CSPB-SCBV 2021
Virtual Meeting

June 7 - 10, 2021

Program and Abstracts

Welcome!

The Canadian Society of Plant Biologists (CSPB-SCBV) meets every year and is intended for students, postdoctoral fellows and faculty with an interest in plant science.

After thorough consideration, and in view of the non-resolved coronavirus pandemic worldwide, the CSPB-SCBV and the Organizing Committee have decided that the 2021 will be held in a virtual format. This decision ensures the continuity of knowledge exchange while at the same time protecting the health and wellbeing of conference participants.

CSPB2021 will include Plenary lectures, Ragai Ibrahim memorial symposium speakers, and oral and e-poster presentations within the frame of 12 concurrent sessions that will cover all aspects of plant science.

The virtual conference program presents the schedule in your respective time zones. All plenary and concurrent talks will be live followed by Q&A session with the speakers. Posters will take place over three days and opportunities for live interaction with presenters. The concurrent session in its entirety will be available for on-demand viewing shortly after the conference is complete.

Scientific Program Committee

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Big thank you to all the Presenters, Session Chairs, Student Presentation Judges, Workshop Organizers and Sponsors!

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Plenary Speakers

Dr. Anja Geitmann, McGill University (Mary Spencer Lecture)

Title: *‘On growth and form’ – From D’Arcy Thompson to micromechanics of plant morphogenesis*

Dr. Geitmann’s research focuses on the cellular processes that are involved in plant reproduction and those that lead to the formation of plant organs and functional tissues. She holds a Canada Research Chair in Biomechanics of Plant Development at McGill University and leads an interdisciplinary team of biologists and engineers. Dr. Geitmann's research combines cell biology with micromanipulation methodology and mathematical concepts to reveal novel aspects of plant functioning. Dr. Geitmann currently serves as President of the International Association of Plant Reproduction Research and she serves on the editorial boards of multiple scientific journals including Cell and Plant Physiology.

Dr. Mark Belmonte, University of Manitoba (CD Nelson Lecture)

Title: ***Driving innovation through discovery while being an invisible minority in science***

Dr. Mark Belmonte is a Professor of Biological Sciences at the University of Manitoba. Mark received his BSc (2001) and MSc (2003) from the University of Calgary before moving to Winnipeg where he obtained his PhD in plant science in 2008. After a brief postdoctoral fellowship at UC Davis, Mark moved back to Winnipeg to start his own lab in the Faculty of Science. Dr. Belmonte's group uses cutting edge next generation molecular tools to improve crop production of some of Canada's most important agricultural crops. Mark has published his work over 55 times, been the recipient of numerous awards, and is devoted to promoting science education and research at outreach events across Canada and takes pride in training the next generation of young scientists.

Dr. Alice Cheung, University of Massachusetts

Title: ***Pollen-pistil Interaction: the tale of three related signaling complexes to enable fertilization***

Dr. Alice Cheung is currently Professor in the Dept. of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, USA. Received her B.A. (Biochemistry) from Smith College and PhD in Molecular Biophysics and Biochemistry, Yale University and postdoc in Plant Biology at the Harvard University. Alice have a long-standing interest in plant reproduction, especially in various male-female interaction processes that lead to fertilization. Particularly interested in understanding the signaling strategies that underlie the cell-cell communicative events during the pollination process. Her work spans molecular and cell biology and also rely heavily in transgenic and biochemical approaches. Our most recent efforts have been on the role of several signaling pathways that mediate pollen-pistil interaction from pollen deposition on the stigma to sperm release in the embryo sac.

Dr. Jacqueline Monaghan, Queen's University

Title: ***Calcium signatures, phosphorylation relays, and protein turnover in the plant immune response***

Dr. Jacqueline Monaghan is an Assistant Professor of Biology at Queen's University, and a Tier II Canada Research Chair in Plant Immunology. Jacqueline studied undergraduate biology at the University of Toronto and was an NSERC graduate scholar at the University of British Columbia. She continued as an EMBO Long-term Fellow and BBSRC Future Leader Fellow at the Sainsbury Laboratory Norwich. Her research group at Queen's University focuses on immune signal transduction and fine-tuning mechanisms with a particular interest in calcium-dependent protein kinases.

Dr. Yang Xu, Michigan State University (Carl Douglas Lecture)

Title: ***Plant and microalgal diacylglycerol acyltransferase: role in lipid biosynthesis and performance engineering***

Dr. Yang Xu is currently a Research Associate in Dr. Christoph Benning's lab at Michigan State University. She got her PhD degree in Plant Science under the supervision of Dr. Randall Weselake from the University of Alberta in 2018 and then worked as a Postdoctoral Fellow in Dr. Guanqun (Gavin) Chen's lab at the University of Alberta for more than two years. Her research focuses on plant and microalgal lipid biochemistry and biotechnology.

Dr. Peter Constabel, University of Victoria (Special Symposium honouring Ragai Ibrahim)

Title: ***Fantastic flavonoids: From enzymes to transcription factors and back again***

Dr. Peter Constabel is Professor and Chair of Biology at the University of Victoria, and former Director of the UVic Centre for Forest Biology. He first became fascinated with secondary plant metabolism during his MSc studies with G. H. Neil Towers at the University of British Columbia. He obtained his doctorate in Molecular Biology from the Université de Montréal working with Normand Brisson, then did post-doctoral research with Clarence Ryan at Washington State University. His research has focused on the biosynthesis, regulation, and function of phenolic secondary metabolites in poplars and other trees. He has published extensively on plant tannins. In 2018, received the CSPB's David J. Gifford Award in Tree Biology.

Dr. Abel Rosado, University of British Columbia

Title: *Bridging the gap – Contact sites and inter-organelle communication in plant cells*

Dr. Abel Rosado is an Associate Professor and Canada Research Chair Tier 2 in Plant Cellular dynamics at the University of British Columbia. He obtained his doctorate in plant stress physiology from Malaga University (Spain) working with Miguel Botella. He discovered his passion for plant microscopy as a Fulbright postdoctoral scholar with Natasha Raikhel at UC-Riverside, and later developed an independent research line in cellular stress physiology as a Marie Curie researcher. His current research uses the model plant Arabidopsis thaliana and aims at understanding how cellular structures known as membrane contact sites regulate the non-vesicular transfer of information during stress episodes.

Day One	**June 7, 2021**

10:30 – 12:00	Plenary Lectures	
10:00	**Dr. Raju Soolanayakanahally** **Dr. Daphne Goring**	Welcome
10:30	**Dr. Alice Cheung** University of Massachusetts Amherst	Plenary 1: CSPB-SCBV Invited Lecture Pollen-pistil Interaction: the tale of three related signaling complexes to enable fertilization
11:15	**Dr. Mark Belmonte** University of Manitoba	Plenary 2: CD Nelson Award Lecture Driving innovation through discovery: what it's like being an invisible minority in plant biology
12:00 – 12:30	Lunch Break	
12:30 – 14:00	Concurrent Sessions 1 to 3	

CS1 Abiotic Stress

CS2 Biochemistry and Metabolism

CS3 Development and Sexual Reproduction

14:00 – 14:15	Coffee Break
14:15 – 16:15	Concurrent Sessions 4 to 6

CS4 Abiotic Stress

CS5 Genomics and Systems Biology

CS6 Biotic Interactions

16:15 – 17:15	Poster Session 1
17:15 – 18:15	Online Networking

Day Two	**June 8, 2021**
10:00 – 10:30	Rapid Poster Talks, Session 1
10:30 – 12:00	Plenary Lectures

10:30 **Dr. Jacqueline Monaghan**
Queen's University

Plenary 3: CSPB-SCBV Invited Lecture
Calcium signatures, phosphorylation relays, and protein turnover in the plant immune response

11:15 **Dr. Abel Rosado**
University of British Columbia

Plenary 4: CD Nelson Award Lecture
Bridging the gap – Contact sites and inter-organelle communication in plant cells

12:00 – 12:30	Lunch Break
12:30 – 14:00	Concurrent Sessions 7 to 9

CS7 Abiotic Stress
CS8 Cell Biology
CS9 Physiology, Nutrient and Human Health

14:00 – 14:15	Coffee Break
14:15 – 16:15	CSPB-SCBV Annual Business Meeting and Awards
16:15 – 17:15	Poster Session 2
17:15 – 18:15	Online Networking

Day Three — June 9, 2021

10:00 – 10:30	Rapid Poster Talks, Session 2	
10:30 – 12:00	Plenary Lectures	
10:30	**Dr. Anja Geitmann** McGill University	Plenary 5: Mary E. Spencer Award Lecture 'On growth and form' – From D'Arcy Thompson to micromechanics of plant morphogenesis
11:15	**Dr. Yang Xu** Michigan State University	Plenary 6: Carl Douglas Prize Lecture Plant and microalgal diacylglycerol acyltransferase: role in lipid biosynthesis and performance engineering
12:00 – 12:30	Lunch Break	
12:30 – 14:00	Ragai Ibrahim Memorial Symposium	
12:30	**Dr. Peter Constabel** University of Victoria	Ragai Ibrahim Memorial Lecture Fantastic flavonoids: From enzymes to transcription factors and back again
		Ragai Ibrahim Memorial Student Presentations
1:15	**Nicole Unterlander** University of Guelph	Flavonol rhamnoside degradation in senescing leaves is linked to α-rhamnosidase activity
1:30	**Kelly Bodding** University of Guelph	Biosynthesis of novel anti-inflammatory compounds from *Cannabis sativa*
1:45	**Danielle Williams** Brock University	Mutagenesis of a plant P450 involved in MIA biosynthesis in *Catharanthus roseus* results in two distinct enzymatic functions
14:00 – 14:15	Coffee Break	
14:15 – 16:15	Concurrent Sessions 10 to 12	
CS10	Molecular Biology	
CS11	Biochemistry and Metabolism	
CS12	Abiotic Stress	
16:15 – 17:15	Poster Session 3	
17:15 – 18:15	Online Networking	

Day Four	**June 10, 2021**

10:00 – 12:30	Workshop #1: Plant Genome Editing

Led by Dr. Sateesh Kagale, National Research Council Canada

13:00 – 15:00	Workshop #2: Canadian Light Source Synchrotron-based Technologies: Shining light on plant science and research

Led by Dr. Chithra Karunakaran, Canadian Light Source
Dr. Teagen Quilichini, National Research Council Canada

13:00 – 15:00	Workshop #3: From the bench to the classroom: Transitioning from research to teaching

Led by Dr. Madoka Gray-Mitsumune, Senior Lecturer at Concordia University
Dr. Marcus Samuel, Professor at the University of Calgary
Dr. Eliana Gonzalez-Vigil, Assistant Professor at the University of Toronto, Scarborough

Concurrent Session 1 Abiotic Stress

1-1	12:30	Lee Marie Raytek	Investigating the functions of in vivo hyperphosphorylation of the glutamate decarboxylase AtGAD1 in phosphate-resupplied Arabidopsis thaliana
1-2	12:45	W.G Duleeka I. Gunawardana	Preliminary Evaluation of the Effect of Environmental Factors (Salt Spray) on the Production of Squamatic Acid in Lichen-Fungus Cladonia uncialis
1-3	13:00	Maria Camila Rodriguez Gallo	Quantitative proteome and PTMome analysis of Arabidopsis thaliana root responses to persistent osmotic and salinity stress
1-4	13:15	Aswini Kuruparan	Assessing the effect of drought stress on flag leaf epicuticular wax composition in old and new bread wheat cultivars
1-5	13:30	Lauren Erland	Integrating metabolomics and ecological niche modelling to predict plant climate change resilience at a permafrost anomaly on Cornwallis Island, Nunavut.
1-6	13:45	Ariana Forand	A shield against stress. Using cell wall structural modifications to overcome abiotic and biotic stress

Concurrent Session 2 Biochemistry and Metabolism

2-1	12:30	Helen Tai	Foliar glycoalkaloids of potato and the herbivorous pest Colorado potato beetle
2-2	12:45	Loïc Soumila	Constitutive defense potential (secondary metabolites) against the wide-spread herbivore Lymantria dispar L. across different conifer genera
2-3	13:00	DHAOUADI Fadoua	Efficient production of therapeutic phytomolecules in the bioengineered diatom Thalassiosira pseudonana
2-4	13:15	Chris White-Gloria	Novel protein phosphatase SLP1 has a vast reach on chloroplast metabolism regulation
2-5	13:30	Jordan VanderBurgt	Production of self-assembling virus-like particles displaying PRRSV epitopes in Nicotiana benthamiana
2-6	13:45	Soheil Mahmoud	Transcription factors controlling monoterpene metabolism in lavender

Concurrent Session 3 Development and Sexual Reproduction

3-1	12:30	André Laroche	Identification of circadian clock regulated immune response genes in wheat
3-2	12:45	Sylvia Silveira	Live-imaging uncovers cellular growth patterns shaping Arabidopsis stamen
3-3	13:00	Harleen Kaur	Auxin biosynthesis and receptors gene expression during mid-phase seed development in Pea
3-4	13:15	Constance Le Gloanec	Cells switch from stochastic to predictable behaviors during leaf development in Arabidopsis
3-5	13:30	Tamara Montoya	Abscission in plants: Structural, chemical and transcriptomic analysis of protective surface layers
3-6	13:45	Maryam Honari	Identifying DNA-protein interactions and promoter function in the candidate apomixis APOLLO gene

