally accepted route through glyoxylate. Elicitation with jasmonates unexpectedly revealed a reprogramming of the TCA cycle. TCA flux in illuminated leaves is noncyclic with citrate mainly exported to the cytosol. Following treatment with methyl jasmonate, the export of citrate decreased, and flux returned to a cyclic pattern resembling nighttime activity. Jasmonate treatment also inhibited photorespiration at the glyoxylate transamination step. Salicylic acid treatment induced the opposite effects in both cases, promoting cyclic TCA flux, export of citrate, and photorespiratory transamination of glyoxylate. The impacts of these defensive phytohormones on terpenoid metabolism are discussed. These findings highlight the hypothesis-generating power of unbiased metabolomics when combined with whole plant isotopic labeling experiments and affirm the antagonistic nature of salicylates and jasmonates in central metabolism.

6.3 - RUBISCO β-elimination supplies pyruvate to the chloroplast 2C-methyl-D-erythritol-4-phosphate pathway

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The 2*C*-methyl-D-erythritol-4-phosphate (MEP) pathway converts pyruvate and D-glyceraldehyde-3-phosphate (GAP) into isopentenyl (IDP) and dimethylallyl diphosphate (DMADP) for isoprenoid biosynthesis in the plastid. While GAP is supplied via the Calvin-Benson cycle, pyruvate cannot produce in the chloroplast through glycolysis, and its source remains poorly understood. Carbon reimports, alternative glycolytic routes, and even Rubisco represent viable alternatives. When plants are fed $^{13}CO_2$, intermediates of the MEP pathway reach labeling plateaus of ~60%. The lack of complete labeling has been attributed to reimport of central intermediates from the cytosol, including phosphoenolpyruvate (PEP) via the PEP/phosphate translocator, which can potentially be converted to pyruvate for terpene synthesis. Here, we performed $^{13}CO_2$ kinetic labeling in intact Arabidopsis and measured ^{13}C incorporation into central metabolites. We observed fast ^{13}C incorporation into pyruvate that precedes PEP labeling kinetics and instead parallels Calvin-Benson cycle labeling, suggesting a PEP-independent source of pyruvate in the chloroplast. The Entner-Doudoroff (ED) pathway, a prokaryotic shortcut through glycolysis, constitutes a third possible source of pyruvate and GAP, but the role of this pathway in higher plants is uncertain Biochemical characterization revealed that plant dehydratases proposed to be involved in the ED pathway actually participate in the branch chain amino acid pathway and cannot supply pyruvate. The kinetic labeling and biochemical results presented here are instead consistent with the Rubisco b-elimination reaction as the most likely source of pyruvate for terpene biosynthesis in the chloroplast. This finding implicates Rubisco directly in terpene biosynthesis for the first time.

6.4 - A lesion-mimic mutant of Catharanthus roseus accumulates the opioid agonist, akuammicine

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Catharanthus roseus is a medicinal plant that produces an abundance of monoterpenoid indole alkaloids (MIAs), notably, anticancer compounds vinblastine and vincristine. While the canonical pathway leading to these drugs has been resolved, the regulatory and catalytic mechanisms controlling many lateral branches of MIA biosynthesis remain largely unknown. Here, we describe an ethyl methanesulfonate (EMS) *C. roseus* mutant (M_2-117523) accumulating high levels of MIAs. The mutant exhibited stunted growth, partially chlorotic leaves, and a lesion-mimic phenotype. The lesions were sporadic and spontaneous, appearing after the first true bifoliate and continuing throughout development. The lesions were also the site of high concentrations of akuammicine, a minor constituent of wild type *C. roseus* leaves. The lesions were further enriched with 25 other MIAs, resulting, in part, from a higher metabolic flux through the pathway. The unique metabolic shift was associated with significant upregulation of biosynthetic and regulatory genes involved in the MIA pathway, including the transcription factors *WRKY1*, *CrMYC2*, and *ORCA2*, and the biosynthetic genes *STR*, *GO*, and *Redox1*. Following the lesion-mimic mutant (LMM) phenotype, the accumulation of akuammicine is jasmonate (JA)-inducible, suggesting a role in plant defence response. Finally, the unique chemical phenotype of this mutant can be a source for akuammicine. This stereochemically complex molecule is a promising medicinal target, acting as a weak opioid agonist with a preference for the non-addictive *κ*-opioid receptor. It is also touted as a potential anti-diabetic drug candidate. Further study of akuammicine biosynthesis and regulation can guide plant and heterologous engineering for medicinal uses.

6.5 - A multi-product farnesyl diphosphate synthase-like protein supplies geranyl diphosphate for cytosolic monoterpene biosynthesis in Pelargonium graveolens

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Monoterpenoids are a principal component of essential oils produced in glandular trichomes of rose-scented geraniums (*Pelargonium spp*). These ten-carbon volatiles can be cyclic or acyclic and are characterized by their pleasant aromas. Biosynthesis of monoterpenoids occurs mainly in the plastid whereby the products of the 2C-methylerythritol-4-phosphate pathway, isopentenyl and dimethylallyl diphosphate (IDP and DMADP), are condensed to geranyl diphosphate (GDP), which serves as the primary precursor to monoterpenoid production. In *Pelargonium*, monoterpene biosynthesis occurs both in the plastid and in a separate pathway that forms exclusively acyclic monoterpene alcohols in the cytosol. However, the source of GDP for the latter is unclear. Here we show that a cytosolic farnesyl diphosphate synthase (FDS)-like protein (FDL2) from Pelargonium graveolens catalyzes the formation of GDP and farnesyl diphosphate (FDP) in a 1:4 ratio in in vitro assays. The highest production of GDP was observed when FDL2 was incubated with DMADP and isopentenyl diphosphate isomerase, which reflects likely in vivo conditions. In contrast, a related sequence identified in the same whole leaf transcriptome, FDL1, produced exclusively FDP. FDL1 and FDL2 are 83% identical at the amino acid level and both localize to the cytosol. Both encode proteins with high similarity to typical FDS sequences from the plant kingdom. The acquisition of partial GDS activity by FDL2 appears to be the result of numerous point mutations following gene duplication of an ancestral FDS sequence. We conclude that FDL2 is the likely source of GDP in the cytosol supplying acyclic monoterpene biosynthesis in Pelargonium.

6.6 - Strategy for High-Yield Expression of Recombinant Proteins in the marine diatom Phaeodactylum tricornutum

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Compared with the bioreactor systems that are currently in use, microalgae are an attractive alternative for the production of pharmaceuticals, recombinant proteins, and other valuable products. In the last decade, the marine diatom *Phaeodactylum tricornutum* has been highly studied for its potential as a platform for metabolic engineering and molecular farming. However, such strategies come with challenges, like proteolytic processing, which is yield-limiting, leading to either partial or complete degradation of recombinant proteins. To overcome these challenges and generate high-expression strain systems, we propose to over-express a putative *P. tricornutum* cystatin (CYS). CYS proteins are cysteine protease inhibitors, which can cooperate and stabilize co-expressed partners by protecting them from endogenous proteolysis, resulting in their accumulation. It has been shown, by the co-agroinfiltration of the tomato SlCYS8 with heterologous genes, that SlCYS8 increased the yield of heterologous proteins. We have identified a putative *P. tricornutum* CYS by blasting as queries 21 plants' CYS amino acid sequences. The Phatr3_EG01374.p1 sequence was selected, presenting 143 amino acids, 34.38% of identity, and 58% of coverage with *Hibiscus syriacus* CYS (XP_038995199.1). The coding sequence was amplified from the diatom cDNA, then cloned under the constitutive promoter *Elongation Factor a*, and tagged with three hemagglutinins. *P. tricornutum* was transformed with/without a plasmid encoding the *yellow fluorescent protein* gene to analyze its production. We put forward this as a novel approach to stabilize and increase heterologous protein production in microalgae as a valuable biofactory platform.

POSTER ABSTRACTS

ABIOTIC STRESS

P1 - Daily changes in the hydration of leaves or roots and hydraulic conductivity in maize grown under simultaneous exposure to the compact soil and to the soil drought

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The aim of our study was to determine changes in a hydraulic conductivity (*K*), water potential (ψ) and water deficit (WD) in roots and shoots of maize hybrids subjected to simultaneous exposure to low or high soil compaction (LI, HI) combined with short or prolonged soil drought (LD, HD). Measurements were carried out four times within 24 hours using a high pressure flow meter (HPFM). Changes in the K_{ROOT} or K_{SHOOT} were dependent on the hybrid stress susceptibility index (SSI) and a time of a day. The lowest *K* were observed at night, while the increase occurred in the morning and the afternoon. In all cases, K_{ROOT} was smaller compared to K_{SHOOT}. A significant decrease in *K* was observed in HI in comparison with plants grown under LI conditions. Moreover, the greatest decrease in *K* was observed in plants grown in high soil compaction and in prolonged drought (21 days). Changes in *K*, especially in the aboveground parts, differed for the sensitive and resistant hybrids, and regulated the water balance. Variartion of *K* in the sensitive hybrid was more distinct. Differences in K_{ROOT} and K_{SHOOT} were accompanied by water potential (ψ) and water deficit (WD), which were significantly correlated with *K*. Also in resistant hybrid a smaller influence of the stresses on dry matter of roots and leaves, membranes injury and specific leaf area index was observed. That may indicate stronger reduction in water loss.

P2 - The relationship between flavonoid metabolism and the drought response of Phaseolus vulgaris

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Throughout the world dry bean (*Phaseolus vulgaris*) production is limited by drought. In other plants, flavonoids accumulate in leaves and roots in response to reduced soil water availability. Dry bean also contains flavonoids, specifically flavonols and isoflavones, and coumestans (e.g., coumestrol). Moreover, coumestrol amasses in the roots of drought-stressed soybean. It is unknown whether similar metabolic events occur in drought-stressed dry bean. To investigate this, two *P. vulgaris* recombinant inbred lines (RILs) varying in their capacity to produce isoflavones with biotic stress (i.e., infection with the causal agent of bacterial blight) were cultivated in a controlled environment room under either 75% relative soil water content (RSWC) or 15% RSWC. Plants from each RIL/water supply treatment combination were sampled periodically over a 12-day period and assessed for water use efficiency and biomass. Regardless of RIL, plant fresh and dry weight were dramatically increased at 75%, RSWC, whereas little change in biomass was evident at 15% RSWC. Water use efficiency was stable and mostly unaffected by the RSWC. A subset of the harvested leaves and roots from each plant were frozen for downstream UHPLC-MS/MS analysis of flavonoid profiles. The metabolite profiling will determine whether cultivation of one or both *P. vulgaris* RILs under 15% RSWC increases the concentrations of specific flavonols and isoflavones in leaves and roots, respectively. The results of this research will provide critical information for future genetic breeding and/or biotechnology strategies aimed at enhancing drought resistance in dry beans.

P3 - Which is better? Exploring cuticular wax and trichome responses to heat and drought in bread wheat

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Canada is one of the largest bread wheat (*Triticum aestivum L.)* producers in the world and the bulk of this production occurs in Western Canada. Climate models have predicted that Western Canada will be particularly vulnerable to rising temperatures and drought events, posing a risk to wheat production. Because of this, there is a need for more drought and heat tolerant bread wheat varieties. Plants have structural defences, such as cuticular waxes and trichomes, to shield them from abiotic stress. These structures can lower leaf canopy temperatures, reflect light, and reduce non-stomatal water loss. Although there have been studies on the cuticular waxes and trichomes of bread wheat varieties outside of Canada, the responses of Canadian bread wheat cultivars are not well understood. To address this gap, the Canadian bread wheat variety AAC Tradition was subjected to a heat and drought experiment. The results revealed that drought stressed samples experienced a significant increase in wax production and trichomes on the adaxial surface. Surprisingly, heat stress and heat in combination with drought did not yield significant increases in wax production. Instead, an increase in the abaxial trichome density was observed. Further research into these two responses can help wheat breeders determine which responses to include into newer varieties, to withstand future climate scenarios.