Concurrent Session 4 Abiotic Stress

4-1	14:15	Dilrukshi Kombala Liyanage	Genotypic response of Canadian short-season soybean cultivars to drought stress
4-2	14:30	Mukund Shukla	The impact of temperature fluctuation on phenology of Hazelnut (Corylus spp.) cultivars
4-3	14:45	Devin Brown	The development of [18F]-3'-F-ABA, a PET Tracer to Monitor ABA Transport in Live Plants
4-4	15:00	K. A Dinithi Kumarapeli	The effect of moderate heat stress on reproductive growth in a wheat RIL population segregating for heat resistance with respect to seed yield
4-5	15:15	Thorsten Knipfer	The water potential curve: A method to determine drought-induced changes in plant-water relations
4-6	15:30	Hai Nguyen	Phytohormone-enhanced heavy metal responses in Euglena gracilis: Ni, Pb and Cd uptake and associated hormone and metabolomic dynamics
4-7	15:45	Keshav Dahal	High-Throughput Screening of Drought Tolerant Potatoes to Achieve "More Crop per Drop”
4-8	16:00	Somaieh Zafari	Alternative oxidase modulates serine metabolism and GABA shunt in tobacco under hypoxia

Concurrent Session 5 Genomics and Systems Biology

5-1	14:15	Yunfei Jiang	Dynamic mRNA-sRNA interactions coordinate gene expression during meiosis in wheat
5-2	14:30	Julia Hooker	Differentially expressed genes involved in low seed protein content in western-Canadian soybeans (Glycine max) identified through transcriptomics
5-3	14:45	Neha Vaid	Genomics Driven Development of Drought Tolerant Wheat Cultivars
5-4	15:00	Marina Cvetkovska	The blind alga: The Antarctic green alga Chlamydomonas sp. UWO241 has a reduced repertoire of photoreceptor genes and an aberrant phototactic response
5-5	15:15	Paul Gamueda	Characterization of guard cell-specific drought-responsive genes in Arabidopsis thaliana
5-6	15:30	Theia Jensen	Cold treatment conditions wheat microspores to respond to Trichostatin A for improved androgenesis
5-7	15:45	Devang Mehta	Data acquisition approaches in proteomics: addressing technological limitations for plant systems biology
5-8	16:00	Yunfei Jiang	Transcriptional dynamics during microspore reprogramming to embryogenesis in wheat

Concurrent Session 6 Biotic Interactions

6-1	14:15	Shawn Clark	Investigating the role of extracellular vesicles in Botrytis infection of tomato
6-2	14:30	Shaun Sharpe	Preliminary Screening Status for Glyphosate-Resistant Kochia in Saskatchewan
6-3	14:45	Hui Liu	Transcriptome analysis of rutabaga (Brassica napus) cultivars indicates glucosinolate-derived nitriles may play a role in inducing plant defense against clubroot disease
6-4	15:00	Brenda C. Salasini	W/Y/L motifs are enriched in core RxLR effectors but only the L motifs are indispensable for pathogenesis: A case study of PpRxLR6 from Phytophthora parasitica var. nicotianae
6-5	15:15	Atta Ur Rahman	Identification of the causal agents of potato early dying in potato plants and fields of Alberta
6-6	15:30	Christie Stephen	Plant host defense peptides as potential tools to reduce crop losses
6-7	15:45	Thomas DeFalco	A conserved module regulates receptor kinase signaling in immunity and development
6-8	16:00	Robert McGee	Improving Tolerance to Environmental Stresses and Yield in Greenhouse-Grown Tomatoes by Engineering the Soil Microbiome

Concurrent Session 7 Abiotic Stress

7-1	12:30	Christian Danve Castroverde	FEELING THE HEAT: The Impact of Temperature on Systemic Immunity in Arabidopsis thaliana
7-2	12:45	Vanessa Shivnauth	Expression Profiling Of The Tomato CBP60g Gene Family During Bacterial Infection And Elevated Temperature
7-3	13:00	Sophia Stone	The ubiquitin ligase XBAT35.2 regulates plant response to biotic and abiotic stresses
7-4	13:15	Aya Hanzawa	Cold stress response in Arabidopsis root is regulated by specific actin isovariant, ACTIN 8
7-5	13:30	Nathan Doner	Identifying new lipid droplet proteins in Arabidopsis thaliana: ERD7 localizes to lipid droplets via its senescence domain
7-6	13:45	Matei Dan-Dobre	Characterization of SPL4 role in drought stress and trichome development in alfalfa

Concurrent Session 8 Cell Biology

8-1	12:30	Diksha Bhola	3D Architecture of Plant Mesophyll Tissues
8-2	12:45	Stuart Macgregor	The role of autophagy in the Arabidopsis self-incompatibility response
8-3	13:00	Chak Chung Kuo	Identification of MOR1 homologs in plants and bioinformatic analysis of putative microtubule-binding motifs
8-4	13:15	Liyong Zhang	Live Imaging of Leaf Spongy Mesophyll Morphogenesis and Microtubule Organization
8-5	13:30	Nathan M Rowarth	Transcriptomic analysis identifies potential regulators involved in programmed cell death and remodelling of lace plant leaves (Aponogeton madagascariensis)

Concurrent Session 9 Physiology, Nutrients and Human Health

9-1	12:30	J. Duncan Giebelhaus	Gibberellin regulation of protein accumulation in developing pea (Pisum sativum L.) seeds
9-2	12:45	Sabine Scandola	Integrating systems-level phenomics and quantitative proteomics to profile Kale cultivars diel pattern
9-3	13:00	Sonia Malik	Essential oil composition of Artemisia vulgaris L. cultivated in Brazil
9-4	13:15	Solmaz Irani	Distinct molecular responses of two ecotypes of the extremophile plant Eutrema salsugineum to low phosphate availability
9-5	13:30	Eduardo Antonio Ramirez Rodriguez	Leveraging phosphoproteomics to uncover mechanisms of cell wall integrity signaling

Concurrent Session 10 Molecular Biology

10-1	14:15	Mohammad Erfatpour	Genetic Control of Hilum Ring in Common Bean (Phaseolus vulgaris L.)
10-2	14:30	Presented by Sheng Wang	Protein levels of several Arabidopsis auxin response factors are regulated by multiple factors and ABA promotes ARF6 protein ubiquitination
10-3	14:45	Nishat Shayala Islam	PvMATE8 is a Multidrug and Toxin Extrusion transporter involved in proanthocyanidin accumulation and postharvest seed coat darkening in common bean
10-4	15:00	Vida Nasrollahi	Characterization of SPL12 role in regulating root architecture, nodulation and nitrogen fixation in Medicago sativa
10-5	15:15	Megan Aoki	Cytokinins beyond plants – Expanding our understanding of the ancient signalling molecules through the social amoeba, Dictyostelium discoideum
10-6	15:30	Ji-Yun Kim	Understanding phloem loading mechanisms through single-cell transcriptomics
10-7	15:45	Nick Schimpf	TURNING THE PEP-TIDE: IN VITRO EVALUATION OF LINEAR HOST DEFENSE PEPTIDES FOR ECTOPIC EXPRESSION IN PLANTS
10-8	16:00	Benjamin P. Brookbank	3’-(Phenyl alkynyl) analogs of abscisic acid: synthesis and biological activity of potent ABA antagonists

Concurrent Session 11 Biochemistry and Metabolism

11-1	14:15	Mackenzie Poirier	Relying on light in a light-limited environment: Chlorophyll biosynthesis in the Antarctic psychrophile Chlamydomonas sp. UWO241
11-2	14:30	Isabel Desgagne-Penix	Characterization of norbelladine synthase and noroxomaritidine reductase, catalyzing the first key steps in Amaryllidaceae alkaloid metabolism
11-3	14:45	Alison Edge	Completion of the vindoline and catharanthine pathways in Catharanthus roseus facilitates characterization of MIA pathways in Ochrosia elliptica
11-4	15:00	Matthew Bergman	Cytosolic geraniol and citronellol biosynthesis mediated by a Nudix hydrolase in Pelargonium graveolens
11-5	15:15	Rebecca Kalinger	Determinants of substrate specificity in a catalytically diverse family of acyl-acyl carrier protein thioesterases from plants
11-6	15:30	Artyom Gritsunov	Investigating Enzymes Involved in Quinate and Chlorogenic Acid Metabolism
11-7	15:45	Michael A. Phillips	Soft ionization GCMS for 13C plant flux studies
11-8	16:00	Brendan O'Leary	Glutamine activates the Target of Rapamycin Pathway signalling pathway in Mature Leaves: Implications for plant nitrogen signalling

Concurrent Session 12 Abiotic Stress

12-1	14:15	Mina Momayyezi	Structural and functional leaf diversity lead to differences in photosynthetic capacity across Juglans regia accessions
12-2	14:30	Gamalat Allam	miR156/SPL network negatively regulates aluminum stress tolerance in Medicago sativa
12-3	14:45	Bridget Murphy	Variation in the timing of autumn cold acclimation in field-grown white spruce under elevated temperatures and reduced water availability
12-4	15:00	Pomona Osmers	Defining Optimum: Growth Conditions Affect Heat Stress Resistance in the Antarctic Extremophile Chlamydomonas sp. UWO241
12-5	15:15	Narendra Singh Yadav	Multigenerational heat-stressed progeny of Arabidopsis displays notable phenotypic, genotypic, and epigenotypic variations
12-6	15:30	Prakash Venglat	The 4Ms (Making, Maintaining, Managing and Mastering Meristems) of plant developmental plasticity from a founder cell perspective
12-7	15:45	Tawhidur Rahman	Dissecting the roles of cuticular wax in plant resistance to multiple environmental stresses
12-8	16:00	Henry Cordoba	Genome-wide association study of transpiration rate in common bean (Phaseolus vulgaris L.) in drying soil

Workshops

1. Plant Genome Editing Workshop

Organizer: Sateesh Kagale, National Research Council Canada

CRISPR/Cas technology has sparked a new revolution in biological research and promises to transform agriculture with its high precision, ease of design, multiplexing ability and low cost. This half-day workshop will focus on CRISPR gene editing principles, and provide an overview of the basic gene editing workflow, consisting of the design of effective guide RNAs (gRNAs), delivery of CRISPR/Cas9 components into host cells, detection of genome modifications and analysis of gene editing efficiency. This workshop is aimed at students/researchers who are familiar with basic molecular biology techniques and are interested in learning about plant genome editing using CRISPR/Cas systems.

2. Canadian Light Source synchrotron-based techniques: Shining light on plant science and research

Organizers: Chithra Karunakaran, Canadian Light Source, Teagen Quilichini, National Research Council Canada

The Canadian Light Source (CLS) synchrotron is a national research facility located on the campus of the University of Saskatchewan that offers innovative and unique-in-Canada infrastructure and support

for research. Synchrotron-based techniques help scientists probe the nature and structure of molecules and materials, making the CLS a valuable tool for both academic and commercial researchers.

This workshop covers two main themes that may be of interests to plant biologists: 1. How synchrotron based 2D or 3D X-ray imaging is advantageous to study internal structures or to characterize structural phenotyping of intact samples in spatial scales from micron to nano scales; and 2. How chemical composition or phenotyping is possible using mid-infrared or X-ray spectroscopy and imaging techniques on intact or sections of pristine samples. Participants will be educated on how to access the CLS for academic research and examples will be shown on sample preparation, data collection and analysis using millet seed and cannabis samples.

3. From the bench to the classroom: Transitioning from research to teaching

Organizers: Madoka Gray-Mitsumune (Teaching stream – Senior Lecturer at Concordia University), Marcus Samuel (Research stream – Professor at the University of Calgary), Eliana Gonzales-Vigil (Research stream – Assistant Professor at the University of Toronto - Scarborough)

In this workshop we will discuss how to write an effective and reflective teaching dossier, explore current topics in teaching and learning, and what to expect in job searches. This workshop is targeted for graduate students, postdoctoral fellows, and early career researchers.

Bridging the gap – Contact sites and inter-organelle communication in plant cells

Abel Rosado[1]

[1]University of British Columbia

Membrane Contact Sites (MCS) are evolutionarily conserved structures where the close proximity between two or more membrane-bound organelles enables direct exchange of molecules and facilitates coordinated inter-organellar adaptive responses without membrane fusion. The presence of MCS in plants has been documented for decades but only recently, advances in plant cell imaging and the development of novel genetic and molecular tools have fueled an emerging field of research devoted to the investigation of their structural organization, dynamics, and physiological functions. Plant MCS are enriched with a variety of protein-protein and/or protein-cytoskeleton tethering assemblies that establish dynamic interactions with membrane phospholipids, and carry out essential cellular functions including, but not restricted to the maintenance of membrane lipid homeostasis, organelle biogenesis, autophagy, endocytosis, and the regulation of Ca2+-dependent stress responses. In this talk, I will highlight advances and current trends in plant MCS research with a focus on processes taking place at the ER-PM interface.