P4 - Palmelloid formation alters the organization of the photosystems which enhances photoprotection in the Antarctic psychrophile, Chlamydomonas priscuii

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Cultures of the obligate, Antarctic psychrophile, *Chlamydomonas priscuii* grown at permissive low temperature (8°C) are composed of flagellated, single cells, as well as non-motile, multicellular palmelloids. The relative proportions of the two cell types are temperature dependent. However, palmelloid formation is not restricted to psychrophilic *C. priscuii* but appears to be a general response of mesophilic Chlamydomonas species to non-permissive growth temperatures. To examine potential differences in photosynthetic performance between single cells versus palmelloids of the psychrophile, a cell filtration technique was developed to separate single cells from palmelloids. Compared to single cells, palmelloids of *C. priscuii* showed a decrease in the abundance of light harvesting complex II (LHCII) proteins with a 2-fold higher Chl a/b ratio and a decrease in both lutein and β-carotene. Chlorophyll fluorescence analyses revealed that isolated palmelloids exhibited lower excitation pressure, measured as 1-qL, but higher yield of PSII (Φ_{PSII}) and 50% higher rates of electron transport (ETR) than single cells exposed to high light at 8°C. This decreased sensitivity to high light in isolated palmelloids compared to single cells was associated with greater non-regulated dissipation of excess absorbed energy (Φ_{NO}) with minimal differences in Φ_{NPQ} in *C. priscuii* in response to increasing irradiance at low temperature. The ratio Φ_{NO}/Φ_{NPQ} observed for palmelloids (1.414 ± 0.036) was 1.38-fold higher than Φ_{NO}/Φ_{NPQ} of single cells (1.021 ± 0.018). The differences in the energy quenching capacities between palmelloids and single cells are discussed in terms of enhanced photoprotection of *C. priscuii* palmelloids against low temperature photoinhibition.

P5 - Feeding our Future: Effects of Elevated CO2 and Temperature on Wheat

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The objective of my project is to quantify how 20 different Canadian wheat varieties respond to elevated temperature and CO2 concentrations. To answer this question, five replicates of each variety were grown in multi-factorial temperature and CO2 treatments over the last growing season, while another replicate will be performed during the next. Wheat is a staple source of calories and protein in the diets of the global population. On a regional and national level, bread and durum wheat are two of the major staple commodity crops produced in Western Canada, comprise over 50% of Saskatchewan's exports, and Canadian wheat contributes heavily to global wheat exports. The goal of this project is to contribute to increasing the resiliency of Canadian wheat production to future climates, increase global food security, promote economic activity in Western Canada, and maintain or increase Canada's competitiveness in the global market. This poster will provide initial insights on how different varieties of Canadian wheat respond to the key drivers of climate change.

P6 - Identification of drought-responsive genes by differential splicing analysis of guard cell transcriptomes

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By the end of the 21st century, the global prevalence of extreme drought is projected to more than double, posing a major threat to global food security and necessitating the development of drought tolerant crops. To investigate the transcriptional response to drought, our lab has generated a guard cell-specific transcriptome using *Arabidopsis thaliana* guard cell nuclear RNA. In addition to identifying thousands of drought-induced differentially expressed genes, we have also demonstrated a widespread role for differential alternative splicing, where the ratio of splice isoforms of the same gene changes under drought stress. While differential splicing doesn't usually alter total gene expression, it has the potential to drastically modify the transcriptome by affecting transcript localization and stability. Interestingly, differentially spliced genes are generally distinct from the differentially expressed population, indicating that gene expression and splicing are largely independent pathways for stress-induced transcriptional remodeling. Using RT-PCR, we confirmed several predicted differential splicing events and demonstrated differences between the alternative transcript populations of guard cell cytoplasmic and nuclear RNA. Since many alternative transcripts are undetectable in the cytoplasm, they are unlikely to be translated and are instead degraded or retained in the nucleus to regulate gene expression. To probe the evolution of gene expression regulation by alternative splicing, we investigated the conservation of alternative introns and their regulatory regions across plant genomes. We found evidence of lineage-specific alternative splicing regulation, suggesting that alternative splicing may be rapidly evolving in plants.

P7 - Differential phosphorylation correlates with activation of the root-specific glutamate decarboxylase-1 (AtGAD1) in phosphate-starved Arabidopsis

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Inorganic phosphate (**Pi**) is a critical, yet limiting macronutrient essential for many aspects of plant metabolism, particularly photosynthesis and respiration. Pi deficient (-Pi) plants induce a 'Pi starvation response', wherein altered gene/protein expression and post-translational modifications enhance Pi acquisition and use efficiency. Using LC-MS/MS, we recently discovered that Pi-resupply to -Pi *Arabidopsis* cell cultures triggered multi-site hyperphosphorylation of glutamate decarboxylase-1 (AtGAD1) (Mehta *et al.* 2021 *Plant J*). Furthermore, the growth and GABA accumulation of –Pi *atgad1* knockout mutants were impaired relative to WT plants (see ERM talk by K. Benedickson). AtGAD1 is a cytosolic Ca^{2+}/calmodulin-dependent, root-specific isozyme that decarboxylates glutamate into 4-aminobutyrate (GABA) as the first committed step of the GABA shunt. Although GAD phosphorylation has been documented in various plant phosphoproteomic studies, the role of this phosphorylation remains unknown. Here, we ask: how does phosphorylation control AtGAD1 and the GABA shunt? Purified native AtGAD1 from both -Pi and Pi-sufficient (+Pi) *Arabidopsis* cells (–P:GAD1 & +P:GAD1, respectively) were analyzed by LC-MS/MS and phosphosite-specific antibodies. The specific activity of purified –P:GAD1 was about 60% greater than that of +P:GAD1 at optimal pH 5.8 (32 ±1.0 and 20 ±0.7 µmol GABA produced/mg, respectively). Pi deprivation or resupply did not alter the diffuse cytosolic localization of AtGAD1-mCherry transiently expressed in *Arabidopsis* cells We hypothesize that AtGAD1 activation by differential phosphorylation mediates increased GABA accumulation and GABA shunt flux, and that this represents an important and novel aspect of the metabolic adaptations of –Pi *Arabidopsis*.

P8 - Activation of a singlet oxygen signaling pathway by competition cues in Arabidopsis thaliana

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Oxidative stress responses of *Arabidopsis* to low red (R) to far-red (FR) signals (R:FR ≈ 0.3), generated by a biological weedy and an artificial source of FR light, were compared with a weed-free control (R:FR ≈1.4). In the low R:FR treatments, induction of the shade avoidance responses coincided with increased singlet oxygen ($^1O_2$) production and decreased levels of superoxide and superoxide dismutase activity. Although the increase of $^1O_2$ was not due to protochlorophyllide accumulation and did not result in cell death, treatments with the $^1O_2$ generator 5-aminolevulinic acid increased sensitivity to cell death. Transcriptome responses minimally resembled those reported in four *Arabidopsis* $^1O_2$ generating systems such that only a few genes (6 out of 1931) were consistently up-regulated supporting the specificity of $^1O_2$ signaling. Moreover, suppressors of jasmonate accumulation including the $^1O_2$-responsive amidohydrolase *ILL6 and* the sulfotransferase *ST2a,* which is involved in the prioritization of elongation growth versus defense were consistently up-regulated. Our data support a model in which photoreceptors link low R:FR light cues to the JA signaling pathway. Repression of bioactive JAs via the amidohydrolase *ILL6* and sulfotransferase *ST2a* may promote shade avoidance (versus defense) and $^1O_2$ acclimation (versus cell death) responses to competition cues.

P9 - Responses of Balsam Poplar to Extreme Weather Events Simulating Late 21st Century Climate

Laura Jones[1], Andrew Yu[1], Oscar Nunez[1], Katharina Braeutigam[1]

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Temperate climates worldwide are shifting toward increasingly frequent and severe heat waves and droughts that put tree survival and forest health at risk. Rising levels of atmospheric CO_2 can mitigate some of the negative impacts of heat and drought on plant performance, but these positive effects are typically modest and decline over time. Much remains to be learned about how trees acclimate to abiotic stress in the context of future atmospheric CO_2. Using *Populus balsamifera* as a model, we seek to understand how seedlings respond to and recover from stress, simulating projected climatic conditions in western-central Canada from 2081-2100. Here, we investigate physiological responses to (i) heat waves and (ii) drought at elevated atmospheric CO_2. Poplar seedlings exposed to a single heat wave eventually resumed growth and reached pre-stress levels of photosynthetic efficiency and evaporative cooling. Seedlings subjected to repeated heat waves survived but accumulated damage over time. They ceased growth, showed a reduction of PSII quantum yield, and a decline in evaporative cooling. When exposed to drought conditions under elevated CO_2 seedlings took longer to show stress symptoms compared with plants grown at ambient CO_2 levels, suggesting that CO_2 fertilization buffers against drought stress. Physiological data will now be complemented with molecular analyses of carefully selected genes coding for stress markers and miRNAs. Linking heat wave- and drought-induced molecular changes to physiological responses will be crucial for understanding climate change responses in trees, and may help predict future resilience of forests exposed to periodic abiotic stress.

P10 - Investigating the role of EPF signaling peptide in regulating drought stress response in Arabidopsis thaliana

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Intercellular signaling mediated by peptide ligands has emerged as a key component of diverse biological processes in plants. Several EPIDERMAL PATTERNING FACTOR (EPF)-family members of small cysteine-rich peptides have been discovered as important signaling molecules controlling various developmental processes including inflorescence growth and stomatal patterning in Arabidopsis. However, the functions of other EPF peptides and their roles in stress management and hormonal responses remain unclear. To reveal biological functions of remaining EPF peptides, EPFL1, EPFL3, EPFL5, EPFL7 and EPFL8, we analyzed the expression patterns of *EPFs* using *EPFspro:GUS* reporters. In addition, we performed *in silico* expression studies and we found potential role of EPF peptide in ABA-mediated biological processes. Thus, we next analyzed transgenic plants overexpressing *EPFs* and loss-of-function mutants to investigate whether EPF peptides modulate ABA-mediated responses. We examined several ABA responses, including seed germination, seedling establishment and root growth in presense of various concentrations of ABA but no obvious differences compared to phenotypes of wild-type were observed. Interestingly, however, we found that the overexpression of *EPF* reduced water loss and markedly enhanced plant survival under drougt stress condition compared with the wild type, suggesting their role in managing drought stress response in Arabidopsis. All EPF-family members characterized to date exert their activities through the ERECTA receptor kinase family consisting of ER, ER-LIKE1 (ERL1), and ERL2. Thus, we next aim to invesitigate whether ERECTA-family receptors act in drought stress responses and these findings may enable us to discover the novel ligand-receptor pair controlling drought stress response in Arabidopsis.