Identification of MOR1 homologs in plants and bioinformatic analysis of putative microtubule-binding motifs

Chak Chung Kuo[1], Geoffrey Wasteneys[1]

[1]The University of British Columbia

The *Arabidopsis* microtubule-associated protein MICROTUBULE ORGANIZATION 1 (MOR1), a homologue of the XMAP215/Dis1 family, contains five Tumor Overexpressed Gene (TOG) domains. These TOG domains catalyze tubulin dimer addition and removal at the microtubule plus end and bind microtubule lattices. We previously reported that MOR1 TOG1-TOG2 showed a higher relative affinity to microtubule polymers than free tubulin dimers, in contrast to the equivalent domains from XMAP215, which showed a higher relative affinity to tubulin dimers than microtubule polymers (Lechner et al. 2012, J. Cell Sci. 125). Using BLAST searches, we identified MOR1 homologues in 1193 eukaryotic species spanning Viridiplantae, algae, animals, yeasts, and protists. Among these homologues, we searched for putative microtubule lattice-binding [KR][ILV][ILV][KR] motifs. Our analysis found that these motifs are located close to the N-terminus in embryophyte homologues but generally located closer to the C-terminus in non-embryophyte homologues. For example, KLLK and RILK motifs are found in MOR1 TOG1, but not in *Xenopus* XMAP215 TOG1 or TOG2, offering support for KLLK and RILK as microtubule-binding motifs. Sequence alignments of non-embryophyte homologues suggest that the N-terminal KLLK motif is found almost exclusively in embryophyte homologues and may have emerged within the streptophyte clade. We hypothesize that the N-terminal KLLK motif evolved for specialized microtubule features in embryophytes as the green lineage transitioned from water to land. Our bioinformatic work has identified key MOR1 amino acid residues for mutagenesis to uncover their functional roles, contributing to a better understanding of microtubule dynamics and organization in *Arabidopsis* and beyond.

Leveraging phosphoproteomics to uncover mechanisms of cell wall integrity signaling

Eduardo Antonio Ramirez Rodriguez[1], Heather E McFarlane [1]

[1]Department of Cell and Systems Biology, University of Toronto

The plant cell wall is a polysaccharide-based extracellular matrix that surrounds and protects plant cells. As highly dynamic structures, cell walls must strike a balance between rigidity for protection and structure, and flexibility to grow and respond to environmental cues. These properties make it an ideal source for renewable materials and bioenergy. However, attempts to overhaul the plant's finely tuned polysaccharide deposition ability by increasing the expression of polysaccharide biosynthetic enzymes in plants have had limited success. These results hint at the existence of an underlying regulatory mechanism, called 'cell wall integrity' (CWI) signaling that perceives changes outside the cell, and in turn, remodels the cell wall and/or regulates plant growth. To date, several cell membrane-bound kinases have been implicated as the signal perception component of CWI, yet the downstream players remain undefined. We employed a proteomics approach by treating plants to induce cell wall stress and analyzing phosphorylated peptides through high resolution mass spectrometry. Altogether, our data from 3 independent experiments (n=9) shows 242 differentially phosphorylated phosphopeptides, corresponding to 241 proteins. We conducted bioinformatic analysis of all 241 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.

Investigating the role of extracellular vesicles in Botrytis infection of tomato

Shawn Clark[1], Ashlyn Parrott[1], Ocean Han[1], Morgan Kirzinger[1], Sabo Hrapovic[1] [1]National Research Council

Botrytis cinerea, commonly referred to as grey mold, can infect over 1000 plant species at virtually all stages of growth and development. Producers rely heavily on the use of fungicides to control this pathogen; however, it is notorious for being able to develop resistance to these treatments and better tools for controlling this pathogen are needed. Botrytis delivers small RNA to the host as virulence factors during infection and plants are also reported to produce small RNA to target fungal genes and limit infection. These observations suggest that RNA movement between host and pathogen plays an important role in disease pathogenesis. Evidence in the literature suggests that this movement is mediated by extracellular vesicles (EVs) and while these organelles have been studied extensively in mammalian biology, our understanding of plant EVs remains limited. To better understand the role of EVs in plant pathology we have isolated EVs from tomato leaf tissue with and without Botrytis infection. Transcriptomic analysis of these samples has been initiated and preliminary results will be discussed. Future work will include proteomic and metabolomics analysis of these samples.

Integrating metabolomics and ecological niche modelling to predict plant climate change resilience at a permafrost anomaly on Cornwallis Island, Nunavut.

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Understanding physiological mechanisms of Northern plant species can predict their viability in the face of changing climates. We are using a novel approach, the integration of metabolomics, growth studies and ecological niche modelling (ENM) to understand species capacity to adapt to changing temperatures. During a botanical field survey near Resolute in the Summer of 2019, we observed an anomaly in the permafrost with temperatures about 10 °C warmer than the surrounding tundra. The plants at the anomaly were different species than the surrounding plant communities. We hypothesized that this anomaly represents a small-scale model of the effects of warming on plant biodiversity. Our objectives were to: (1) Determine community composition at and adjacent to the anomaly; (2) Perform ENM of the species; (3) Chemically characterize the species by metabolomics; (4) Identify relationships between metabolomics data and plant communities and (5) Test hypotheses generated in *in vitro* cultures of field collected longstalk starwort (*Stellaria longipes* Goldie), the species with greatest abundance at the anomaly. Plant species with higher kurtosis and more negative skew had highest abundance at the warmest points in the anomaly. Metabolomics analysis identified unique chemical fingerprints indicative of climate exposure. *S. longipes* was found to have distinct responses to environmental stimuli including temperature, light and exposure to volatile organic compounds. These results demonstrate the potential for identification of biomarkers of climate change vulnerability and resilience through integration of multidisciplinary approaches.

Dissecting the roles of cuticular wax in resistance to multiple environmental stresses

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Cuticular waxes are a mixture of very-long-chain fatty acids and their derivatives accumulated in the uppermost layers of the plant cuticle. Numerous studies have defined the role of cuticular wax in plant resistance largely based on non-stomatal water-loss under drought. Our lab has been investigating the role of cuticular wax in reducing low-temperature and dehydration stress in plants using model and crop plant species. *Arabidopsis thaliana* mutants and transgenic genotypes with abnormal formation of cuticular wax has been studied. *cer3-6*, a known *Arabidopsis* wax-deficient mutant (with distinct reduction in aldehydes, n-alkanes, secondary n-alcohols, and ketones compared to wild type (WT)), was most sensitive to water loss; while *dewax*, a known wax overproducer (greater alkanes and ketones compared to WT), was more resistant to dehydration compared to WT. Furthermore, cold-acclimated *cer3-6* froze at warmer temperatures, while cold-acclimated *dewax* displayed freezing exotherms at colder temperatures compared to WT. GC-MS analysis identified a characteristic decrease in the accumulation of certain waxes (e.g. alkanes, alcohols) in *Arabidopsis* cuticles under cold acclimation, which was additionally reduced in *cer3-6*. However, the *dewax* mutant showed a greater ability to accumulate waxes under cold acclimation. Our data from *Arabidopsis* indicate cuticular alkane waxes along with alcohols and fatty acids can facilitate avoidance of both frost formation and leaf water loss, and are promising genetic targets of interest. Attempts have been undertaken to identify *dewax* and *cer3-6* orthologs from corn, wheat, and canola, and study their role in reducing freezing and drought stress.

Soft ionization GCMS for 13C plant flux studies

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Gas and liquid chromatography – mass spectrometry (GCMS and LCMS) have become indispensable tools for metabolite profiling in plant metabolomics research. The inclusion of stable isotopes enables measurements of flux and pool size simultaneously, and 13CO2 whole plant labeling is currently the most informative technique for monitoring global carbon flow in plant metabolic networks. GCMS is generally the preferred method to analyze plant metabolites due to its high chromatographic resolution and reproducibility, but the hard ionization of electron impact produces significant fragmentation of analytes and complicates accurate quantification of isotopic label. Here we describe an ammonia chemical ionization technique that combines the chromatographic advantages of GCMS with a soft ionization method reminiscent of electrospray ionization that simplifies label incorporation calculations by preserving the entire molecular ion cluster. Molecular ions of metabolites with molecular weights up to 1,000 Da are uniformly preserved using this technique with <1% fragmentation in most cases. The technique can operate in positive [M+H]+ and negative [M-H]- mode and provides unambiguous molecular mass information in addition to accurate secondary isotope measurements useful for labeling calculations. In a polar Arabidopsis extract, we observed 13C incorporation into ~80 primary metabolites in time course labeled rosette tissue that includes carbohydrates, photorespiratory intermediates, amino acids, citric acid cycle intermediates, and phytosterols. These data provide inputs into flux models such as isotopically nonstationary metabolic flux analysis as well as new insights into carbon partitioning between primary and secondary metabolism.

Physiological responses of doubled haploid wheat lines differing in grain yield under rainfed and irrigated environments

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Understanding the physiological mechanisms contributing to high grain yield is important for early generation selection in breeding, either by directly measuring physiological traits or by selecting for markers to those traits. A six year study of a doubled haploid (DH) bread wheat population “B0767&” (Carberry/ AC Cadillac) in the field showed significant grain yield variation under rainfed and irrigated conditions. Four DH lines with contrasting grain yield were further investigated in the field and greenhouse for various physiological mechanisms associated with water stress response. Analysis of flag leaf stomatal traits from two independent experiments revealed significantly low stomatal density ($p < 0.05$) and conductance (gs) ($p<0.05$) in the high yielding line ‘B0767&AG075’ (registered as AAC Goodwin) and ‘B0767&AX125’ under water stress but not under irrigation. The lines B0767&AG075 and ‘B0767&AX125’ also showed a high $\Delta 13C/\delta 18O$ ratio, a cooler canopy, and higher NDVI compared to the lines B0767&AD028 and B0767&AH156 with low grain yield. Greenhouse studies for gas exchange parameters, the maximum rate of carboxylation (Vcmax), photosynthetic electron transport rate (J), dark respiration (Rd), and mesophyll conductance (gm) showed contrasting reaction of these DH lines during water stress. Taken together, our results suggest that diverse physiological mechanisms and traits related to carbon fixation contribute to the grain yield variation in DH lines during water stress. The DH lines such as ‘AAC Goodwin’ with high yield potential and contrasting physiological mechanisms hold great promise to develop wheat germplasm for dryland environments.

Identifying DNA-protein interactions and promoter function in the candidate apomixis APOLLO gene

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Apomixis is a natural form of asexual seed production in plants, and is of potential importance to agriculture due to its ability of fixing of hybrid vigor. The Apollo gene is a candidate apomixis factor identified through differential gene expression in sexual vs. apomictic ovules in the plant genus *Boechera,* a wild relative of Canola (Corral *et all*.,2013). Sexual individuals are homozygous for the Sex-alleles ,while apomicts are heterozygous for the Apo/Sex-alleles. I hypothesized that a 20 bp Apo-insertion/Sex-deletion in the 5′ UTR of Apo-alleles corresponds to specific transcription-factor binding sites (TFs), and a yeast one-hybrid assay conducted for sexual and apomictic *Boechera* showed two different sets of TFs.
To learn about the regulation of APOLLO *in planta*, the function of the APOLLO promotor was studied in transgenic lines. Apomictic *Boechera* were transformed with a 2kb Apo promoter and demonstrated tissue-specific GUS activity in the stigma of different developmental stages, while the 2kb sex promoter did not show any GUS activity. Finally, five different synthetic ~2kb promoter-swap constructs were made to test Apo- vs. Sex-specific promoter components. The data showed that changes to the APOLLO promoter causes shifts in tissue- and developmental-stage specificity.

Cytokinins beyond plants – Expanding our understanding of the ancient signalling molecules through the social amoeba, *Dictyostelium discoideum*

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Cytokinins (CKs) encompass a family of evolutionarily significant growth regulating hormones, most well-known for their many roles orchestrating plant growth and development. CKs were once thought to be unique to plant taxa; however, increasing evidence suggests that these hormones are pervasive in a wide variety of organisms, such as: bacteria, algae, fungi, insects, fish, and mammals. Using the social amoebae, *Dictyostelium discoideum*, we are expanding our understanding of the roles of CKs beyond plants. Owing to its distinctive life cycle, *D. discoideum* is a unique model in which we can study the role of CKs at both single- and multi-cellular states. We have shown that *D. discoideum* produces 6 types of CKs throughout its life cycle. Furthermore, we have been targeting key CK biosynthesis and metabolism genes in *D. discoideum* – which include isopentenyltransferases (key CK biosynthesis enzymes), putative candidates for phosphoribohydrolases (CK activating enzymes; also referred to as LOG in plants), and a putative candidate for discadenine synthase (novel CK biosynthesis enzyme unique to *D. discoideum*). Through targeted experiments, involving gene knockout or heterologous expression, we aim to obtain a broader picture of the specific functions of CKs at the single cell level and beyond, into multicellular organization and development. Our results will offer insight as to how CKs have evolved as signaling molecules.

Novel protein phosphatase SLP1 has a vast reach on chloroplast metabolism regulation
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Plants, like all eukaryotes, employ reversible protein phosphorylation mediated by protein kinases and phosphatases, for signaling and regulation of cellular processes. Despite the model plant A. thaliana having over 1000 protein kinases and 217 protein phosphatases, few are experimentally localized to the chloroplast. Not only is chloroplast protein phosphorylation fundamental to the regulation of the organelle's metabolism (e.g. photosynthesis, starch and lipid synthesis) but altering phosphorylation in this compartment also has profound effects on plant physiology. Recent steps in assembling a chloroplast protein phosphorylation network have led to the identification of key new chloroplast protein kinases, such as CK2. CK2 is fundamental in controlling multiple aspects of chloroplast biology. The protein phosphatases in this organelle, playing the antagonistic role to protein kinases, have been neglected despite being equally as crucial in maintaining chloroplastic homeostasis. Recent bioinformatic studies from our group discovered Shewanella-like phosphatase 1 (SLP1), which we have since confirmed as one of the few protein phosphatases residing in the chloroplast. To clarify the function of SLP1, our group performed mass-spectrometry based quantitative phosphoproteomics with wildtype and slp1 knockout plant tissue to reveal protein phosphorylation profiles of cellular proteins. Astonishingly, from greater than 18,000 cellular phosphorylation sites, 193 phospho-sites from 171 chloroplast proteins were identified as hyperphosphorylated in the absence of the phosphatase and therefore represented putative target proteins of SLP1. Consequently, these data hint at an extensive role for SLP1 in chloroplast biology, laying groundwork for SLP1 to emerge as a key player in plant cell biology.

Production of self-assembling virus-like particles displaying PRRSV epitopes in *Nicotiana benthamiana*
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Porcine reproductive and respiratory syndrome (PRRS) is a disease leading to spontaneous abortions and stillbirths in sows and lowered life quality and expectancy in growing pigs. PRRS is a major issue for the swine industry as it is prevalent worldwide and has significant economic impacts. PRRS is caused by a small virus, and the only vaccine currently on the market is an inactivated live vaccine that has occasionally been shown to revert to virulence and spread the disease instead of providing protection. Therefore, there is a need for further measures to control PRRS. Co-expression of the two most abundant proteins in the viral outer envelope, the matrix protein (M) and glycosylated protein 5 (GP5), were shown to produce a neutralizing immune response for the virus providing a potentially effective subunit vaccine against the disease, but they are difficult to express. The aim of this research was to create a subunit vaccine candidate for PRRS by displaying portions of the M and GP5 proteins on the surface of an unrelated virus-like particle, the Tobacco Mosaic Virus. This construct was expressed in *Nicotiana benthamiana* leaves as they can produce high levels of recombinant proteins and complex glycosylation marks, which are important for the M-GP5 epitope. Results of accumulation, purification, and assembly of the nanoparticles will be presented and discussed. This work provides a foundation for investigating this candidate vaccine’s immunogenicity and effectiveness in preventing PRRS infection. Producing protein-based vaccines in plants could provide a cost-effective method of administration to livestock through feeding.

Expression and properties of aconitate isomerase in maize (Zea mays L.)

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Aconitate isomerase (EC 5.3.3.7) catalyzes the reaction of interconverting *cis*- and *trans*-isomers of aconitic acid. We studied the expression of aconitate isomerase gene and the properties of partially purified enzyme in maize (*Zea mays* L.) leaves. It was shown that aconitate isomerase is induced by white and by red light and suppressed by far-red light, which indicates phytochrome involvement in its regulation. The induction takes place at the level of gene expression. The enzyme is localized exclusively in the cytosol of maize leaf cells. It was partially purified and its properties were investigated. The Km value was determined as 0.75 mM with *cis*-aconitate and 0.92 mM with *trans*-aconitate, pH optimum was 8.0–8.2 with both substrates, citrate and malate suppressed its activity. We conclude that aconitate isomerase actively participates in the interconversion of *cis*- and *trans*-aconitate in leaves during photosynthesis, which provides a possibility of utilizing the pool of trans-aconitate for the regulation of the tricarboxylic acid cycle activity and for the facilitation of citrate/isocitrate supply for biosynthetic and signaling purposes in photosynthetic cells in the light.

Live-imaging uncovers cellular growth patterns shaping *Arabidopsis* stamen

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One of the fundamental questions in biology is how organ shapes are acquired during the development of multicellular organisms. Precise understanding of organogenesis requires measurements of cellular behaviors over space and time. In plants, such a quantitative approach has been successfully used to dissect organ development both in leaves and external floral organs such as sepals. However, the observation of floral reproductive organs is hampered as they develop inside tightly enclosed floral buds, difficult to access for imaging. Here we develop a new confocal time-lapse imaging method that allows, for the first time, a full quantitative characterization of the development of stamens, male reproductive organs, from their initiation until the final shape is established post-anthesis. Lineage tracing reveals the early specification of the filament and the anther. Formation of the anther lobes is associated with a temporal increase of growth at the lobes with simultaneous repression of growth in the notches. Filament elongation passes through three phases: (1) Intense anisotropic growth and high proliferation right after specification; (2) Restriction of growth and proliferation to the filament base, which elongates relatively slowly and thickens; (3) Resumption of intense and anisotropic growth, displaced to the distal portion of the filament, without cell proliferation. The full quantitative analysis of growth achieved here provides a solid framework for future studies into stamen development and its adaptation to environmental perturbations.

Analysis of *Arabidopsis thaliana* RING-type ubiquitin ligases using protein-protein interaction datasets

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Eukaryotes utilize an essential post-translational modification system, called ubiquitination. Ubiquitination regulates protein localization, activity, and abundance within the cell. Ubiquitination occurs by the ubiquitously expressed protein ubiquitin being carried along an enzyme cascade, from a

ubiquitin-activating enzyme (E1) to a ubiquitin-conjugating enzyme (E2) to a ubiquitin ligase (E3) which transfers the ubiquitin to a target protein. Ubiquitinated proteins are determined by the E3s and are utilized in different cellular functions or recognized by the 26S proteasome for degradation. In the plant Arabidopsis, RING (Really Interesting New Gene)-type E3s are a group of approximately 500 E3s that are known to play important roles in plant growth, development, reproduction, and response to abiotic and biotic stresses. However, most of these RING E3s are uncharacterized and their target proteins unknown. This study provides an analysis of Arabidopsis RING-type E3s via the identification of potential target proteins using an interactome built from high-throughput protein-protein interaction databases and low-throughput studies. To further identify potential substrates, the interactome was cross-referenced with a merged ubiquitinome dataset curated from 13 independent high-throughput studies. Our results reported 1,551 interactions between 210 RING E3s and 842 unique proteins. RING E3s were versatile and found mostly interacting with multiple unique proteins. 191 of the 842 proteins were also found to be ubiquitinated from the merged ubiquitinome and therefore supported as potential target proteins. Further study of the RING E3s and the proteins they are ubiquitinating, could widen our understanding of the molecular mechanisms for plant stress response, growth, and development.

Over-expression of AINTEGUMENTA-LIKE7 (AIL7) in *Arabidopsis* alters morphological characteristic and heat stress response

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Members of the AINTEGUMENTA-LIKE (AIL) family of transcription factors act as master regulators of growth and organ development in plants. There are 8 *AIL* genes in Arabidopsis, and while most of them have been well-characterized in the context of meristematic development and shoot phyllotaxy, the precise function of *AIL7* has yet to be elucidated due to a high level of functional redundancy among *AIL* members. In the current study, we aimed to improve our understanding of the molecular and physiological role of *AIL7* through the generation and assessment of constitutive over-expression Arabidopsis lines. *AIL7* over-expression lines were found to exhibit a significant increase in plant height, rosette size, stem width and leaf biomass compared to wild type control lines. Furthermore, over-expression of *AIL7* also led to a significant delay in flowering compared to wild type. We also evaluated the response of over-expression lines to several types of abiotic stress and found that thermotolerance was enhanced in transgenic Arabidopsis seedlings compared to wild type controls. Further evaluation of Arabidopsis *AIL7* constitutive over-expression lines for tolerance to abiotic stresses at different stages of plant growth, as well as the unravelling of potential mechanisms driving differential responses, is currently in progress. Such knowledge not only provides evidence for additional roles of *AIL7* in plant development, but could also prove valuable for the development of crop cultivars with superior stress tolerance in the future.

Auxin biosynthesis and receptors gene expression during mid-phase seed development in Pea

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Developing pea (*Pisum sativum* L.) seeds contain high levels of the auxins, 4-chloroindole-3-acetic acid (4-Cl-IAA) and indole-3-acetic acid (IAA), a plant hormone class involved in seed growth and development. To further understand auxin biosynthesis and response dynamics in developing pea seeds, we determined the transcript abundance patterns of the auxin biosynthesis (*TAR* and *YUC*) and receptor (*TIR*/*AFB*) genes in seed tissues over mid-seed development (12-18 days after anthesis; DAA). The transcript abundance patterns of three *TAR* and four *YUC* genes indicate that *PsTAR2* and *PsYUC10* are highly expressed in the seed coat and embryo tissues during this developmental period. Auxin perception is mediated by *TIR*/*AFB* receptors that bind auxins to modulate tissue-specific auxin responses. Transcript expression of *PsAFB2* in the seed coat (8-18 DAA), endosperm and embryo (12 DAA), and embryo axis (16-18 DAA) and *PsTIR1b* and *PsAFB4* in the cotyledons (16-18 DAA) contributed to the transcript pool of auxin receptors in the seed. Overall, our data indicates that temporal- and tissue-specific modulation of auxin biosynthesis and receptor gene expression is part of the mechanism involved in regulating auxin-mediated seed growth and development.
TAR, TRYPTOPHAN AMINOTRANSFERASE RELATED; TIR1/AFB, TRANSPORT INHIBITOR RESPONSE 1/AUXIN-SIGNALING F-BOX proteins; YUC, YUCCA flavin monooxygenase

Transcriptome analysis of rutabaga (*Brassica napus*) cultivars indicates glucosinolate-derived nitriles may play a role in inducing plant defense against clubroot disease

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Clubroot, caused by the obligate biotrophic protist *Plasmodiophora brassicae*, is one of the most damaging diseases of the Brassicaceae. Glucosinolates (GSL) are a group of defense-related secondary metabolites found in species of this family, and their hydrolysis products (isothiocyanates, thiocyanate, nitriles) are implicated in plant defense processes against many pathogens and herbivores. We analyzed the GSL pathway in a database from a recently published study (Zhou et al., 2020 Int J Mol Sci 21, 8381) where the authors compared transcriptomic profiles of two rutabaga (*Brassicae napus* subsp*. rapifera*) cultivars which showed resistant ('Wilhelmsburger') and susceptible ('Laurentian') responses to *P. brassicae* inoculation. The results indicated that several genes that lead to production of nitriles along the indolic GSL degradation pathway are more highly upregulated 7 days after pathogen inoculation in 'Wilhelmsburger' than in 'Laurentian'. Nitriles serve as defensive compounds against plant pathogens because of their toxic nature and are also proven to elicit defense response pathways in plants. These data suggests that GSL-derived nitriles may play a role in inducing enhanced plant defense against the clubroot pathogen in the rutabaga cultivar 'Wilhelmsburger'. Further research to understand the role of GSLs in defense against *P. brassicae* in cruciferous plants is warranted.

Essential oil composition of *Artemisia vulgaris* L. cultivated in Brazil

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Artemisia vulgaris L., commonly known as mugwort, is one of the important plant species of the genus *Artemisia*, which is usually known for its volatile oils. It possesses a broad spectrum of therapeutic properties, such as anti-malarial, anti-inflammatory, anti-hypertensive, antioxidant, anti-tumoral, immunomodulatory, hepatoprotective, anti-spasmodic and anti-septic. These properties are mainly ascribed to volatile compounds present in essential oils including α- pinene, camphor, caryophyllene, camphene, germacrene D, 1,8-cineole, and α-thujone. These chemical compounds and their composition differ depending upon the environmental conditions, geographic source, and plant growth stage. Knowing the exact chemical composition of mugwort oil is vital to identify its biological properties. This talk will present an overview on the essential oils from *A. vulgaris* cultivated in Brazil and their biological activities. The essential oils from *A. vulgaris* showed fungicidal and bactericidal activities against *Candida albicans*, and *Staphylococcus aureus* respectively. Anthelmintic activity against *Haemonchus contortus* was absent in this essential oil. The results indicate the potential *A. vulgaris* essential oil as disinfectants and preservatives against micro-organisms.