P11 - Chlorophyll fluorescence as a tool for tracking photosynthetic phenology in spruce genotypes

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White spruce is a cornerstone tree species of temperate-boreal forests. Spruce populations are experiencing increasingly unfavorable environmental conditions due to changing climates. Here we aim to track the variation in photosynthetic phenology and identify performance by assessing photosynthetic traits of different spruce genotypes under contrasting environmental conditions. We measured chlorophyll fluorescence and pre-dawn water potential to estimate photosynthetic phenology and performance of spruce genotypes. Field data were collected from 90 trees representing 30 white spruce genotypes from two experimental field sites established by Natural Resources Canada, including one in Pickering, Ontario (warm and dry) and in Pintendre, Quebec (cold and humid), from July to November 2022. Preliminary results show that changes in chlorophyll fluorescence correspond with differences in pre-dawn water potential. Variations in chlorophyll fluorescence over time and among genotypes reflected environmental conditions at the two sites. In general, most spruce genotypes in Pintendre showed a higher quantum efficiency of Photosystem II (F_v/F_m) during summer, and an earlier downregulation of photosystem II photochemistry (ΦPSII) during autumn and winter, compared to the warmer and drier Pickering field site. In Pintendre, we found that twenty-two genotypes showed a decrease of F_v/F_m from summer to winter, while eight genotypes showed constant performance through the considered period. In summary, our results indicate that chlorophyll fluorescence (i) can be used to track photosynthetic phenology in different white spruce genotypes and further suggest that chlorophyll fluorescence (ii) can be used to quickly screen white spruce breeding populations to assess genotype performance under different environmental conditions.

P12 - Characterizing Real-Time Sensors of Cell Wall Stress in Arabidopsis thaliana

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The plant cell wall is a dynamic structure crucial for the structural integrity and environmental sensing of the cell. Plants react to biotic and abiotic stresses, including cell wall stress, by turning on signaling pathways and responding to stress through differential gene expression. We generated constructs for the promoters of 12 cell-wall stress-responsive genes in *Arabidopsis thaliana* fused to the reporter luciferase. Then, 18-hour time course assays were conducted to characterize the behaviour of these cell wall stress reporters under the cell wall stress inducer isoxaben. Various other types of stress elicitors, including other forms of cell wall stress, osmotic stress, and pathogen-associated elicitors, have also been investigated to evaluate crosstalk between cell wall stress and other signaling pathways. Several reporters were identified that are either specific reporters of cell wall stress responses, or that generally respond to a variety of stresses., These reporters provide potential tools to track cell wall stress responses in real time for future study of cell wall signaling pathways.

P13 - lncRNA mediated LRR-RLK regulation in Arabidopsis thaliana

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Plants have evolved a multitude of cell surface receptors to perceive signals from their environments and alter their growth or defense strategies accordingly. An expanded family of cell surface receptors, called the Leucine-Rich Repeat Receptor-Like Kinases (LRR-RLKs), are known to play important roles in growth and defense. Due to the high cost of immune system activation, plants must have mechanisms to regulate the expression of LRR-RLKs in order to maximize growth in the absence of pathogens and activate an immune response only when pathogens are present. Several factors suggest non-coding RNAs are key regulators of LRR-RLKs, and in my work I focus on a subclass called natural antisense transcripts (NATs), which are encoded on the antisense strand of a receptor. The objective of my research is to uncover how receptor-NATs regulate the expression of their cognate receptor, and the downstream effects on plant immunity in *Arabidopsis thaliana*. I have begun characterizing the NAT targeting the immune receptor MIK2 using a variety of immune assays and gene expression analysis in NAT overexpression lines. I have shown that NAT expression positively regulates MIK2 receptor transcript abundance and I am working to determine the significance of this in regard to plant immunity. In the future I will investigate the functional effect of MIK2 expression regulation and expand my work to study other NAT-receptor pairs.

BIOTIC STRESS

P14 - Caterpillar detoxification of plant specialized metabolites: Battle of the sexes!

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In response to insect herbivory, plants have evolved several strategies to protect themselves. One strategy is by chemical defenses that act directly, as a noxious compound or antifeedant for the insect herbivore, or indirectly, by recruiting predators or parasitoids of the herbivore. These specialized metabolites can be constitutive or induced. In response to attack, phytohormone signaling pathways result in the production of plant specialized metabolites. In particular, chewing herbivores, such as caterpillars, activate the biosynthesis of jasmonates that lead to saponin biosynthesis in the legume *Medicago truncatula*. Here, we compare the effect of two jasmonates, 12-*oxo*-phytodienoic acid (OPDA) and jasmonic acid (JA), on the development, pupal biomass and mortality of two caterpillar species, the beet armyworm *Spodoptera exigua* (a generalist) and the cabbage looper *Trichoplusia ni* (an adaptive specialist that prefers to feed on Brassicaceous plants). The results indicate that there are differences in the detoxification abilities between these two species and between the sexes of each species. In *M. truncatula*, *S. exigua* caterpillars fed less on plants that had a higher level of oleanolic acid-derived saponins. Through antifeedant studies, preliminary studies with *T. ni* show that saponins may act as an attractant and suggest there also may be sex-related differences.

P14 - Investigation of Flavonoid Synthesis in M. truncatula during S. meliloti Inoculation Based on Co-Evolutionary History

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Given the ecological and economic importance of symbiosis between legumes and nitrogen (N) fixing bacteria known as rhizobia, understanding the mechanisms underlying symbiosis establishment is critical. While both flavonoid secretion by the plant and NodD perception by the rhizobia are known to play an important role in initiating a symbiotic relationship, it remains unclear whether hosts plastically respond to the strain of rhizobia present in the rhizosphere by modulating the identities and/or relative abundance of the flavonoids they secrete. In particular, whether hosts respond differently to symbiont genotypes for which they share an evolutionary history compared to a novel symbiont, remains untested. I hypothesize that a shared history between partners will impact flavonoid secretion, resulting in a more beneficial outcome for both partners. To test my hypothesis, I will conduct a manipulative experiment using one line of the model legume *Medicago truncatula* inoculated either with coevolved or novel *Sinorhizobium meliloti* strains obtained from a previous study that passaged these strains on the same (coevolved) or different (novel) host lines. I will extract root exudates and analyze them using an HPLC-UV to identify and quantify the flavonoids secreted. If flavonoid secretions are impacted by coevolutionary history, then this suggests that prior recognition of symbionts could aid in beneficial symbiotic establishment. This work will lead to greater insights on flavonoid secretion in coevolved species and can be used to develop inoculants for crop production, providing a more sustainable method of N supplementation in agriculture.

P16 - To eat or not to eat? Surveying resistance to insect herbivores in the wild tomato Solanum habrochaites

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Trichomes serve as a chemical defence barrier against herbivory in many plants. Glandular trichomes, found in many *Solanum* spp., produce and store a variety of chemical compounds such as terpenoids, acyl sugars, and methyl ketones. While the composition of these compounds is variable across *Solanum*, this diversity has been lost in cultivated tomato (*S. lycopersicum*). Wild relatives of tomato, such as *S. habrochaites,* harbour striking chemical diversity in their trichomes. Zingiberene, a predominant sesquiterpene in several *S. habrochaites* accessions, is known to reduce insect performance. However, not much is known about the effects on herbivory relative to trichome chemical diversity. To address this gap, 17 accessions of *S. habrochaites* and two cultivars of *S. lycopersicum* were exposed to *Trichoplusia ni* (Lepidoptera: Noctuidae) larvae for three days. Insect performance (larval weight and consumed leaf area) was collected and will be compared to plant chemical profiles (volatile terpenes and cuticular waxes). Preliminary data indicates significant differences in insect performance among *S. habrochaites* accessions and cultivated tomatoes. Next, the performance will be compared as a function of the terpene and wax profiles in these plants.

P17 - Tomato specialized metabolites can modulate the insect-gut microbiome

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From the first contact with plants, insects are targeted by plant antiherbivore defences including specialized metabolites. These can constrain insect development and reduce plant damage. Yet there is another biotic factor that can modulate plant-insect interactions – bacteria living inside the insect gut. However, there is little evidence on how plant defensive compounds affect the insect-gut bacteria; hence, a thorough exploration of the effect of specialized metabolites on the insect-gut microbiome is needed. In this study, we explore how the cabbage looper (*Trichoplusia ni*) gut bacterial communities are affected by plant defensive metabolites from tomato (*Solanum lycopersicum*). We reared cabbage loopers on several defence-deficient tomato mutants and their corresponding wildtype lines. Larval weight, insect-associated bacterial communities from frass (insect feces) and leaf, and foliar biochemical profiles were analysed. Larval weight varied among plant hosts, confirming the effects of specialized metabolites on insect performance. Comparisons of leaf and insect-associated bacterial communities suggest that the insect microbiome is diverse and not acquired entirely from the host plant. Representatives of the Enterobacteriaceae and Pseudomonaceae families were widespread across all samples. However, insect bacterial community abundance and diversity substantially varied relative to plant genotype. Paired with foliar biochemical profiles, these results suggest that plant defensive metabolites can modulate herbivore insect-associated bacterial communities. This study provides a critical step in understanding gut bacterial communities as a component of plant-insect interactions and can be used to develop novel pest biocontrol strategies.

P18 - Tomato defence hormone responses to the foodborne pathogen Salmonella under elevated temperatures

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Climate change has many negative impacts on the agricultural sector, including potentially increased risk and incidence of fresh produce outbreaks associated with human pathogens such as *Salmonella enterica* (*S. enterica*) and *Escherichia coli* (*E. coli*). Although they do not cause disease in plants, previous research has shown that human pathogens have adapted to survive, penetrate into and colonize plant tissues, causing serious food-borne illnesses and threatening overall food safety. Research on plant immune responses against plant pathogens is well-established; however, how plants respond to human/animal pathogens remains unclear and largely unexplored at a mechanistic level. Elevated temperatures have been previously shown to regulate various components of plant stress hormone pathways in response to canonical plant pathogens, including salicylic acid (SA), jasmonate (JA), abscisic acid (ABA), and ethylene. Here we show that expression of the SA marker gene PATHOGENESIS-RELATED PROTEIN (*PR1*) was downregulated in tomato plants upon *Salmonella* infection at 28°C compared to those grown at 23°C. Additionally, expression of the ethylene marker gene ETHYLENE RESPONSE 1/2 (*ETR1/2*) and ABA marker gene *LE4* were downregulated at 28°C at 5 and 7 days post-inoculation, respectively. Finally, the JA marker gene Lipoxygenase D (LOXD) does not seem to be temperature-sensitive following *Salmonella* infection. Overall, warming temperatures differentially affect tomato hormone pathways following foodborne pathogen infection, reflecting certain trends observed upon plant pathogen attack. These insights aim to narrow major knowledge gaps surrounding human/animal pathogens on plants (HPOP/APOP) under changing temperatures, potentially shedding light on climate-resilient plant disease prevention strategies and food safety strategies.

P19 - Diverse interactions between Solanum lycopersicum and beneficial Canadian soil-borne bacteria regulate increased immunity

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In present agricultural systems, disease is responsible for up to 40% of crop loss, with climate change predicted to aggravate these losses further. Increasing temperatures can promote the spread of pathogens and elevate sources of abiotic stress, reducing the resilience of plants against pathogen attacks. We currently rely on agrochemicals, such as fungicides and pesticides, to combat this issue, but they too can have severe environmental consequences. Thus, finding alternative ways to protect plants and secure food production is of utmost importance. The microorganisms present in the soil directly present in the rhizosphere can promote plant growth and immunity, therefore presenting a unique system to leverage for agricultural use. Colonization of roots by some non-pathogenic bacteria can trigger induced systemic resistance (ISR), a type of systemic immunity. Some of these bacteria can also out-compete others for nutrients and resources, limiting pathogens from the rhizosphere through anti-microbial secretions and successful colonization of the plant root through biofilm formation. However, the factors that regulate bacterial colonization patterns are not well-understood. This project identified soil-borne bacteria beneficial for crop health by screening the Canadian Soilborne Bacteria library using *Solanum lycopersicum* for enhanced resistance against the fungal pathogen, *Botrytis cinerea*. We identified 16 bacterial strains that increased plant immunity. Interestingly some induce ISR, but others exhibit direct anti-botrytis activity, indicating diverse mechanisms to protect plants among these strains. Focusing on bacterial morphology, colonization, and growth phases, we investigated how these strains interact with the host plants and ultimately increase their resistance to fungal pathogens.