Genotypic response of Canadian short-season soybean cultivars to drought stress

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Soybean [*Glycine max* (L.) Merr.] is the main legume crop in the world. It fixes atmospheric nitrogen through the symbiotic rhizobia bacteria that inhabit root nodules. Drought stress limits plant growth, yield, and symbiotic nitrogen fixation (SNF) in soybean. The main objective of this study is to identify genotypic response among short-season soybean cultivars for plant physiological parameters, phenotypic traits, and SNF under drought stress. A diversity panel of 100 early-maturity Canadian soybean varieties was used in this study. A greenhouse pot experiment was conducted to determine the various plant physiological, phenotypic traits, and SNF under drought stress. Soybean seedlings were inoculated with *Bradyrhizobium japonicum* USDA 110, and the initial soil moisture content was maintained at 80% field capacity. The drought treatment was imposed after 3-weeks of plant growth, where half of the plants were maintained at 30% field capacity (drought) and the rest at 80% field capacity (well-watered) until maturity. In general, drought stress reduced the stomatal conductance, number of pods, number of seeds, and seed yield, while increasing leaf chlorophyll content. Genotypic variability was observed in leaf chlorophyll content, stomatal conductance, photosynthesis, number of pods, number of seeds, and seed yield. The % nitrogen derived from the atmosphere (%NDFA) will be measured using the 15N-dilution technique. A genome-wide association study will be performed to identify allelic variation associated with SNF under drought stress and provide molecular markers that will be useful in future soybean breeding programs.

Alternative oxidase modulates serine metabolism and GABA shunt in tobacco under hypoxia
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We have studied possible involvement of the nonenergy-conserving alternative oxidase (AOX) pathway of plant mitochondria in modulation of the phosphorylated (non-photorespiratory) pathway of serine synthesis and the γ-aminobutyric acid (GABA) shunt. The nitric oxide (NO) production and relative expression of phosphoglycerate dehydrogenase (PGDH), phosphoserine aminotransferase (PSAT), phosphoserine phosphatase (PSP), glutamate decarboxylase (GAD), and γ-aminobutyrate transaminase (GABA-T) were directly estimated using real-time PCR in the lines of tobacco (*Nicotiana tabacum* L.) plants differentially expressing AOX incubated in the nitrogen atmosphere. NO production started upon oxygen depletion and was the highest in the overexpressing lines and lowest in the knockdown AOX lines. This corresponded to the level of expression of class 1 phytoglobin and *S*-nitrosoglutathione reductase that participate in NO scavenging. Biochemical methods were used to quantify the activities of nitrate reductase (NR), phosphoglycerate kinase (PGK), and GAD in the wild type (WT) and transgenic tobacco plants with different levels of AOX under hypoxia. We found that the plants overexpressing AOX exhibited an increased transcript abundance of the genes encoding PGDH, PSAT, and PSP during the first (3-12) hours of oxygen depletion, while expression of the genes and enzyme activities involved in the GABA shunt exhibited the increase after 24-48 h of hypoxia. Based on our results, we suggest that possible AOX involvement in NO turnover results in the activation of the non-photorespiratory serine metabolism and later of the GABA pathway as the important processes linking carbon and nitrogen metabolism and maintaining cellular energy levels under hypoxic conditions.

Actin-mediated developmental process in Arabidopsis is regulated by redundant function of RIC2 and RIC4
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Cell cytoskeleton component, actin regulates plant growth and development through modulating numerous cellular activities including cell division, cell elongation and protein trafficking. Actin filaments are formed by assembly of monomeric actin, called G-actin, to filamentous actin, called F-actin. Also, actin filaments are continuously remodelled through actin polymerization and depolymerization, to form the dynamic network of functional actin. This actin remodeling process is regulated by a complex pathway requiring participation of actin polymerization and depolymerization factors. One of the major groups of proteins that regulate this process are RICs, a member of ROP-INTERACTIVE CRIB MOTIF-CONTAINING PROTEIN. Previous studies revealed that RICs regulate pollen tip growth and leaf development through modulating actin tread milling. However, the functional roles of RICs in modulating the whole plant development remain to be determined. To understand the role of RICs in plant development, we firstly focused on root and analyzed the *RICs* expression pattern in Arabidopsis root. Among the *RIC*s, *RIC2* and *RIC4* were found to be highly expressed in the root. Interestingly, amino acid sequence similarities and phylogenetic analysis placed RIC2 and RIC4 in the same group. For functional analysis of RIC2 and RIC4, we characterized *ric2*, *ric4* and *ric2ric4* mutants. *ric2* and *ric4* do not exhibit any obvious developmental defect in the overall plant development. Interestingly, *ric2ric4* shows slow-growing phenotypes for all organs and at all developmental stages. Collectively, these

results suggest that RIC2 and RIC4 function redundantly and play a major role in plant development by affecting the actin assembly in Arabidopsis.

Biosynthesis of novel anti-inflammatory compounds from *Cannabis sativa* Kelly Bodding[1], Eric Soubeyrand [2], Tariq Akhtar[3] [1]Guelph, [2]Researcher, [3]Principal Investigator

The use of *Cannabis sativa* as a natural therapeutic has gained mainstream international appreciation. While the medicinal properties that are associated with the plant have long been attributed to Δ9-tetrahydrocannabinol and the pharmacologically-related cannabinoids, a growing body of evidence points to other specialized metabolites that may contribute to Cannabis' therapeutic potential. One such class of compounds are the bibenzyls, which are potent dual inhibitors of the inflammation pathways in our bodies. These compounds are synthesized via a novel branch point from the core phenylpropanoid pathway in Cannabis in a three step sequence, which we describe herein. Together, the identification and characterization of Cannabis enzymes involved in bibenzyl synthesis provides a valuable contribution to the growing 'parts prospecting' inventory for the rationale metabolic engineering of natural product therapeutics.

The impact of temperature fluctuation on phenology of Hazelnut (*Corylus* spp.) cultivars Mukund Shukla[1], Murali-Mohan Ayyanath[1], Praveen Saxena[1] [1]University of Guelph

Hazelnuts (*Corylus* spp.) typically flower over a range of 2-3 months depending on the genotype, environment, and location. In Southern Ontario, cold winter followed by a short spring promotes flowering at the end of winter, lasting several weeks. The flowering events such as female flower ratio, bud position, and catkin dehiscence were studied during the growth period of hazelnut cultivars. Our observations revealed that each bud, irrespective of its position on the stem, has an inherent capacity to result in a female flower. We hypothesized that fluctuating weather patterns may impact the female flower ratio, catkin dehiscence, and eventually the seed setting. Warmer winter days during flowering improved the rate of style protrusion and catkin dehiscence. However, while the female flowers suspend or slow phenological changes during sudden freezing conditions, the catkins do not, resulting in no pollen for the season. The catkin elongation and pollen dehiscence occurred in a span of 2 days during the year 2019, which was sufficient to pollinate the flowers without affecting nut yields. In 2018, pollen dehiscence and pollination were affected by warmer February followed by sudden freezing conditions in March, which resulted in poor crop yield. Our results indicated that the precipitation received in the form of snowfall assists in reproductive phases through a gradient generated by conduction/convection during snowmelt, especially in the sourced cultivars. This also appears to be critical for improving the percentage of female flowers and prolonging the receptivity of the female flowers in hazelnut cultivars included in this study.

Cold treatment conditions wheat microspores to respond to Trichostatin A for improved androgenesis

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Obtaining doubled haploids (DH) plants from isolated microspores is a valuable tool for plant breeders. Many years can be shaved from the breeding process through the ability to obtain homozygous lines in a single generation. For success, microspores must be coerced away from pollen development for entry into androgenesis. Most crops require some form of stress pre-treatment for this induction. Here we report RNA-seq analysis of cultured microspores that have been subjected to a three-week cold treatment and exposure to the histone deacetylase inhibitor Trichostatin A (TSA). After cold treatment we see upregulation in transcription, cytoplasmic turn over, signal transduction through kinase cascades, and chromatin remodelling supporting a change in cell fate. Upregulation is also observed for BRCA1 associated ring domain protein 1 which is involved in DNA damage repair, ubiquitination, and transcriptional regulation maintaining genomic stability. HYPOXIA UP-REGULATED PROTEIN 1 was downregulated in stressed material, which is associated with accelerated apoptosis. RNA-seq at 0, 3 and 48 hours after isolation point to dramatic transcriptionally reprogramming after cold pre-treatment. These cold-stressed microspores are then able to respond to TSA treatment by an early and transient genome-wide upregulation of several thousand genes, something not seen in unstressed microspores. Pathways impacted by this upregulation include mitotic cell cycle phase transition genes, transcription, and DNA recombination and repair. These pathways may explain the increase in androgenesis seen after application of TSA. Our results shed light on the behavior of wheat microspores in culture and pave the way for improved androgenesis and DH production.

ATP Binding Cassette protein ABCG36 is not a cadmium transporter

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Heavy metal Cadmium (Cd) is an environmental pollutant with harmful effects on most organisms. It is a potential threat to human health as it enters in the food chain from the contaminated soil through the crops. To clean the contaminated soil, a potential technique is “Phytoremediation”, which uses plants’ ability to uptake and sequester pollutants. To use the technique, the transport mechanism of particular metal needs to be understood. Hence, elucidation of the mechanism of Cd uptake is necessary to develop plants for phytoremediation purpose. Previously it was demonstrated that ATP Binding Cassette protein, ABCG36 is a Cd efflux transporter. Since ABC transporters can transport multiple substrates, we hypothesized that there might be other ABC transporters capable of transporting Cd. To find the new Cd transporters, we screened a large number of ABC mutants using T-DNA insertion lines. During this screening, we found that previously reported *abcg36* T-DNA mutant does not show any altered response to CdSO4. To confirm the result, we generated new mutant of ABCG36 using CRISPR-Cas9, and also used another allele of T-DNA insertion line. All three mutants showed hypersensitivity to Indole-3-butyric acid (IBA)-induced root growth inhibition, a typical response of *abcg36* mutant supporting the notion that ABCG36 functions as IBA transporter. Interestingly all three alleles of ABCG36 showed wild-type like response to Cd. Taken together, these results suggest that ABCG36 is not a Cd transporter.

W/Y/L motifs are enriched in core RxLR effectors but only the L motifs are indispensable for pathogenesis: A case study of PpRxLR6 from *Phytophthora parasitica* var. nicotianae

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Phytophthora species engineer a constant evolution of their RxLR effector repertoire to stir an arms-race with host resistance-genes towards pathogen success. This attribute lies within a variable C-terminal region with conserved W/Y/L domains observed in about 44% of RxLR effectors. With this wide distribution, one would expect their occurrence in RxLR effectors translates to an essential pathogenetic role. In fact, most functional assessments on RxLR effectors in *Phytophthora* pathogenesis have targeted these domains. However, contrasting outcomes from these assessments have been observed living us in doubt on their role in pathogenesis. To gain insight on the potential role(s) of W/Y/L domains, we turned to the evolutionary conserved, termed 'core' RxLR effectors implicated in essential roles of *Phytophthora* pathogenesis. Our focus addressed three questions: Are W/Y/L domains enriched in core RxLR effectors? If they are, what is their potential role in pathogenesis? And does this role in pathogenesis confer pathogen benefit(s)? We exploited *in silico* analytical platforms and identified, seven clusters of orthologous groups containing, 100 putative core RxLR effectors with evolutionary conservation across five *Phytophthora* spp. of diverse hosts. The W/Y/L domains were enriched in 'core' RxLR effectors of these *Phytophthora* spp. Further, *in planta* functional analyses of an evolutionary conserved W/Y/L domain in a putative core RxLR effector, PpRxLR6, from *P. parasitica* reviewed an indispensable role in plant immunity and pathogenesis. PpRxLR6 elicits cell death in *Nicotiana benthamiana* and contributes to pathogenesis. Our results implicate evolutionary conservation to align an essential role in pathogenesis of W/Y/L domains in PpRxLR6.

Phytoblobin 1 is involved in hypoxia-induced root bending

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R3T2N2 Mohamed M Mira, Robert D Hill, Claudio Stasolla
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Root growth is influenced by the availability of oxygen within the soil. While in well aerated soils root growth is mainly gravitropic, oxygen shortage typical of water saturation, causes an anti-gravitropic bending of the root system. This "avoidance" growth response is reversible in that gravitropic growth can resume after the re-establishment of normoxic conditions. It is well established that root growth and bending are mediated by auxin and Phytoglobins (Pgbs), plant proteins scavenging NO, are able to alter auxin synthesis and flow. Here we show that hypoxia-induced root bending in Arabidopsis is accentuated by the over-expression of *Pgb1* and mitigated by the suppression of the same gene. These effects were mediated by the group VII ethylene response factor (ERFVII) RAP2.12, known to inhibit root bending. Relative to wild type, the transcript levels of *RAP2.12* were lower in hypoxic roots over-expressing *Pgb1*, and higher in roots down-regulating Pgb1. Furthermore, the exacerbation of hypoxic root bending observed in the *rap2.12* mutants was attenuated in the *pgb1-rap2.12* double mutant, suggesting Pgb1 acts upstream of RAP2.12 in root bending. The effects of Pgb1 on hypoxic root bending were mediated by profound changes in the establishment of auxin maxima at the root tip and alterations in PIN localization patterns. Taken together these studies suggest Pgb1 might be an important regulator of root growth during conditions of reduced oxygen availability.

Differentially expressed genes involved in low seed protein content in western-Canadian soybeans (*Glycine max*) identified through transcriptomics

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Soybean [*Glycine max* (L.) Merr.] is among the most important agronomic crops in Canada with widespread uses in human consumption, animal feed, and biotechnology. The capacity to fix atmospheric nitrogen into biologically available forms gives soybean an important role in sustainable agricultural practices (i.e., reducing the need for nitrogen fertilizers). Understanding the effect environmental variation has on gene expression in Canadian soybean is valuable for developing sustainable agricultural systems. The Canadian Grain Commission has reported lower seed protein content from soybeans grown in western Canada compared to eastern Canada, regardless of genotype. This project will uncover key genes responsible for differences in seed protein content across Canada. Here we use a transcriptome-wide approach to identify differences in expression of genes which contribute to seed protein content, and to study the effect of environmental variation on geographically-dependent gene expression (West vs East). Ten soybean lines ranging low to high in seed protein content are growing in four locations over four years across western and eastern Canada. Using RNA sequencing, differential transcript analysis of each line is compared between West and East to determine key genes responsible for lower seed protein content in western soybeans. Analysis of first year data has identified three genes encoding cupins and 93 lipid-related genes. This research will provide novel information about the best geographically-fitting soybean cultivars to grow across Canada's growing regions. The findings of this research will be used to develop allele-specific markers, assisting breeding programs to develop high protein soybean cultivars for western Canada.

Chloroplast Ultrastructure and Thylakoid Architecture enhances Photosynthetic Efficiency under challenging environments in *Amaranthus* spp.

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Photosynthesis occurs in the chloroplast containing thylakoid membranes. A stack of thylakoids forms a granum, many stacks are known as granal lamellae or appressed thylakoids. Adjacent grana are connected by unstacked thylakoids known as stromal lamellae or non-appressed thylakoids. All thylakoid membranes within the chloroplast are known as thylakoid architecture and contain components involved in the electron transport chain. However, these components are not distributed evenly within the thylakoid architecture resulting in what is referred to as lateral heterogeneity, thought to provide flexibility of the photosynthetic apparatus to respond to challenging environments. The objective was to evaluate chloroplast ultrastructure and determine the effect of thylakoid architecture to explain differential rates of photosynthetic efficiency (FappO2) and tolerance to photoinhibition observed between red and green vegetable varieties of *Amaranthus*, grown at high light (500 µmol m-2 s-1). Tolerance to photoinhibition was 54% greater in the red than green variety. The red variety also exhibited greater values of FappO2 (18%) than the green variety. In the bundle sheath cells, the red variety was greater than the green variety in: granal index (length of appressed thylakoids as a percentage of total thylakoid length) by 50%, the ratio of appressed to non-appressed thylakoid length by 68%) and the ratio of thylakoids per granum by 55%. Chloroplast ultrastructural analyses revealed the presence of a peripheral reticulum, cytoplasmic protrusions and crystalline inclusions in various cell types of the green variety. Overall, chloroplast ultrastructure and thylakoid architecture may play a role in enhancing (FappO2) and tolerance to photoinhibition.

Genetic Control of Hilum Ring in Common Bean (*Phaseolus vulgaris* L.)

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The hilum ring is a specialized area in common bean (*Phaseolus vulgaris* L.) seed coat that marks the previous point of attachment of the developing seed to the placenta. The presence or absence of hilum ring colour is a quality characteristic of beans that is controlled by duplicate dominant epistasis between two Mendelian loci, *Z* and *J*. Individuals with genotypes *Z_jj* and *zzJ_* have seeds with a coloured hilum ring, and *zzjj* individuals have colourless hilum rings. *J* is associated with the gene *Phvul.10G130600* encoding a R2R3 MYB transcription factor. *Z* is associated with a STS marker, OAM10S490, mapped on chromosome *Pv*03 at a distance of 1.4 cM. The current research aims to deepen our understanding of the genetic control of hilum ring colour in common bean. A population of 128 F6 recombinant inbred lines, derived from a cross between 'Wit-rood boontje' (without hilum colour) and '1533-15' (with hilum colour), was genotyped with BARCBEAN6K_3 BeadChip and phenotyped for the presence or absence of hilum ring colour. A linkage map was constructed using 2,710 informative SNPs arranged into 11 linkage groups and spanned 1232.4 cM. Two major hilum-associated QTLs were identified on linkage groups *Pv*03 and *Pv*10 which explained 37.8% and 17.3% of the phenotypic variation for the trait, respectively, that included candidate genes such as two UDP-glucosyl transferases, a Mate efflux family protein, and a MYB transcription factor. Amplicon sequencing will be used to identify the genetic variants associated with the presence of hilum ring colour in common bean.

Genomics Driven Development of Drought Tolerant Wheat Cultivars

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Drought stress can have devastating effects on plant growth and poses a major threat to agriculture globally. The role of plant hormone abscisic acid (ABA) in limiting the loss of water through stomata under drought stress has been well studied. Our lab has also identified a regulatory relationship between ABA and brassinosteroid (BR) biosynthesis pathways and has found that inhibition of BR biosynthesis could improve drought tolerance in several model and crop species. We have utilized this knowledge and subjected EMS mutagenized M2 wheat population to a chemical and have identified several candidate drought-tolerant wheat mutants. We have conducted detailed phenotyping of these mutants in the greenhouse and validated their performance under multi-year field trials. These analyses have identified mutant lines with higher water-use efficiency, biomass, number of tillers, and seed yield under drought stress. Using Illumina 90k wheat SNP platform, various candidate SNPs and associated target genes have been identified in the mutant lines, including genes known to impart stress tolerance in model plants and key genes in hormone biosynthesis pathways. We are currently validating the identified candidates for their role in imparting drought stress tolerance and working toward the identification of molecular markers associated with stress tolerance in wheat. In addition to providing drought-tolerant wheat lines that can be integrated into the ongoing wheat breeding programs, this study will further our understanding of drought tolerance mechanisms in wheat. The work also forms a template for the generation of drought-tolerant cultivars in other economically important crops.

Distinct molecular responses of two ecotypes of the extremophile plant *Eutrema salsugineum* to low phosphate availability

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The halophyte and extremophile plant, *Eutrema salsugineum*, is a close relative of Arabidopsis and Brassicaceae crops. Two Eutrema ecotypes frequently compared originate from the Yukon, Canada, and Shandong, China. Yukon plants are reported to be tolerant of low-phosphate (Pi) conditions, likely facilitated by the glycolytic bypasses, including elevated phosphoenolpyruvate carboxylase (PEPC) activity that avoids the use of ATP by pyruvate kinase and releases Pi. Here we report that Shandong plants do not tolerate low-Pi conditions, their biomass and root development were impaired relative to Yukon seedlings. We hypothesized that tolerance to low-Pi in Yukon Eutrema associates with higher expression of *High-affinity Phosphate Transporters* (*PHT*s) as they play important roles in Pi uptake, translocation and remobilization. However, gene-specific RT-qPCR results showed no compelling evidence for this association in Yukon plants grown with low-Pi. Moreover, genes encoding several PHT family members were highly upregulated in Shandong seedlings under low-Pi despite comparatively poor growth. The Eutrema genome encodes three plant-type *Phosphoenolpyruvate Carboxylase* (*PEPC1; 2; 3*) genes and two bacterial-type *PEPC* (*PEPC4.1; 4.2*) genes, all but one bacterial-type *PEPC* gene are expressed in both ecotypes, albeit differentially. Even when Pi is present, *PEPC1* and *PEPC2* are expressed at higher levels in shoots of Yukon compared to Shandong seedlings. Thus the exceptional capacity for Yukon Eutrema plants to cope with low-Pi availability is likely not conferred by an augmented Pi transport capacity but rather in the ability to constitutively engage metabolic bypasses to reduce use of ATP and retrieve internal Pi for reuse.

Silver and Symbiosis: Detrimental Effect of Silver Nanoparticles on Soybean (*Glycine max*) Symbiosis with *B. japonicum*

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Due to their antimicrobial properties, silver nanoparticles (AgNPs) have become more popular in commercial products and industry, leading to increasing agricultural and environmental concentrations. These increased concentrations could lead towards detrimental effects on not only plants, but the microbes, and relationships that plants and microbes hold. Using a 96-well plate and a liquid culture, growth of *Bradyrhizobium japonicum USDA 110* was assessed when subjected to 10 µg/mL AgNPs, finding that no significant growth occurred at this concentration compared to exponentially growing control bacteria. Plants were grown in hydroponics within a growth chamber and either inoculated or axenic (no symbiont given), growth of inoculated plants was significantly decreased at 2.5 µg/mL AgNPs by 50% compared to inoculated controls, while axenic control had no significant difference at 2.5 µg/mL AgNPs, pointing to problems of AgNPs effecting with nodulation, and not only growth. Effects on nodulation are confirmed by TEM images showing treated (2.5 µg/mL AgNPs) plant nodules absent of bacteroids compared to healthy control nodules, and also confirmed by plants given 0.5-2.5 µg/mL AgNPs that result in 40-65% decreased nitrogen fixation compared to control. In conclusion, I determined that AgNPs not only interfere with plant-microbe relations (e.g., decreased nitrogen fixation and absent bacteroids) but also with general plant and bacterial growth, and that we should be mindful of not releasing them to the environment and agricultural land.

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

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Understanding the genetic bases of complex traits including yield in strategic crops such as common bean (*Phaseolus vulgaris* L.) is the most sustainable way to address the growing global food demand in the near future. However, yield and its related traits are controlled by various genes with major and minor allele effects. Several selection strategies can be implemented to increase the annual yield genetic gain in a common bean population. For this study, 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 four field environments. The distribution of all the traits, days to 50% flowering, days to maturity, and yield was continuous and showed some transgressive segregation compared to the parental lines in each environment. In order to test different selection strategies, two population were created based on phenotypic selection of twenty-five RILs with the lowest and highest productivity in the tested population. The results indicated that a yield gain of 6.3 % can be achieved by phenotypic selection from the NAM population. In the future, genomic and QTL index selection strategies will be compared with the phenotypic selection method to evaluate their efficiency for increasing the overall genetic gain for yield from the NAM population.

Tree seed germination under stress

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Tree seed germination is critical for the maintenance of healthy natural forests and the regeneration of disturbed sites. Successful germination is strongly influenced by environmental conditions, and this might especially be true for wind-distributed seeds with relatively little or no endosperm at maturity. As a consequence of climate fluctuations and anthropogenic activities such as mining or certain irrigation practices, soil salinity has increased, and this has removed thousands of hectares from use for agriculture and agroforestry in Canada. At the same, little is known about the effect of salinity on seed germination in several of the tree species native to Canada. Here, we study the effect of increased salinity on seed germination in poplars, which represent prominent trees in Canadian forests and are of economic importance. *Populus deltoides* seeds were exposed to increasing concentrations of salt under controlled environmental conditions. Threshold concentration for delayed development as well as lethality were determined. We also introduce the criterion of maximum harm concentration that discriminates between germination and early seedling development through seedling damage post successful germination. Moreover, effects on biomass production were analyzed. Finally, the effects of salinity on sex of the seedling in this deciduous species were studied. The findings contribute to our mechanistic understanding of susceptibility during germination and potential use in regeneration efforts of a native deciduous tree.

Can biofortification safely be used to alleviate selenium deficiency and reduce cadmium uptake by plants grown on marginal lands?

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Consumption of crops grown on marginal land with elevated cadmium concentrations poses a risk to consumers. However, selenium, an essential micronutrient for animals, has been shown to decrease plant cadmium uptake. Previous reports attribute this to selenium-induced cell wall thickening, which could provide more binding sites for cadmium. Selenium deficiency is problematic in some regions due to low soil levels, but biofortification can be used to prevent adverse health effects. Whether selenium biofortification is an option to mitigate nutritional deficiency while simultaneously improving the safety of crops grown on cadmium-containing soil was investigated in 5 crop species: canola, corn, lettuce, sorghum, and wheat.

Experiments were conducted using the most bioavailable form of selenium for crops, selenate. Since selenate is taken up by plants by sulphate transporters, it is in competition with sulphate. Thus, selenate and cadmium were applied to the hydroponic growth medium of crop species with both adequate and excess sulphate levels. Total lignin contents of plant roots were measured as a proxy for cell wall thickness, but no differences were found among treatments. As predicted, excess sulphate decreased selenate uptake. However, contrary to expectations, concentrations of cadmium and selenate in the shoot tissue were positively correlated in three of the species tested. Consequentially, biofortification with selenium on marginal lands with elevated cadmium concentrations is not supported as a safe option, particularly in soils with high sulphur levels.