P20 - Involvement of two cyclic nucleotide-gated ion channel subunits in jasmonic acid-mediated immune signaling

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Upon pathogen infection, myriads of changes occur in plants. One notable early cellular response is the rapid, and transient influx of apoplastic calcium ions, which act as a powerful signal to activate downstream signaling events. Among many calcium channels identified to date, cyclic nucleotide-gated ion channels (CNGCs)—a well-characterized calcium channel family with 20 members—facilitate calcium influx to regulate various physiological functions including development, and immune signaling. Our bioinformatics study revealed that many CNGCs show co-expression during specific responses. Specifically, we found that two CNGCs are co-expressed in response to infection with the necrotrophic fungal pathogen, *Botrytis cinerea*, suggesting the involvement of these CNGCs in necrotrophic pathogen—or more broadly, jasmonic acid (JA)-related—immune response. To investigate this further, single, and double knockout mutants of these CNGCs were created to elucidate the individual and collective role of these channels in immunity. Results show that the double mutant, but not single knockout mutants, displays enhanced susceptibility against *B. cinerea* compared to wildtype, which is consistent with the co-expression pattern previously observed for these channels. Subsequent studies revealed diminished reactive oxygen species (ROS) and calcium signal activation upon chitin (a fungal elicitor) treatment, as well as the tendency of these mutants to have higher insect-susceptibility, further implicating both of these CNGCs in JA-related immune signaling. Overall, this study highlights the specificity as well as versatility that CNGCs have in maintaining plant health.

P21 - Resistance to bacterial brown spot in adzuki bean

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Adzuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi] is cultivated on approximately 15,000 acres across Ontario, mostly for export to Japan. Bacterial brown spot caused by *Pseudomonas syringae* pv. *syringae* van Hall *(Pss)* can result in in yield losses of up to 40 %. There is currently no resistant commercial adzuki variety. The hypothesis of this research is that the differences between the molecular interaction of *Pss* and adzuki bean that results in resistance to bacterial brown spot can be identified and utilized to create molecular markers for breeding bacterial brown spot-resistant varieties. The objectives are to: (a) characterize differences between highly virulent and less virulent strains of *Pss* (b) develop a screening methodology for bacterial brown spot in adzuki bean and (c) identify single nucleotide polymorphisms associated with quantitative trait loci (QTL) for resistance to bacterial brown spot using a population of adzuki recombinant inbred lines (RILs). To date, the varieties used as parents of the RIL population and a subset of the RILs have been screened with *Pss.* The results show that the RILs are segregating for resistance to bacterial brown spot. Bacterial cultures that may be *Pss* have been isolated from adzuki seed lots. In the future, 16S rRNA sequencing will be used to identify these isolates and virulence factors will be identified from whole genome sequences of *Pss* strains. Host resistance/susceptibility candidate genes targeted by these virulence factors will be identified in the QTL for resistance in the adzuki genome.

P22 - Investigation of 13 Immunity-Priming Bacterial Strains from the Canadian Soilborne Bacteria Library with Respect to their Plant Growth Promoting Effects

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The ability of soil microorganisms to protect plants has been known for a century. Our goal is to harness microbe/microbiota-associated protection (MAP) for agriculture. However, minimal understanding of the complex nature of rhizospheric plant-microbe interactions limits microorganism use in agricultural settings. MAP occurs in the rhizosphere, a narrow region of soil surrounding plant roots housing a thriving microbial community. Beneficial rhizospheric bacteria provide robust defense against pathogens via direct competition and a phenomenon known as Induced Systemic Resistance (ISR). ISR confers broad-spectrum resistance against pathogens and is induced by non-pathogenic soil microorganisms. Studies show that ISR-inducing bacteria often also promote host plant growth, classifying them as both ISR inducers and Plant Growth Promoting Rhizobacteria (PGPRs). Recently, our team identified 13 bacterial strains from our Canadian Soilborne Bacteria Library. These strains conferred resistance to a broad spectrum of pathogens, including fungal *Botrytis cinerea* and bacterial *Pseudomonas syringae*. This current study investigates these strains from a PGPR point-of-view to observe whether these strains confer growth benefits on their hosts, specifically increasing lateral root number and shoot fresh weight. Additionally, this study aims to elucidate the involvement of hormones and hormonal signaling pathways by which this PGPR effect is conferred to the host, focusing primarily on auxin signaling. Furthermore, the interplay between growth and immune signals will be explored. Ultimately, this study aims to answer whether these 13 strains both induce ISR and promote plant growth, thereby expanding the repertoire of bacterial strains that can be used as both biopesticide and biofertilizer.

P23 - Characterization of Effector Binding Elements present on genes from Phaseolus vulgaris L. targets of the Transcription Activator- like Effectors from Xanthomonas species

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Xanthomonas phaseoli pv. *phaseoli* and *Xanthomonas citri* pv. *fuscans* are the causal agents of common bacterial blight (CBB) disease in *Phaseolus vulgaris* L (common bean). These pathogens harbor remarkable effectors called Transcription Activator-like Effectors (TALEs) which are injected into host cells via the Type III secretion system (T3SS). These proteins migrate to the nucleus and mimic eukaryotic transcription factors to modulate host cell gene expression. TALEs recognize and bind to specific promoter regions in the host genome call Effector Binding Elements (EBEs). Some common bean varieties have genes for CBB resistance and molecular markers associated with CBB resistance loci, the most significant being SU91 on chromosome 8. A few genes near this molecular marker have been identified, including several R genes and a Nieman Pick gene, that may be related to mechanisms of resistance in the host. The identification and characterization of the EBEs for TALEs proteins in the promoter regions of genes close to the SU91 molecular marker is the main goal of this work. Used bioinformatic tools such as CLC Genome Workbench to identify and compare promoter sequences of the potential resistance genes that might be regulated by TAL effectors. In addition, other online platforms were utilized to predict and identify the DNA target preferences of TALE proteins. Genes associated with resistant responses contain binding sites for more than one TALE protein and the number of EBEs in the promoter regions are different in each genotype, meaning their expression could be dependent upon the action of *Xanthomonas* effectors.

P24 - Identifying proteins that interact with DIR1 during Systemic Acquired Resistance (SAR) using an estrogen-inducible SAR system and LC-MS/MS-based detection methods

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In response to localized infections, mobile signals including Defective In Induced Resistance 1 (DIR1) protein accumulate in the phloem of initially-infected leaves and travel to distant leaves where their perception induces broad-spectrum Systemic Acquired Resistance (SAR) to secondary infection. During SAR induction, a DIR1-containing high molecular weight complex is detected in the phloem. To identify proteins that interact with DIR1 that could be part of the long-distance SAR signaling complex, the Cameron lab has generated plant lines to facilitate immunoprecipitation and identification of proteins bound to FLAG-tagged DIR1 using FLAG antibody matrices and LC-MS/MS. These plants overexpress DIR1-FLAG and carry a system to induce SAR easily in many leaves by spraying with estrogen. The XVE estrogen-inducible promoter controls expression of the *Pseudomonas* effector AvrRpt2, so spraying with estrogen induces expression of AvrRpt2. Recognition of AvrRpt2 by endogenous RPS2 receptors in the Col-0 'SAR+' line (35S:DIR1-FLAG/XVE:AvrRpt2/Col-0) initiates effector-triggered immunity (ETI) and SAR, while in the *rps2* 'SAR-' line (35S:DIR1-FLAG/XVE:AvrRpt2/*rps2*) AvrRpt2 recognition and SAR induction does not occur. In the SAR+ line but not the SAR- line, estrogen spray treatment efficiently induced SAR and the accumulation of proteins including DIR1 in phloem exudates collected from treated leaves. The strength of SAR displayed by SAR+ plants and total protein levels measured in phloem exudates were similar to plants induced for SAR with *Pst (avrRpt2)*. This knowledge will be used to design large-scale phloem exudate collection experiments to obtain sufficient phloem sap for the immunoprecipitation of DIR1-FLAG complexes and LC-MS/MS-mediated identification of interacting proteins.

P25 - Genetic Expression of the Tomato CBP60g Gene Family in Response to Bacterial Infection and Elevated Temperature

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Given the current climate crisis, plant disease is an important factor to mitigate for ensuring adequate crop yields. Transcription factors CBP60g and SARD1 contribute to the biosynthesis of the defense hormone, salicylic acid (SA) in *Arabidopsis thaliana.* My project investigates SA production in relation to pathogenic exposure and elevated temperature by determining the gene expression profiles of 11 *CBP60g* homologs in the *Solanum lycopersicum* (tomato) Castlemart cultivar. Leaves were infiltrated with either mock ($MgCl_2$), or *Pseudomonas syringae pv. Tomato* (*Pst*) DC3000, incubated at 23°C (control temperature) or 32°C (elevated temperature) and then collected. RNA was extracted from the tissues and used as a template for qRT-PCR. Though there was varied expression among the 11 homologs, there were some found to be significantly expressed under each of the treatments. These findings contribute to our understanding of molecular defense mechanisms in tomatoes and will aid in the process of genetically engineering crops resilient to the effects of climate change.

P26 - Production of Cannabis (Cannabis sativa L.) Synthetic Seeds for In vitro Clonal Mass propagation and Germplasm Conservation

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Biologically active components of cannabis provide medical benefit leading to rapid expansion of global cannabis cultivation in recent years. Despite decriminalization of cannabis in over 70 countries, existing methods are lacking in vitro cannabis mass propagation, germplasm conservation, and the ability for simplified international trade. Synthetic seeds provide an efficient clonal propagation and storage technique for meeting the growing needs of commercial cannabis cultivation. This study focused on media supplementation, explant sources, low temperature, and light to expand the viability of synthetic seeds during storage. Cannabis nodal segments (5 mm) were immersed in 3% sodium alginate followed by 75 mM calcium chloride in Murashige and Skoog (MS) medium. After 150 days storage, regrowth rates of 70% and 90% were observed in synthetic seeds from *in vitro* and *in vivo*-derived sources, respectively, when stored at 6 °C and exposed to light. Furthermore, acetylsalicylic acid (ASA) supplementation into the encapsulation matrix not only delayed precocious germination of synthetic seeds stored at room temperature (22 °C), but also improved the regrowth rate of *in vivo*-derived synthetic seeds to 100% when stored at 6 °C under light. Exposure to light during storage significantly enhanced shoot length of regrown synseeds when compared to those stored in darkness. The difference in shoot length observed between light and dark stored synseeds was reduced when synseeds were supplemented with 25 µM acetylsalicylic acid. All regenerated plantlets were rooted and acclimatized in sterile rockwool plugs without morphological changes.

CELL BIOLOGY AND DEVELOPMENT

P27 - Elucidating the role of RKF1 interactors in post-pollination responses in Arabidopsis thaliana.

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Plant reproduction is a complex, multistep process following pollination and ultimately leads to pollen tubes growing into ovules for fertilization and the initiation of seed development. The dialogue between pollen and the pistil is essential for successfully mediating this process. Understanding this process is necessary for potentially improving yields in crops such as canola where seeds are harvested for seed oil. A new subgroup of Receptor-Like Kinases (RLKs), the Leucine-Rich Repeat (LRR) VIII-2 RLKs, have been identified as key players in the pistil for the early stages of this dialogue. The *Receptor-Kinase in Flowers 1* (*RKF1*) gene cluster was found to be involved in the stigma to promote compatible pollen hydration, as knocking out this gene cluster resulted in decreased wild-type pollen hydration. The *RKF1* gene cluster participates with other *RLK*s to support compatible pollen tube growth through the upper pistil. Using the cytosolic kinase domain of RKF1 as bait, potential binding partners were identified through a yeast-2-hybrid screen of an *Arabidopsis* flower cDNA library (Hybrigenics Services). Here, we work to confirm these interactions and identify their roles in post-pollination processes. Top candidates identified from this screen included the Related to Apetala (RAP) 2.12 and 2.3 transcription factors which belong to the Ethylene Response Factor VII (ERF-VII) group. Various interaction combinations were tested between RKF1 and the candidate proteins to identify interaction domains. In addition, phenotypic analyses of ERF-VII T-DNA knockout lines are also been conducted. These novel interactions will potentially illuminate undescribed pathways regulating the pollen-pistil dialogue.

P28 - Investigating downstream regulators in the self-incompatibility pathway of transgenic SI A. thaliana

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Members of the Brassicaceae family possess an intraspecific mating barrier, known as the self-incompatibility system, that allows the pistil to recognize and reject self-pollen. The SI system is initiated by the *S*--haplotype specific interactions between a pollen cysteine-rich protein ligand, SCR/SP11, and a pistil-specific receptor kinase, SRK. This interaction is followed by the activation of ARC1, a member of the plant U-Box family of E3 ubiquitin ligases. Molecular events downstream of this pathway cause self-pollen rejection by inhibiting the stigmatic responses required for pollen hydration, germination, and pollen tube growth. *Arabidopsis thaliana* has lost its SI system due to the pseudogenization of the *SCR/SP11, SRK*, and *ARC1* genes. Efforts in investigating the SI system in *Arabidopsis* has been made possible through the introduction of functional *SCR/SP11* and *SRK* transgenes into *A. thaliana*, though the degree of the SI response varies between accessions. SI was established in the Col-0 accession by transforming the *SCR/SP11-SRK-ARC1* genes from *Arabidopsis halleri,* and this research is now investigating the role of a J domain protein (JDP1) which was previously found to be an ARC1 interactor in *Brassica*. However, SI was also established in the C24 accession by only transforming *SCR/SP11-SRK* from *Arabidopsis lyrata* and so an unidentified ARC1 related factor is hypothesized to be involved. The role of two ARC1 homologues, PUB16 and PUB17, are now being investigated in the C24 SI pathway. Overall, this research will further elucidate the role of putative downstream signalling proteins in the transgenic *A. thaliana* SI pathway.

P29 - Investigating the role of LRR-VIII-2 Receptor-Like Kinase genes in intra- and inter-species pollinations in Arabidopsis thaliana.

Laura Canales Sanchez[1], Daphne Goring[1]

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Successful fertilization in *Arabidopsis thaliana* requires a series of tightly controlled interactions between compatible pollen and the pistil. As a mechanism to prevent interspecies hybridizations, flowering plants have also evolved reproductive barriers that prevent fertilization by incompatible interspecies pollen. Most research thus far has focused on the later stages of compatible pollen tube growth and ovular reception in the pistil, identifying many key players belonging to the large family of predicted Receptor Like Kinases (RLKs) which serve as primary sensors that perceive and translate extracellular signals. As well, a knowledge gap remains on the signalling proteins responsible for establishing pre-zygotic interspecies barriers. Previous work in the Goring lab has identified a group of *Leucine-Rich Repeat (LRR) VIII-2 Receptor-Like Kinase* (*RLK*) genes that function in the upper part of the female reproductive tract to support compatible pollen hydration and pollen tube growth. Here, I further explore the function of these *LRR-VIII-2 RLKs* in the reproductive tract of the pistil for promoting compatible pollen responses and establishing an interspecies barrier.

P30 - Investigating the role of NAC transcription factors in Arabidopsis thaliana seed coat development

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In *Arabidopsis thaliana* the seed coat plays one of the first forms of physical and biochemical defence of the developing plant embryo against biotic and abiotic stress. Seed coat functions include regulation of water and nutrient transport, defence against pathogens, establishment of dormancy, and desiccation tolerance. The NAC transcription factors (TFs) belong to one of the largest plant-specific TF families with function in processes such as stress response and fruit ripening, yet the functions of most of its members are largely uncharacterized.

Recent work in the Gazzarrini lab has identified two NAC TFs preferentially expressed in the seed coat, which regulate seed coat permeability and mucilage accumulation. Through in silico gene expression analysis, I have identified additional NAC TFs that are preferentially expressed in the seed coat. I selected multiple homozygous mutants carrying T-DNA insertions in these genes and characterized their role in seed coat permeability and mucilage secretion. I am currently generating double mutants and overexpression lines to complement these findings.

Overall, I aim to characterize the function of these NACs through genetic, molecular, and phenotypic analyses of these mutants and transgenic lines. This study will shed light on the role of a subfamily of uncharacterized NAC TFs in seed coat development in Arabidopsis.

P31 - Investigating the role of ubiquitination in cell wall signal transduction

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Plants monitor the state of their cell walls and induce intracellular responses to regulate cell wall composition during growth through the cell wall signalling pathway. Recent studies have implicated several receptors in the plasma membrane that are required for appropriate responses to cell wall stress. However, the mechanisms of intracellular signal transduction downstream of these receptors remain unclear. Considering that protein post-translational modifications could actively participate in these processes, we produced a proteomics dataset using Arabidopsis seedlings with short-term cell wall stress conditions (induced by isoxaben, a small molecule inhibitor of cellulose synthesis) and generated a list of the most significantly differentially ubiquitinated proteins, including UBIQUITIN6 (UBQ6).
T-DNA insertion affecting *UBQ6* displayed resistance to cell wall stress, while other UBQ-family members did not, and complementation with fluorescently-tagged UBQ6 could restore the mutant to wild-type levels of cell wall stress sensitivity. Spinning disk microscopy analysis of cellulose biosynthesis machinery revealed differences in *ubq6* compared to wild type, particularly under cell wall stress treatment. We are currently investigating the subcellular localization of UBQ6 to further characterize its role in cell wall stress responses. Our results suggest that ubiquitination may negatively regulate plant growth under cell wall stress conditions and that UBQ6 might be particularly important in this process.

P32 - Characterizing immunity and physiological phenotypes in temperature-sensitive and -resilient accessions of Arabidopsis thaliana

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Temperature has a significant impact on plant innate immune responses, but relatively little is known about the mechanisms underlying natural variation in temperature signal transmission to defense pathways. Previous research in the model plant species *Arabidopsis thaliana* revealed that high temperatures suppress the production of salicylic acid, which is a key plant hormone that mediates immune responses against numerous pathogens and pests. The widely investigated accession of *A. thaliana*, Col-0, has provided much of our understanding of temperature-regulated plant immunity and trade-offs between defenses and growth. However, the molecular mechanism of phenotypic variation in *Arabidopsis* immunity and physiological responses to high temperatures is not well understood. To fill this significant knowledge gap, we aim to characterize natural *Arabidopsis* accessions from various geo-climate origins at two different temperatures, 23℃ and 28℃. In contrast to the temperature-sensitive accession Col-0, we previously established candidate accessions with potentially temperature-resilient immunity based on bacterial disease resistance assays. We are currently conducting immune gene expression analyses and salicylic acid quantification in these temperature-resilient accessions. Unlike the reference accession Col-0, temperature–resilient accessions are anticipated to maintain defense gene expression and SA biosynthesis at elevated temperatures. In addition, to assess the physiological variation of *A.thaliana* accessions, we will also measure photosynthetic and non-photosynthetic parameters using a PhotosynQ. Our expected findings will hopefully aid in understanding the fundamental molecular and genetic underlying temperature-sensitive plant immunity. Overall, this study can potentially identify molecular strategies to engineer disease-resistant and climate-resilient plants, with far-reaching implications for agricultural and natural ecosystems.

P33 - Functional analysis of the N-terminal intrinsically disordered region of an immune kinase

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The receptor-like cytoplasmic kinase BIK1 is considered a major hub protein for plant immune signaling. It interacts with and is a direct substrate of multiple immune receptors at the plasma membrane. Here we focus on the role of the N-terminal intrinsically disordered domain of BIK1. This region of approximately 70 amino acids is co-translationally lipidated and post-translationally phosphorylated and ubiquitinated on multiple residues. We systematically tested the role of these modifications. Our results suggest that they contribute to the electrostatic regulation of BIK1 membrane localization, which has multiple effects on its function.

P34 - A cell-based system to study gene expression and protein function: Arabidopsis mesophyll protoplasts

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Emerging plant cell-based gene expression systems are a versatile tool for the comprehensive analysis of cellular components and signaling pathways. Among such systems, *Arabidopsis* protoplasts provide a unique and easily accessible way to examine gene and protein function including subcellular localization or molecular interactions in the context of a plant cell. Here, we aim to set up a robust *Arabidopsis* mesophyll protoplast system for the molecular analyses in both native and heterologous context. Leaf tissue was treated with a fungal enzyme cocktail consisting of cellulase and macerozyme to decompose plant cell walls and release protoplasts. Next, a cell viability assay was set up using the lipophilic fluorescein diacetate, which emits a fluorescence signal when hydrolyzed inside living cells. Cell counts and viability stain confirm consistently high yields of live *Arabidopsis* mesophyll protoplast. In addition, time series show lasting survival of protoplast for up to three days after extraction. Subsequent steps will focus on proof-of-principle transfection by PEG-calcium treatment using GFP as an expression marker. The system will then be used to study key regulatory enzymes from both *Arabidopsis* and woody species. This work will then be used as basis to generate protoplast from woody species that have proven difficult to transform. This will further enable studies on protein structure, function, and interaction partners in these species at higher throughput and shorter times, as studies that involve the traditional generation of transgenic lines are both time- and labour-intensive.

P35 - Investigating the role of subgroup IV calcium-dependent protein kinases (CDPKs) across the plant lineage

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Calcium-dependent protein kinases (CDPKs) are a unique family of integrated Ca^{2+}-sense/response proteins with diverse functions in plants. Within the plant kingdom, CDPKs cluster into four subgroups (I, II, III & IV). In *Arabidopsis thaliana,* there are 3 members in subgroup IV, with AtCPK28 being the most highly expressed among them. AtCPK28 is a major regulator of immune homeostasis and growth in multiple angiosperm plants including tomato, rice, cotton, and *Arabidopsis*. This project aims to investigate the conserved and/or diverged functions of subgroup IV CDPKs from extant lineages that represent crucial points over the evolutionary history of plants. Our results suggest that the function of subgroup IV CDPKs is well conserved across over 450 M years of evolution.

P36 - Investigating the mechanistic role of Arabidopsis HSP90.7 in auxin-mediated plant development

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The *Arabidopsis* HSP90.7, an ortholog of GRP94, reportedly modulates pollen tube germination and ER stress tolerance. The HSP90.7 knock-down mutant *shepherd* (*shd*) displays defects in pollen tube germination and compromised root and shoot development, demonstrating the critical role of HSP90.7 in meristem regulation and male fertility. We identified and characterized a new HSP90.7 knockout mutant (*hsp90.7-1*) to further investigate its role in plant development. Unlike the *shd* mutant, *hsp90.7-1* exhibits seedling lethality, accentuating the importance of HSP90.7 in early plant development. To investigate the underlying mechanism behind the arrested growth of *hsp90.7-1* mutants, we conducted a comparative transcriptome analysis to gain insight into the global gene expression in the *hsp90.7-1* mutant. Interestingly, we observed significant down-regulation of genes involved in auxin signalling and transport in the mutant, suggesting a potential role for HSP90.7 in regulation of cellular auxin homeostasis. Using the RNA sequencing analysis to guide our investigation, we studied the expression of representative marker proteins and found that PIN1GFP is significantly suppressed in root tissues. Additionally, expression of auxin responsive reporter lines DR5GUS and DR5GFP in *hsp90.7-1* seedlings suggested a reduction in cellular auxin content in tissues that typically accumulate high concentration of auxin. With auxin being a key regulator of the shoot and root apical meristems, altered expression of HSP90.7 likely induces alterations in the local auxin signalling pathways resulting in abnormal growth. It is anticipated that this study will shed light on the mechanistic role of HSP90.7 in plant development and its importance in cellular auxin homeostasis.