Identification of the causal agents of potato early dying in potato plants and fields of Alberta

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Potato early dying (PED) complex causes premature plant senescence and can decrease potato marketable yield as much as 50%. This infectious disease is a major limiting factor in profitable potato production in Canada. Several pathogens are involved in PED in other potato growing regions: the soilborne fungi *Verticillium,* the root-lesion nematode *Pratylenchus penetrans,* the black dot fungus *Colletotrichum coccodes*, the soft rot bacteria *Pectobacterium*, and possibly others. The severity of PED depends on inoculum density of the pathogens in field soils and the virulence of the resident strains. Knowledge of the factors contributing to PED is crucial for the development of effective disease management strategies. Hence, the goal of the current project is to determine inoculum density of *V. dahlia* and *P. penetrans* in Alberta's agricultural soils and to isolate the resident strains involved in PED. Soil samples were collected from 30 fields in fall of 2020. *V. dahlia* and *P. penetrans* were quantified in the soil using both traditional plate counting method and qPCR. Soil samples were collected again in spring of 2021 prior to planting to quantify the pathogen levels at the beginning of the cropping season. Plant tissue samples will be collected throughout the growing season to isolate and identify pathogens colonizing plant tissues and possibly contributing to PED. Pathogen-specific DNA primers have been selected and validated to ensure accurate species identification. At harvest, plants will be assessed for disease severity and tuber yield to determine region-specific thresholds for disease development.

Douglas-fir LEAFY COTYLEDON1 (PmLEC1) expression in *Arabidopsis* induces somatic embryogenesis in the lec1-1 null mutant but not in wild type plants

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Somatic embryogenesis (SE) is the most promising method for rapid propagation of superior plant genotypes. Douglas-fir (*Pseudotsuga menziesii*) is one of the world's most economically and ecologically important lumber species due to its desirable wood qualities and its ability to withstand climate change. However, the application of SE to conifers remains challenging due to limited knowledge about the genes involved in embryogenesis and the processes that lead to somatic embryo formation. We previously isolated the Douglas-fir homolog of the angiosperm embryo-regulatory gene, *LEC1*. In this study, we evaluated the potential of Douglas-fir *PmLEC1*, to induce SE in vegetative cells of a heterologous host, Arabidopsis. *PmLEC1* complemented the *Arabidopsis thaliana lec1-1* null mutant and led to a variety of phenotypes ranging from normal morphology to developmental arrest at various stages in the first transgenic generation (T1). *PmLEC1* did not affect the morphology of wild type Arabidopsis T1 plants. More profound results occurred in the second transgenic generations (T2). *PmLEC1* expression induced formation of recurrent somatic embryo-like structures in vegetative tissues of the rescued *lec1-1* mutant but only led to loss of apical dominance (bushy phenotype) in wild type plants. The activation of embryonic programs in the *lec1-1PmLEC1* T2 plants was confirmed by the presence of the embryo-specific transcripts, *oleosin* and *cruciferin*. In contrast, no embryo-like structures, and no *oleosin* or *cruciferin* were observed in *PmLEC1*-expressing bushy wild type T2 plants.

Can Drone Based Vegetation Indices Track Photosynthetic Phenology in Forest Ecosystems?

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Forests play an important role in the terrestrial carbon sinks. Changes in forest vegetation greenness and the length of growing season have been widely reported recently on a global scale. A longer growing season will in turn affect climate through water and carbon cycle. The northern hemisphere forests are sensitive to climate change, so accurately estimating their photosynthetic activity is important to understand vegetation themselves as well as their responses and feedbacks to changing climate. Phenology can provide a lot of information about tree species and its resilience to climate change and can be measured from leaf-level to ecosystem-scale. Eddy-Covariance towers (Flux towers) can provide relatively accurate Gross Primary Productivity. However, such measurements are time consuming and require sophisticated long-term monitoring infrastructure and hence lack flexibility. Drone-based remote sensing data can provide high resolution spatial and time-repeat information, which can greatly enhance the efficiency and the level of spatial and temporal details of vegetation monitoring. In this study, we aim at developing a methodology that can using drone to access phenology in forest and use drone images to validate satellite data outside the footprint of Flux tower. First, we will validate the drone-based vegetation indices by spectral reflectance sensors installed on the EC tower, as well as GPP obtained by tower measurements. Then, use drone-based vegetation indices to validate satellite data. We focus on the comparison of drone-based different vegetation indices using correlations and models to identify a more accurate approach to track the seasonal variations in canopy-level in forests.

3’-(Phenyl alkynyl) analogs of abscisic acid: synthesis and biological activity of potent ABA antagonists

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We report here the synthesis and biological testing of 3’-(phenyl alkynyl) abscisic ABA analogs, a new class of potent ABA antagonists. These ABA analogs incorporate a rigid framework of eight carbon atoms attached at the 3’-carbon atom of ABA that prevents folding of the ABA analog-bound receptor required for ABA signalling. The two-step synthesis is based upon the optimized conversion of natural (S)-ABA to 3’-iodo ABA which can be coupled to phenyl acetylenes using Sonogashira conditions, or to styryl compounds through Suzuki chemistry. The parent 3’-(phenyl alkynyl) ABA analog 7 was obtained in 29% yield, 74% yield based on recovered starting material. In a lentil seed germination assay, compound 7 was found to have more potent activity than other known 3’-substituted ABA antagonists to date. In a structure activity study parasubstituted phenyl alkynyl analogs had comparable activity to the analog 7 while the 3’-styryl ABA 18 was only slightly less active. Analog 7 overcame ABA inhibition of germination and seedling growth in a wide range of mono and dicot plant species, including canola, lentil, soybean, rice, wheat, barley, cannabis and canary seed. 3’-(Phenyl alkynyl) ABA analogs have numerous potential practical agricultural applications including promoting ripening of crops, dormancy breaking of seeds and woody perennials, as well as promoting seed germination, and growth under stress conditions as demonstrated in this report.

Identification of circadian clock regulated immune response genes in wheat

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The plant circadian clock functions to optimize cellular processes and physiological reactions with respect to light/dark cycles and seasonal changes within a 24 h cycle. Diverse physiological pathways are connected to the circadian clock and increasing evidence indicates that the plant immune responses are also connected to the circadian clock. Maximal jasmonic acid (JA) and salicylic acid (SA) accumulations occur at midday and midnight respectively. Cross talk between JA and SA signalling is an important part of plant defence against different pathogens. The plant must be ready to respond with peak expression of defence genes concordant with the time of pathogen attack. Regulation of the other plant growth regulators (PGFs) ABA, IAA, BA, cytokinin, ethylene and GA also occur during the 24 h cycle. We are studying gene expression using RNA-Seq over 12 times points from ZT1 to ZT23 within a 24 h cycle in the wheat cultivar Fielder to determine the expression of core clock genes and other transcripts that may be regulated by the circadian clock with the overall goal of modulating the clock for improving wheat resilience to changing climatic conditions. As part of this gene expression study, we will present the response of genes of the biosynthetic pathways in the above eight PGFs to see the potential role of the circadian clock on the plant growth regulation and response in wheat.

Metabolic Maestros: Synchronized response by mitochondria and chloroplasts during growth at elevated CO2

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Plants will experience an elevated atmospheric concentration of CO2 (ECO2) in the future. Since CO2 is a rate-limiting substrate for photosynthesis, ECO2 will likely have profound and pervasive effects on carbon and energy metabolism. I am examining how ECO2 influences the metabolic interactions that occur between photosynthesis and respiration. To do so, I analyze metabolic components of chloroplasts and mitochondria that are hypothesized to maintain carbon and/or energy balance in response to environmental change. These include a chloroplast glucose 6-phosphate (G6P)/phosphate translocator called GPT3, a chloroplast G6P shunt associated with the Calvin cycle, chloroplast cyclic electron transport pathways, and a mitochondrial non-energy conserving terminal oxidase called alternative oxidase (AOX). Experiments indicate that AOX protein amount in leaves increases over time at ECO2 but not at ambient CO2. Additional experiments indicate that, along with AOX, GPT3 also responds dynamically to both short-term and long-term changes in growth CO2 concentration. Subsequent experiments aimed at manipulating the sugar and/or phosphate status of excised leaves indicate that both AOX and GPT3 gene expression are highly responsive to carbohydrate and phosphate levels, however more investigation is required to determine the functional significance of these responses. Further research will use AOX knockdown and overexpression plants to investigate the potentially complementary roles of mitochondrial respiration and chloroplast G6P transport in maintaining carbon and energy balance during growth at ECO2.

Efficient production of therapeutic phytomolecules in the bioengineered diatom *Thalassiosira pseudonana*

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Plants are factories of bioactive molecules used in many medicinal practices since the dawn of human history. Such bioactive molecules are often derived from specialized metabolism. After the last discoveries of new therapeutic targets, specialized metabolites have become more interesting in the pharmaceutical field. However, the production of these molecules in plants is often limited in yield and purity, hence the interest in finding a heterologous system for their production. Microalgae are privileged candidates for the bioengineering of plant bioactive molecules due to their homology for some cellular structures as well as for plant synthetic pathways. In our project, we aim to produce specialized metabolites in a microalga of the diatom family, *Thalassiosira pseudonana*. Our methodology consists of the incorporation of genes coding for enzymes catalyzing the formation of specialized metabolites in the microalgae. Our main goal is to produce a diatom strain capable of producing them at levels comparable to those of the original plant. To develop this tool, the microalga is transformed by plasmid insertion using the two transformation techniques, bacterial conjugation, and electroporation. After genetic transformation, the positive transformants are characterized by different molecular tools (colony PCR, sequencing, Western blot...) and compared between them for their production of metabolites of interest. Thus, this platform can be used as a proof of concept for the bioengineering of various other metabolic pathways.

Identification of macronutrient-stress-induced proteins involved in phloem-mediated long-distance signaling

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In plants, Nitrogen (N), Phosphorus (P) and Potassium (K) are essential macronutrient and consequently, main constituent of fertilizers required for plant growth, development and sustainable agriculture output. The phloem translocation stream functions in the delivery of photosynthate, amino acids, and essential mineral nutrients to developing tissues and organs of the plant. Recently studies have raised the question as to whether the phloem evolves the capacity to deliver specific stress-signaling molecules for control over plant development and growth when plants face nutrient deficiency. Here, our group is profiling phloem-mobile proteins as potential long-distance macronutrient-stress-associated signaling agents. Heterografting between cucumber and watermelonwas established to elucidate systemic signaling mechanism(s) under N, Pi and K-stress conditions. To verify spatial features of protein profiles along the phloem, phloem exudate was collected from the unloading (apical sink region) and shoot–root junction regions of recipient watermelon stems of heterografted plants under N, Pi and K-stress conditions and processed for phloem-mobile proteomes. Here, we address the role of the phloem long-distance signaling in mediating the processes involved in photosynthate reallocation under Pi-stress conditions. To this end, we characterized a phloem-borne cucumber protein, CsPPSR (*Cucumis sativus* Phloem Phosphate Stress Repressed), which responded early to an imposed Pi-stress. The mechanism involved in this Pi-stress-induced decrease of CsPPSR in central source region (CSR) STS involved 26 S proteasome-mediated degradation. This process was controlled by the phosphorylation status of CsPPSR; a threonine residue was shown to be critical for protection against 26 S proteasome-mediated degradation.

Variation in the timing of autumn cold acclimation in field-grown white spruce under elevated temperatures and reduced water availability

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Temperature and photoperiod are critical environmental cues for regulating phenology of photosynthesis in boreal conifers. While photoperiod will be unaffected by climate change, it is unclear how future warming and reduced water availability will impact the regulation of autumn cold acclimation. Genetic variation may also affect the timing of cold acclimation due to local adaptations to photoperiod. The goal of our project is to understand the impact of warming and the relative importance of photoperiod on the timing of cold acclimation in two latitudinally distinct white spruce (*Picea glauca)* genotypes when grown under similar environments. The experimental design consisted of four treatments in replicate field plots: (1) control, (2) reduced soil moisture, (3) warming and (4) warming combined with reduced soil moisture. Across all time points, there were strong genotype, treatment, and genotype by treatment effects on photosystem II (PSII) efficiency, non-photochemical quenching (NPQ), photochemical reflectance indices (PRI), and chlorophyll carotenoid indices (CCI). Genotype and treatment differences became evident in late autumn as temperatures decreased and photoperiods shortened. The southern genotype showed higher PSII efficiency, PRI, CCI and dynamic NPQ, compared to the northern genotype, characteristic of delayed cold acclimation. Control and drought treatments had lower PSII efficiency, PRI, CCI, and higher sustained NPQ compared to the warming and warming combined with reduced soil moisture treatments. Our findings provide evidence that

delays in the downregulation of photosynthesis and the upregulation of sustained NPQ occur under warming and are greater in southern white spruce genotypes compared to northern genotypes.