P37 - Functional studies of Arabidopsis MAPK phosphatases reveal novel regulators of chloroplast biogenesis

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Mitogen-activated protein kinases (MAPK) are group of protein kinases that play important roles in a wide range of environmental and developmental responses. As terminal components of sequential phosphorylation events, the activity of MAPKs is up-regulated through MAPKK-catalyzed phosphorylation and down-regulated through dephosphorylation catalyzed by phosphoprotein phosphatases. The *Arabidopsis* genome contains 5 MAPK phosphatases (MKPs), MKP1, MKP2, DsPTP1, IBR5 and PHS1, but relatively less is understood about their biological roles, especially in plant growth and development, compared to their substrate, MAPKs. Other than the *mkp1* mutant exhibiting stomatal defects, we found that none of other single *mkp* mutants displayed obvious growth differences compared with the wild-type. Thus, we next systematically generated different combinations of *mkp* higher-order mutants to reveal the potential functional redundancy and discovered the *mkp* mutants which exhibit a distinct albino phenotype, eventually leading to seedling lethality. Consistent with their albino phenotype, the chlorophyll contents were reduced significantly in *mkp* mutants and electron microscopy further demonstrated that the structure of thylakoids were disorganized and plastoglobules are higly accumulated. In addition, expression analysis revealed that plastid-encoded polymerase-dependent and nuclear-encoded genes involved in chlorophyll biosynthesis and photosynthesis were substantially down-regulated in the *mkp* mutant. Taken together, these data suggest that proper regulation of MAPK activity by phosphatases play a critical role in early chloroplast development in Arabidopsis.

GENOMICS, SYSTEMS BIOLOGY AND TECHNOLOGICAL INNOVATIONS

P38 - miR156/SPL12 modulates nodulation, nitrogen fixation and root regeneration in Medicago sativa by Silencing AGAMOUS-LIKE 6

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The root system architecture in plants is critical because of its role in controlling nutrient cycling, water use efficiency and resistance to biotic and abiotic stresses. We previously showed that transgenic *Medicago sativa* (alfalfa) plants overexpressing *microRNA156* (*miR156*) had increased nodulation, improved nitrogen fixation and longer roots. In alfalfa, transcripts of 11 *SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE* (*SPL*), including *SPL12*, are targeted by miR156. Thus, association of each target *SPL* gene to a trait or set of traits is essential for developing molecular markers for alfalfa breeding.

We determined the role SPL12 in root architecture and nodulation by investigating the phenotypic changes associated with altered expression of *SPL12* and by determining SPL12 targets. In this study, we used three *SPL12*-silencing and -overexpression alfalfa plants to investigate the role of SPL12. Furthermore, we conducted transcriptomics analysis of *SPL12* RNAi alfalfa roots and identified differentially expressed genes. Phenotypic analysis showed that alfalfa plants with reduced *SPL12* level had an increase in nodulation and root regeneration. Illumina next-generation sequencing-based transcriptomics in root tissues of *SPL12*-silenced genotypes also revealed SPL12 effects on genes involved in nodulation and nitrogen assimilation pathways. In addition, a gene encoding the transcription factor, AGAMOUS-like MADS box protein 6 (AGL6), was also identified as being directly silenced by SPL12 based on Next Generation Sequencing-mediated transcriptome analysis and chromatin immunoprecipitation assays, suggesting that AGL6 may be involved in regulating alfalfa nodulation. The present findings suggest that SPL12/AGL6 module regulates root development and nodulation, as well as nitrogen uptake and assimilation.

P39 - Comparative Analysis of Different Phenotypic and Genotypic Selection Strategies to Increase the Yield Genetic Gain using Nested Association Mapping Population in Dry Bean

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Increasing the rate of crop improvement for important crops, such as common bean (*Phaseolus vulgaris* L.), is the most sustainable way to decrease the global food insecurity caused by the current exponential increase in the human population. However, yield and its related traits are controlled by multiple genes with major and minor allelic effects. Therefore, several selection strategies are being considered for implementation in bean breeding. In the current work, a Nested Association Mapping (NAM) population of F4:5 recombinant inbred lines (RILs) was created with the cultivar Ex Rico 23 as the common parent, and 10 founder lines that span the genetic diversity of Ontario Mesoamerican germplasm. The NAM population was evaluated for different agronomic traits including yield, days to 50% flowering, and days to maturity in the field in four environments. In addition, the susceptibility and resistance to Anthracnose, an important biotic stress in bean, was evaluated in the greenhouse. The distributions of all the traits (days to 50% flowering, days to maturity, yield, and disease severity) showed some transgressive segregation compared to the parental lines for each environment. In order to have a comparative analysis of different selection strategies, two populations were created, containing of twenty-five RILs that had the lowest and highest means scores in the test population. The results indicated the genetic gain for higher yield was 3.2% and the genetic gain for low yield was 6.2%. A comprehensive comparison of the relative effectiveness of genomic selection and QTL index selection is in progress.

P40 - Diversity, function, and regulation of the immunity-associated CALMODULIN-BINDING PROTEIN 60 (CBP60) family in Solanum lycopersium (tomato) under biotic and abiotic stresses

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Molecular genetic analyses in *Arabidopsis thaliana* have demonstrated the roles of CALMODULIN-BINDING PROTEIN 60 (CBP60) proteins in growth, stress signaling, and immune responses. Two *Arabidopsis* paralogs CBP60g and SARD1 are functionally redundant transcription factors regulating various drivers of the immune system, including biosynthetic enzymes for defence metabolites salicylic acid and N-hydroxypipecolic acid. We have previously identified 1052 CBP60 homologs across 62 plant genomes. However, their function, regulation and diversification in most taxa remain unclear. Here we report the characterization of 11 members of the *Solanum lycopersicum* (tomato) CBP60 gene/protein family. Our phylogenetic analyses revealed that these 11 tomato CBP60 proteins can be divided into 2 major clades, due to differences in N- and C-terminal regions. Conserved domain analyses showed that these proteins possess putative CAM-binding domains, potentially connecting them to tomato Ca^{2+} signalling. AlphaFold-predicted structural analyses led to a hierarchical clustering of protein structural similarity. Additionally, gene expression analyses indicate that most tomato *CBP60* genes are induced during biotic stress by the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000. In terms of abiotic stress (warm temperature), tomato *CBP60* genes exhibited both temperature-resilient and -sensitive expression profiles. To potentially explain these differential expression patterns, we predicted distinct and overlapping transcription factors that could regulate these 11 genes. Finally, we are currently phenotyping mutants in three tomato *CBP60* genes most homologous to *Arabidopsis CBP60g/SARD1*. Taken together, we performed genomewide identification of putative Ca^{2+}-sensing plant regulators in tomato plants and provide evidence on their potential function and regulation during stresses.

P41 - A Genetic Suppressor Screen to Identify New Alleles and Downstream Targets of SMAX1

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Striga hermonthica is an obligate root parasitic plant of the Orobanchaceae family that infests many crops in Sub-Saharan Africa resulting in $7 to $10 billion in annual crop losses. *Striga hermonthica* seeds lay dormant in the soil for many years undetected, making eradication of *Striga* difficult. To infest host crops, *Striga* has evolved to utilize the alpha/beta hydrolase receptor termed, *HYPOSENSITIVE TO LIGHT/KARRIKIN INSENSITIVE 2 (HTL/KAI2)* to preceive strigolactones emitted from the host plants' roots. This receptor and subsequent signaling pathway is conserved in *Arabidopsis thaliana* to promote germination through the degradation of *SUPPRESSOR OF MAX2 1 (SMAX1)*, a negative regulator of germination. *SMAX1* has weak homology to *HEAT SHOCK PROTEIN 104 (HSP104)*, but much is unknown of the molecular mechanism of *SMAX1*. Utilizing a genetic suppressor screen to find mutants that germinate in the dark likewise of the loss of function *SMAX1* mutant (*smax1-2*), may uncover new alleles of *SMAX1* and downstream targets of *SMAX1*. In my research, I have identified and functionally characterized new *SMAX1* alleles from the genetic screen to elucidate *SMAX1* function. My data also suggests a new regulation component involved in *SMAX1* degradation.

P42 - Importance of the development of a co-dominant marker in the introgression of novel traits through marker-assisted backcrossing

Sajida Noor[1], Peter K. Pauls[1]

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Common beans (*Phaseolus vulgaris* L.) are a rich world resource of biodiversity with two centers of domestication (Andes and Central America) and over 10 major market classes cultivated globally (Singh et al.,1991; Voysest,2000). Dry bean production has increased by about 60% since1990, whereas the area harvested only increased by 36% in the same period (FAO, 2022). These statistics indicate that bean production increases are due to increases in area under cultivation but also because of the improved agronomic practices and novel dry bean breeding techniques. Continued introgression of germplasm from common bean genebanks provides new sources of genetic diversity to enhance food security and combat malnutrition (Siddiq et al., 2022).

The current research focuses on the introgression of a novel non-darkening seed coat trait from the non-darkening genotype Wit-rood into elite cranberry beans through marker-assisted backcrossing. Post-harvest seed coat darkening is a highly undesirable trait associated with lower nutritional quality, increased cooking time, and decreased palatability. The current study converted the single nucleotide polymorphism (SNP), found in the coding region of the MYB gene *Phvul.010G130600 which* has been shown to be tightly linked to the *J* locus for seed coat colour (Erfatpour and Pauls, 2020), into a co-dominant Kompetitive allele-specific PCR (KASP) marker which enables early identification of heterozygous (*Jj*), homozygous recessive (*jj*) and homozygous dominant individuals (*JJ*) in segregating populations. The marker can be used to identify heterozygotes carrying the non-darkening *j* allele in backcross breeding populations used to introgress this trait into elite cranberry bean background.

P43 - Facilitating High Throughput Screening of Common Beans (Phaseolus vulgaris L.) for Anthracnose Resistance Genes

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Common beans (*Phaseolus vulgaris* L.) provide important calories, fiber and protein globally but yield can be greatly reduced by disease. Screening for markers linked to known disease resistance genes provides useful information to improve this valuable food source. The KASP (Competitive Allele Specific PCR) assay is a quick and affordable genotyping technique that can be used for genetic screening to accelerate breeding programs. Several KASP markers have been developed for genes involved in resistance to bean anthracnose (*Colletotrichum lindemuthianum*) which disproportionately affects small farmers who save seed and can lead to yield loss of up to 100%. The objective of this undergraduate research was to conduct KASP assays for anthracnose resistance genes in common beans then facilitate the high throughput application of KASP assays in McGill's pulse breeding laboratory through automation. Elite lines were screened for four validated anthracnose resistance KASP markers linked to the genes *Co-1*, *Co-3* and *Co-4*^{2} both by hand and with the use of the Opentrons OT-2 liquid handling robot. To implement the OT-2, a protocol was written for DNA extraction and KASP assay thermocycling. Inoculation of bean anthracnose differential cultivars containing known resistance genes was used to further confirm the results of both methods. Using the OT-2, information from KASP assays can be accumulated with little active time and no need for time consuming steps such as running agarose gels. Results suggest that KASP can easily be applied as an effective genetic screening tool for quick genotyping at disease resistance markers.

BIOCHEMISTRY AND METABOLISM

P44 - Heterologous expression of non-proteolyzed glutamate decarboxylase-1 (AtGAD1) from Arabidopsis thaliana

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Glutamate decarboxylase (GAD) catalyzes the first committed step of the γ-aminobutyrate (GABA) shunt, a partial bypass of the mitochondrial Krebs' cycle. AtGAD1 (AT5G17330) is a root-specific, Ca^{2+}/calmodulin-activated cytosolic isozyme that became *in vivo* hyperphosphorylated at multiple, conserved serine residues located near its N-terminus 48 h following Pi resupply to Pi-starved (–Pi) *Arabidopsis thaliana* cell cultures (Mehta *et al.* 2021 Plant J). *In vivo* phosphorylation of AtGAD1 and its orthologs at analogous, conserved residues has been reported. However, the impact of reversible phosphorylation on plant GAD function has not yet been determined. Our ultimate objective is therefore to compare the kinetic properties of WT vs. phosphomimetic AtGAD1 mutants. Recombinant His_6-AtGAD1 (rAtGAD1) was heterologously expressed in *E. coli* and purified to homogeneity. However, SDS-PAGE and immunoblotting demonstrated that partial proteolysis of AtGAD1's C-terminal region occurred during its extraction and purification. A wide range of protease inhibitors and cocktails were tested and found to be ineffective in suppressing this unwanted proteolysis. However, inclusion of the cysteine protease inhibitor 2,2-dipyridyl disulfide (2 mM) effectively blocked rAtGAD1 proteolysis *in vitro*, but also abolished AtGAD1 activity. The metalloprotease inhibitor 1,10-phenanthroline (33 mM) also prevented partial rAtGAD1 degradation, but interfered with the metal-affinity chromatography purification step. Work is in progress to optimize approaches for the efficient purification of non-proteolyzed and active WT vs. phosphomimetic rAtGAD1 mutants. Results of this research will contribute to our understanding of the biological significance of reversible phosphorylation in the post-translational control of GAD and the GABA shunt in the plant kingdom.

P45 - Investigating the function of chloroplast chaperone HSP90C C-terminal extension in abiotic stress resistance

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Plants are sessile organisms and require adaptation to various stress conditions with the assistance of molecular chaperones. HSP90Cs are plastid stroma localized HSP90 family chaperones that facilitates protein import into the chloroplast as well as transport across the thylakoid membrane. While C-terminal extension (CTE) of cytosolic HSP90s in cochaperone binding and that of ER HSP90 in ER retention have been elucidated, the role of the HSP90C CTE is largely unknown. The HSP90C CTE disordered region has a highly conserved D-P-W triplet in all land plants and some green algae. This might indicate a functional constraint and prompted us to investigate the role of this region. Overexpression of a GFP fusion of the client protein PsbO1 caused protein translocation stress and albino cotyledon phenotype, which was rescued by HSP90C overexpression. Yeast two hybrid assays between the HSP90C clients PsbO1 and LHCB2.1 with HSP90C CTE deletion mutants demonstrated that HSP90C CTE is required for interaction with clients. Size exclusion chromatography confirms that the dimerization capacity is not affected in the CTE deletion mutants. Using the gHSP90C genomic DNA and introducing point mutations to generate premature stop codons, HSP90C CTE deletion mutant Arabidopsis were obtained. The CTE deletion mutant was able to rescue the embryonic lethality of HSP90C knockout mutant and developed like wild-type, therefore abiotic stress test and photosynthetic capacity analysis will be done. Further chaperone activity assay and ATPase activity assay will be performed to compare the general chaperone function between wildtype and mutant HSP90Cs.

P46 - What is the role of phosphorylation of the cytosolic glucose-6-phosphate dehydrogenase isozyme AtG6PD6 in response to phosphate nutrition of the model plant Arabidopsis thaliana?

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Inorganic phosphate (Pi) is a limiting macronutrient critical for plant development. Our phosphoproteomics study indicated that the cytosolic glucose-6-phosphate dehydrogenase isozyme AtG6PD6 became hyperphosphorylated at multiple N-terminal serine residues following Pi-resupply to Pi-starved (–Pi) *Arabidopsis* cell cultures (Mehta *et al*. 2021 Plant J). *In vivo* AtG6PD6 phosphorylation at similar residues was detected in various *Arabidopsis* phosphoproteomic studies. However, the impact of reversible phosphorylation on the function of any plant cytosolic G6PD remains unknown. G6PD is a tightly regulated enzyme that catalyzes the first committed step of the oxidative pentose phosphate pathway (OPPP) which plays a pivotal role in generating reducing power (NADPH) and C-skeletons (e.g., ribose-5-P) needed for anabolism and cell growth. Our aim is to test the hypothesis that phosphorylation activates AtG6PD6 to enhance OPPP flux during recovery from Pi-deprivation. Immunoblotting with a phosphosite-specific antibody revealed that AtG6PD6 from Pi sufficient (+Pi) or Pi-resupplied, but not –Pi, *Arabidopsis* cells or seedlings was phosphorylated at Ser18. This correlated with increased G6PD activity following Pi-resupply to –Pi *Arabidopsis* cell or seedling (i.e., root) extracts. Although the native enzyme is highly unstable *in vitro*, a partial purification of phosphorylated G6PD6 was achieved from +Pi suspension cells which could be rapidly dephosphorylated by incubating with exogenous λ-phosphatase. Studies are underway to compare kinetic properties of purified phospho- versus dephospho-AtG6PD6. Studying the interplay between Pi nutrition and AtG6PD6 phosphorylation may contribute to our understanding of the post-translational control of G6PD and the OPPP in plants, while identifying targets for developing Pi-efficient crop varieties.

P47 - Highlighting the in vitro impact of chalcone isomerase-like on legume-specific chalcone biosynthesis

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Across all lineages, plants utilize diverse catalogues of specialized metabolites for (a)biotic stress response, cellular signalling, and plant-microbe interactions. Specialized metabolites are also often extremely valuable for human applications, from medicine to agriculture. Isoflavonoids are a class of specialized metabolites that are fundamental for the formation of plant-microbe nodules in legumes and can be used as antimicrobials or phytoestrogenic nutraceuticals for human use. Specific isoflavonoid derivatives occur in small concentrations, in complex cocktails, and only under stress conditions. Therefore, their utility is limited by costly and complex purification steps. By reassembling the plant pathway in engineered microbes, such as *E. coli* or Baker's yeast (*Saccharomyces cerevisiae*), large-scale bioproduction of isoflavonoids can be accessed. When these pathways are reconstituted *ex planta*, the absence of auxiliary or regulatory players can derail the canonical pathway and the formation of by-products, reducing the yield of the desired product(s). Chalcone isomerase-like (CHIL) is a non-catalytic player that has shown an ability to correct early chalcone biosynthesis prior to the formation of the isoflavone scaffold. My research investigates the ability of CHILs from across plant lineages to interact and maintain the activity of flavanones using *in vitro* enzymatic studies with a specific interest in legume-specific downstream pathways. Understanding how CHIL assists in chalcone biosynthesis emphasizes the importance of auxiliary components when manipulating plants or engineering systems for isoflavonoid synthesis.

P48 - A waxy coat makes poplars popular in Northern Hemisphere

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The cuticle is the first line of defence against the environment**,** and is composed of a cutin polymer and a mixture of lipids referred to as cuticular waxes. The cuticle plays significant roles in environmental adaptation, protecting plants against biotic and abiotic stress. The aliphatic lipids of the cuticle are derivatives of very-long-chain fatty acids that are functionalized as distinct chemical classes. In this study, we examine the wax and cutin diversity in stems and leaves of *Populus trichocarpa* and *P. balsamifera,* two sister species found under different environmental conditions in Canada using gas chromatography-mass spectrometry. This survey found variation in composition depending on the accession, tissue, and developmental stage. Different chemical classes of wax were identified including alkanes, alkenes, alcohols, fatty acids, aldehydes, phenolics, triterpenoids and alkyl hydroxycinnamates. Some of these compounds had tissue-specific accumulation, for example, alkenes accumulated only on the leaf. Others were species-specific, e.g. aldehydes were only detected in *P. trichocarpa*. Unlike the large chemical variation observed in waxes, cutin composition was rather uniform. The most abundant monomers in cutin were hydroxy fatty acids, followed by fatty acids, phenolics and diacids, with their concentration increasing as stems aged. Surprisingly, even though phenolic compounds were significantly more abundant in cuticular waxes of *P. balsamifera*, their contribution to cutin was similar in both species. Due to poplars' long life-span and widespread range of distribution, the metabolic diversity observed in these accessions might be an adaptation to the local environment.

P49 - A combinatorial bioengineering approach aimed at enhancing the accumulation of α-eleostearic acid-containing neutral storage lipids in Nicotiana benthamiana leaves

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Plant seed oils have numerous commercial applications, ranging from foods to biofuels. While most oil-lipids primarily consist of five basic fatty acids, some plants accumulate high amounts of a single unusual fatty acid. One example is the tung tree (*Vernicia fordii*), whose seed oil contains up to 80% *a*-eleostearic acid (ESA; 18:3Δ9*cis*,11*trans*,13*trans*), a conjugated fatty acid with industrial drying uses. Due to societies' ever-growing demand for petroleum-based resources, numerous bioengineering strategies have been developed to produce alternative fuels and chemical feedstocks in plant vegetative biomass. Amongst these are the so-called "Push, Pull, Package and Protect" approaches, which involve the up/down regulation of genes associated with fatty acid or storage lipid biosynthesis ("Push" and "Pull"), storage lipid compartmentalization ("Package") into lipid droplets (LDs), and/or storage lipid turnover ("Protect"). Generally these approaches have been more effective together, but the combination of genes required for the synthesis and hyperaccumulation of single fatty acids is not well understood. Here we present the findings of our ongoing efforts aimed at enhancing the accumulation of ESA in neutral storage lipids in leaves using a "Push, Pull, Package and Protect" bioengineering strategy. Candidate *V. fordii* genes were transiently expressed in *Nicotiana benthamiana* leaves and expression confirmed with RT-PCRs. Furthermore, fluorescence microscopy was used to confirm proper subcellular localization of *V. fordii* "Package" proteins, the influence of candidate gene expression on LD size/abundance, and the potentially, aberrant side effects of ESA synthesis on leaf tissue/cellular morphology. Preliminary results of GC-FID confirming ESA synthesis within leaves are also discussed.

P50 - Mapping the local proteome of the endoplasmic reticulum and chloroplasts membrane contact sites using biotin proximity labeling

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Acyl lipid biosynthesis in plant cells is a complicated and highly regulated process, involving extensive lipid and fatty acid import/export between the endoplasmic reticulum (ER) and chloroplasts. While it has been proposed that this transfer of lipids occurs via membrane contact sites (MCSs), the protein players and cellular mechanisms involved in ER-chloroplast lipid trafficking are largely unknown. However, a previous study indicated that the *Brassica napus* chloroplast lipase 1 (BnCLIP1) localizes to ER-chloroplast MCSs and, therefore, may be an important factor in lipid trafficking. As such, BnCLIP1 is an attractive candidate for mapping the local proteome of the ER-chloroplast MCSs through biotin proximity labeling, a method that uses biotin ligase (TurboID) to biotinylate proximal proteins and facilitate their purification through an affinity-based streptavidin pull-down assay and their subsequent identification using mass-spectrometry-based proteomics. The current study aims to map the proteome of ER-chloroplast MCSs by overexpressing a BnCLIP1-TurboID fusion protein under the constitutive *UBQ10* promoter, both stably in *Arabidopsis thaliana* and transiently in *Nicotiana benthamiana* leaves. This poster showcases some of our results to date, including confocal microscopy studies confirming the subcellular localization of a BnCLIP-TurboID-YFP (yellow fluorescent protein) fusion protein to putative ER-chloroplast MCSs in transiently transformed *Nicotiana benthamiana* leaves. Also shown are the results of dual protease digestions of intact chloroplasts isolated from transgenic Arabidopsis lines stably overexpressing BnCLIP-TurboID, as well as preliminary biotinylation optimization experiments.

P51 - Investigation of the metabolism and biological activity of (+)-tetralone ABA, an ABA analog, in Arabidopsis

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Agricultural productivity is negatively affected by environmental stress, including heat, cold and drought. Abscisic acid (ABA) plays a critical role in regulating plant stress responses. Structural analogs of ABA have been designed to improve biological activity *in planta*. An efficient and practical synthesis of (+)-tetralone ABA has been developed to replace the planar vinyl methyl portion of ABA with an aromatic ring. In plants, the levels of ABA are primarily regulated by hydroxylation of the 8'-methyl group to form 8'-hydroxy ABA, which cyclizes to form less active phaseic acid (PA). Similarly, (+)-tetralone ABA is converted into 9'-hydroxy-tetralone ABA, which cannot be further converted into a PA-like compound due to the presence of the aromatic ring. To investigate the persistence of (+)-tetralone ABA and 9'-hydroxy-tetralone ABA, a metabolism study was performed to Arabidopsis. The results revealed that 9'-hydroxy-tetralone ABA accumulated in plants, consistent with the hypothesis that 9'-hydroxy-tetralone ABA is more persistent than PA. To confirm the analog's potency, chemical complementation assays of (+)-tetralone ABA were performed in Arabidopsis ABA-deficient *aba2-2* mutants. (+)-Tetralone ABA restored the stunted growth phenotype of *aba2-2* mutant to a greater extent at all concentrations compared to ABA, suggesting that (+)-tetralone ABA exhibits a more potent ABA activity than ABA itself. Thus, the (+)-tetralone ABA possesses enhanced biological activity *in planta* and can serve as a potential plant growth regulator for agricultural studies in which ABA lacks persistence.

P52 - The role of phosphorylation in regulating AROGENATE DEHYDRATASE 2 function in Arabidopsis thaliana

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Phenylalanine, an essential amino acid, is synthesized *de novo* in *Arabidopsis thaliana* predominantly via the arogenate pathway. The final step of the arogenate pathway is catalyzed by an arogenate dehydratase (ADT). ADT2 differs from the remaining six ADT isozymes in *Arabidopsis* as it has been shown to function as a PDT and has a unique moonlighting role during chloroplast division, forming a ring at the division plane. Phosphorylation is a post-translational modification known to affect protein activity and localization and may explain the differences observed between ADT2 and the rest of its enzyme family. The phosphorylation status of ADT2 will be established by checking its ability to interact with 14-3-3 protein SGF14I. Once the interaction is confirmed, *adt2* sequences will be generated by introducing point mutations that code for amino acids that either mimic phosphorylation or prevent it. Expression clones containing the altered *adt2* sequences will be generated to identify the effect of phosphorylation on ADT2 function. These altered adt2 proteins will be analyzed using confocal microscopy to determine if there are differences in interaction with SGF14I and in subcellular localization. The constructs will also be transformed into two *Saccharomyces cerevisiae* strains, *pha2* and ACAI, to identify changes in ADT and PDT activity. Examining the effects of phosphorylation on the enzymatic activity and subcellular localization of ADT2 will provide insight into how phosphorylation is involved in differentially regulating enzymes within an enzyme family. Overall, this study will improve our understanding of phenylalanine biosynthesis and chloroplast division in *Arabidopsis*.

P53 - Probing interacting regions of lipid biosynthetic enzymes from flax

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Plant oils in the form of triacylglycerols (TAGs) are economically valuable agricultural products, with applications ranging from food-grade vegetable oil to biofuels, cosmetics, and synthetic polymers. TAG rich in polyunsaturated fatty acids (PUFAs) is particularly valuable due to the role of PUFAs in human health. Some plant species such as flax (*Linum usitatissimum* L.) can accumulate a large amount of PUFAs in their seed oils but how PUFAs are enriched in these plants requires further exploration. Recently, we found that flax TAG biosynthetic enzymes acyl-CoA:diacylglycerol acyltransferase 2 (DGAT2), acyl-CoA:lysophosphatidylcholine acyltransferases (LPCAT) and phosphatidylcholine diacylglycerol cholinephosphotransferase (PDCT) were able to interact with each other through membrane yeast two-hybrid (MYTH) assay and bimolecular fluorescence complementation (BiFC) in *Nicotiana benthamiana,* which may contribute to PUFA enrichment in TAG. However, the molecular mechanisms of their interactions are currently unknown. Here, we predicted the structures of these flax transferase interactomes using AlphaFold2, an emerging machine learning-based prediction program for protein-protein interactions. Several potential interacting regions and sites were identified through AlphaFold2, including a helix near the C-terminus of LPCAT containing residues predicted to interact with DGAT2 or PDCT. We are currently performing site-directed mutagenesis and deletion mutagenesis to introduce mutations to the predicted interaction regions/sites and testing their relevance to interaction capability using MYTH and BiFC assays.

P54 - Subcellular localization of enzymes in the isoflavonoid pathway

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Plants are found in various ecological niches, displaying diverse morphological and chemical adaptations to their environment. For example, plants produce lineage-specific metabolic profiles with networks of low molecular weight compounds, referred to as specialized metabolites. One such class of compounds is isoflavonoids, which are characteristic of legumes (Fabaceae). This research focuses on isoflavonoid derivatives classed as phytoalexins, occurring in the forage crop *Trifolium pratense* (red clover). They create a chemical line of defence, produced "on-demand," protecting against microbial pathogens and herbivory. The biosynthesis of phytoalexins is often restricted to distinct tissue types under stress conditions. Understanding the tissue and subcellular localization of the protein machinery responsible for producing those compounds can shed light on their regulation and induction. I have used Gateway cloning to add a yellow fluorescent protein (YFP) fusions tag to several isoflavonoid enzymes. Subsequently, I transiently transformed vectors encoding the fusion proteins into *Nicotiana benthamiana* using *Agrobacterium tumefaciens*-mediated infiltration. Co-infiltration was also performed with vectors containing subcellular markers for specific organelles (tagged with CFP). Leaves expressing the tagged proteins were visualized with fluorescence confocal microscopy. Determining the subcellular localization of proteins can help us deduce function and proximity to substrates and products, bringing us closer to the reconstitution of such pathways in heterologous hosts (e.g., yeast). Synthetic bioproduction of plant-protective phytoalexins could be scaled reliably and sustainably, with applications for the agrochemical industry.

P55 - Interaction analysis between chloroplast molecular chaperone HSP90C and subunits of the thylakoid SEC translocase

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Plastid stromal localized molecular chaperone HSP90C works to maintain optimal chloroplast proteostasis and to assist in protein translocation. Previous works have shown that HSP90C directly binds to Sec translocase-dependent client pre-protein PsbO1 and the SecY1 subunit of the thylakoid membrane-bound Sec translocase channel system. However, whether HSP90C directly binds and affects the function of the vital extrinsic homodimeric Sec translocase subunit, SecA ATPase, remains elusive. We propose to study whether and how HSP90C interacts with the SecA. The behaviour of purified SecA in solution has shown a tendency to form an equilibrium between oligomeric forms ranging from monomers to tetramers. Upon characterizing the behaviour of quiescent-state SecA dimer form with ATP, our data shows a higher rate of monomer formation. Under nucleotide-deprived conditions, we did not see any interaction between HSP90C and the SecA monomer. However, SecA's binding behaviour to the chaperone in its oligomeric forms and the influence of nucleotides remain to be further investigated. We anticipate that HSP90C and SecA interactions are ATP stimulated, but ADP inhibited. We aim to connect this interaction to better understand HSP90C's role during pre-protein translocation into the thylakoid and to reveal chloroplastic SecA's binding events before and during pre-protein translocation.

P56 - Characterizing the targeting pathway of chloroplast outer membrane protein OEP18

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Approximately 95% of chloroplast proteins are encoded in the nucleus and are post-translationally targeted to the organelle. An estimated <55% of the 138 currently known or predicted chloroplast outer membrane proteins are thought to use one of four canonical targeting pathways. A recently described fifth novel pathway, referred to as the Toc159-like pathway, relies on a C-terminal reverse transit peptide-like sequence. The exact mechanisms of targeting and membrane association of the Toc159-like pathway is not fully characterized and understood. Here we investigate the targeting signal for one Toc159-like protein, outer envelope protein 18 (OEP18). OEP18 is predicted to contain a C-terminal reverse transit peptide-like sequence with an immediately upstream predicted β-hairpin. Transient expression in both onion epidermal and *A. thaliana* protoplasts show that OEP18 constructs containing the β-hairpin, with and without the reverse transit peptide-like sequence are able to localize to plastids, with the protoplasts showing outer membrane localization. This is also true for an OEP18 construct that lacks the β-hairpin but contains the reverse transit peptide-like sequence. These results suggest that the β-hairpin and the reverse transit peptide-like sequence both play a role in localization and may suggest a bipartite signal. It is hypothesized that the reverse transit peptide-like sequence gets OEP18 to the chloroplast outer membrane region and the β-hairpin is involved in inserting or anchoring the protein to the membrane. This provides more information about the scantily understood Toc159-like pathway and suggests a possible mechanism for membrane anchoring.

P57 - TOC159 Receptors are Targeted to the Chloroplast Outer Membrane Using a Bipartite Targeting Signal at the C-Terminus

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Plastids are a dynamic group of organelles in plant cells that serve a wide variety of roles in different tissues. They can also transition between types in response to different developmental and environmental cues. The most well-studied plastids are the chloroplasts, which house the machinery for photosynthesis. Chloroplast biogenesis and function rely on the targeting of chloroplast preproteins, a well understood process by which N-terminal chloroplast transit peptides (TPs) direct preproteins to the translocon at the outer membrane of the chloroplast (TOC complex), where they are recognized by TOC33 and TOC159 receptors and imported by the TOC75 translocation channel. Less understood is how chloroplast outer membrane proteins, like TOC159 receptors, are targeted. Previous studies have shown that TOC159 targeting relies on the C-terminus. Here, we refine this description, providing evidence that TOC159 is targeted to the chloroplast outer membrane by a bipartite signal at the C-terminus. Fluorescent targeting assays in Allium cepa and Arabidopsis thaliana demonstrate that TOC159 targeting is mediated by a β-strand and enhanced by a reverse transit peptide-like sequence. Truncation of the β-strand abolished targeting of fluorescent fusion proteins to the chloroplast outer membrane. Further, sitedirected mutagenesis targeting highly conserved residues in the β-strand and replacing them with alanine also abolished targeting. Recent evidence suggesting TOC159 receptors are anchored in the chloroplast outer membrane by a β-barrel support these findings, suggesting the β-strand is related to the β-signal that targets β-barrel proteins of the outer membranes of mitochondria and gram-negative bacteria. A deeper understanding of how TOC complex components, like TOC159, are targeted to the chloroplast outer membrane and assembled into functional TOC complexes is an essential step in our understanding of plastid proteome targeting pathways and their regulation, which ultimately dictate plastid transitions essential to plant growth, development, and adaptability.

List of Attendees

Last name	First name	Institution
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Balasubramaniam	Anchalya	University of Toronto
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