Phytoglobin 1 overexpression improves water stress tolerance in soybean (*Glycine max*)
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Soybean (*Glycine max*) is an economically important crop and abiotic stress, especially drought, compromises growth and reduces yield. Among factors participating in plant responses to conditions of abiotic stress are phytoglobins (Pgbs), ubiquitous heme-containing proteins capable of scavenging nitric oxide, a signal molecule involved in the regulation of many plant processes. The current study reveals that over-expression of *GmPgb1* enhances tolerance to drought stress. Imposition of drought, mimicked by applications of polyethylene glycol (PEG), depressed several gas exchange parameters including photosynthesis, stomatal conductance and transpiration, and induced extensive foliar injury. These effects were attenuated by over-expression of *GmPgb1*, and exacerbated by suppression of the same gene. Relative to WT plants, leaves of water stressed plants over-expressing *GmPgb1* exhibited higher transcript levels of ABA biosynthetic genes and a reduction of transcripts of ABA catabolic enzymes. This was in contrast to leaves suppressing *GmPgb1*, characterized by a transcriptional induction of ABA catabolic enzymes following water stress. Irreversible inhibition of the catabolic enzyme ABA 8'-hydroxylase by foliar applications 8'acetylene ABA improved drought tolerance in the WT and *GmPgb1* suppressing plants. In natural germplasm a strong correlation was found between that expression of *GmPgb1* in leaf tissue and the ability of soybean plants to tolerate drought stress. Collectively this study suggests alteration of *GmPgb1* expression enhances plant tolerance to soybean water stress and could be an effective marker in selecting for drought tolerance.

Flavonol rhamnoside degradation in senescing leaves is linked to α-rhamnosidase activity
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Flavonol rhamnosides (e.g., kaempferitrin) occur in various vascular plants. In Arabidopsis, kaempferitrin (kaempferol 3-*O*-α-rhamnoside-7-*O*-α-rhamnoside) limits auxin-mediated growth processes and functions as an anti-herbivory compound. This metabolite accumulates in young leaves, and at high levels during abiotic stress, but losses are apparent following the recovery from abiotic stress. Here, we provide biochemical evidence for flavonol rhamnoside degradation in leaves during on-the-plant senescence and with postharvest storage at low temperature. HPLC analysis of flavonol extracts prepared from leaves of Arabidopsis and silver linden revealed kaempferitrin levels fluctuate with development, whereas elevated concentrations of its putative catabolites (i.e., kaempferol 7-*O*-rhamnoside, kaempferol 3-*O*-rhamnoside and kaempferol) occurred with senescence. UHPLC-MS/MS analysis evidenced similar catabolite profiles in postharvest radish leaves chilled for 8 days. HPLC analysis of *in vitro* assays containing clarified leaf extracts demonstrated that Arabidopsis, radish and silver linden have α-rhamnosidase activities that hydrolyze kaempferitrin to kaempferol 3-*O*-rhamnoside and kaempferol 7-*O*-rhamnoside to kaempferol. Kaempferitrin α-rhamnosidase activity was most prominent in leaves of 8-week-old Arabidopsis relative to earlier developmental stages; in senescent (i.e., yellow) silver linden leaves this activity was approximately 1.2-fold that of their green counterparts. Radish leaf α-rhamnosidase activity increased up to 135% after 5 days of postharvest

storage at 5℃. A 38 kDa flavonol rhamnoside α-rhamnosidase activity from postharvest radish leaves was purified to homogeneity. Current efforts are focused on establishing the kinetic properties and the genetic identity of flavonol rhamnoside a-rhamnosidase activity in plants. This research will facilitate biotechnological strategies for boosting flavonol levels in agriculturally relevant crops.

Integrating systems-level phenomics and quantitative proteomics to profile Kale cultivars diel pattern.

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Diel plant cell regulation plays a major role in plant physiology, greatly modifying plant behavior to allow development through seasons and during day and night cycles. About 1/3 of plant genes are impacted by the circadian clock, involving major hormonal pathways and stress response to the environment. New studies on Brassicas show that knowledge of diel fluctuations can have a major impact on cultivation practices. Kale (Brassica Oleracea var. acephala) is well- known for its rich nutrient content and for being well suited for indoor growing facilities that enable accessibility to fresh food to isolated populations. Despite the growing amount of publication on Kale, differences between cultivars and their diel molecular profile remain understudied. Using our real-time phenomics platform and a newly developed mass spectrometry acquisition workflow, this study aims to establish diel differences and systems-level connections between Kale species grown under precision LED lighting systems.

How will climate change affect the CO2 dynamics of Canada's boreal forests?

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Boreal forests are an important reservoir of terrestrial organic carbon and a key component of the global carbon cycle. Rising atmospheric carbon dioxide (CO2) levels are expected to warm high latitude regions, including boreal zones, by up to 8ºC by 2100. Despite this, we have relatively few data on the physiological responses of boreal forests to climate change. My goal is to explore how foliar photosynthesis and respiration in Canadian boreal trees respond to growth under high temperatures and CO2. I will grow five North American boreal tree species (black spruce, white spruce, tamarack, jack pine and paper birch) under ambient (400 ppm) or elevated CO2 (750 ppm) at either ambient temperatures or with +4ºC or +8ºC warming. After 4-5 months, I will characterize photosynthetic capacity (as the maximum rates of Rubisco, *V*cmax, and electron transport, *J*max), dark respiration (*R*dark), biomass allocation and various leaf biochemical and morphological traits. I expect to see widespread reductions in *V*cmax, *J*max and *R*dark (measured at a standard temperature) in line with acclimation. The results of my project will improve the accuracy of our Earth system models, provide insight on forest productivity in the future and further our understanding of thermal and CO2 acclimation in plants.

The development of [18F]-3'-F-ABA, a PET Tracer to Monitor ABA Transport in Live Plants

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Abscisic acid (ABA) is a signaling molecule that regulates plant responses to abiotic stress. ABA regulates transpiration to maintain water status in drought conditions and induces expression of genes/proteins required for adaptation to abiotic stress. Positron emission tomography (PET) is well suited for imaging ABA trafficking to understand the mechanisms of ABA transport *in vivo*. PET imaging is sensitive, non-invasive and can be employed on any plant without the use of transgenics, leading to new strategies for breeders to develop drought tolerant crops. We first assessed a known derivative, 3′-F-ABA, and determined it would be a suitable analog for PET imaging and has similar biological activity as natural ABA. We compared metabolism of ABA versus F-ABA in plants using stably labeled analogs and metabolites in feeding studies with destructive analysis by HPLC/MS/MS. We found that 3´-F-ABA and ABA elicited similar biological responses in plants e.g. *in-vivo* stability and gene/protein expression. With this confirmation we then synthesized 3′-18F-ABA as a PET tracer and performed the first ever imaging studies in two plant systems: *Arabidopsis* and *Brassica napus*. When applied to leaves, 3'-18F-ABA was transported to adjacent leaves as well as roots and shoots confirmed by autoradiography, gamma counting and PET. Additionally, HPLC shows that 80% of the radiotracer signal is due to the 3'-18F-ABA, consistent with MS studies. 3'-18F-Phaseic acid was confirmed as the major catabolite. ABA transport studies comparing stressed plants with non-stressed plants will be undertaken in the next phase of the project.

Comparative physiological, biochemical and transcriptomic assessments of two subspecies of *Medicago sativa* exhibiting differential levels of drought resilience

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Alfalfa (*Medicago sativa* L.) is one of the most extensively grown perennial forage legumes worldwide; however, its productivity is hindered by drought, which is expected to become increasingly problematic in coming years due to climate change. Since the demand for meat and milk is expected to expand considerably in the foreseeable future as a result of our growing population and escalating affluence, the need for highly productive alfalfa cultivars with superior drought tolerance will be critical. The aim of this study is thus to identify novel sources of genetic variation for the downstream generation of alfalfa germplasm with improvements in drought tolerance through comparative analyses between alfalfa (*M. sativa* subsp. *sativa*) and a close relative (*M. sativa* subsp. *falcata*), which exhibits superior drought tolerance. Responses to drought in terms of performance, physiological and biochemical characteristics were assessed in both genotypes, and RNA-Seq was also carried out in an attempt to elucidate possible mechanisms behind the differences in drought resilience between the two subspecies. Several morphological differences between the two subspecies, including a significant reduction in leaflet area in the 'falcata' subspecies, may have contributed to their distinct drought response. In addition, numerous genes were differentially regulated in the two subspecies under both well-watered and water deficient conditions. The results of this study further our understanding of

drought tolerance in *M. sativa*, and may facilitate the development of novel alfalfa germplasm in the future.

The effect of moderate heat stress on reproductive growth in a wheat RIL population segregating for heat resistance with respect to seed yield

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Rising global atmospheric temperature can negatively affect the reproductive development of wheat plants by decreasing grain yield. This study was carried out using selected heat-sensitive and heat-resistant recombinant inbred lines (RILs) derived from a 'CDC GO' (heat-sensitive) X 'Attila' (heat-resistant) RIL population where the parents segregate for heat resistance with respect to seed yield. Plants were subjected to heat stress (35°C for 6h per day for 8 days) at approximately the BBCH 37 stage (when flag leaf just visible) or they were maintained at control temperatures (20°C/17°C, day/night, 16 h photoperiod). Heat stress either did not change or reduced spike and spikelet length in the heat-resistant RILs, while no change occurred in the heat-sensitive RILs, with one exception. In one heat-sensitive RIL, heat stress increased the spike and spikelet length, and ovule size, along with reducing pollen viability and seed number and seed weight at maturity. Anther length decreased with heat stress in both parents, and most heat-resistant and heat-sensitive RIL lines, with reduced pollen viability occurring in specific RILs in both heat-sensitive and resistant lines as a result of heat stress. These data suggest that heat stress affects anther, pollen, and ovule/spikelet development, with reduced growth of these organs under heat stress conditions associated with the heat-resistant RILs with respect to grain yield.

Interaction between the parasitic lichen *Ochrolechia frigida* and its moss substrate

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The crustose lichen, *Ochrolechia frigida* (Sw.) Lynge, colonizes a variety of organic substrates in the subarctic and arctic-alpine bogs. It is thought to parasitize other organisms, typically mosses, before becoming lichenized. *O. frigida* was recently found growing on hummocks in a coastal bog of Newfoundland. Hummocks are formed by a variety of mosses, on which lichens and vascular plants may grow, providing *O. frigida* with many substrates to colonize. Since little is known about substrate specificity and the parasitic nature of this lichen, the objectives of this study were to investigate 1) substrate preference, and 2) colonization pattern. *O. frigida* preferred a substrate of *Dicranum leioneuron* to *Sphagnum fuscum* or *Sphagnum rubellum,* and did not grow on *Racomitrium lanuginosum* despite its dominance in the area. Hummock moisture showed a relationship with lichen preference for specific moss species. The colonization pattern of *D. leioneuron* by the lichen was studied microscopically along the gradient of the developing interaction, by sampling from three positions on the hummock: moss uncolonized by fungus, moss colonized by fungus at the margin of the interaction; and moss colonized by lichen. The mycobiont appeared to be initiating the interaction by attaching to and suppressing the moss apex and spreading to the surrounding leaves. During the lichenization, algal pockets developed gradually in the forming thallus, while the moss tissues gradually died. These findings provide new information about the substrate preferences and colonization strategy of *Ochrolechia frigida*, and suggest future directions for study of this unusual lichen.

Preliminary Screening Status for Glyphosate-Resistant Kochia in Saskatchewan

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Kochia (*Bassia scoparia*) is an invasive, early emerging, halophytic, allelopathic tumbleweed and a troublesome weed on the Canadian Prairies. Kochia has evolved resistance to Group 2 herbicides, as well as dicamba (Group 4), and glyphosate (Group 9), with multiple-resistant biotypes present in Saskatchewan. Previous surveillance activities for Saskatchewan in 2011/2012 identified the presence of glyphosate-resistant kochia in nine municipalities. The study objective was to determine the current occurrence of glyphosate-resistant kochia at 300 sites within central and southern Saskatchewan. A post-harvest survey was conducted in the fall of 2019. The experimental design was a stratified-random survey, with site selection stratified by proportional cultivated land area per ecodistrict. A composite sample of twelve plants were gathered at each location. Seed was threshed and screened in the greenhouse with glyphosate (900 g ae ha-1). From the 300 sites, 152 populations from 92 municipalities have been screened. Ninety percent of tested populations demonstrated some degree of glyphosate-resistance, with 30% demonstrating low-level resistance (1 to 20% survival), 38% demonstrating moderate resistance (21 to 60% survival), and 22% demonstrating high resistance (61 to 100% survival). While results are preliminary, substantial increases in glyphosate-resistant kochia populations were detected across a larger geographical area. Continued reliance on glyphosate to control kochia will result in further resistant-biotype shifts. Integrated kochia patch management and monitoring ditches and field margins is advisable. Good herbicide stewardship practices including applying chemistry at proper staging, using full label rates, and rotating modes of action will be critical to mitigate ongoing resistance evolution.

Plant host defense peptides as potential tools to reduce crop losses

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Over the next few decades we need to maximize crop productivity on existing farmlands to meet global food demand in a sustainable manner. To meet this challenge it is essential to develop environmentally friendly strategies to protect plants against major diseases, which currently account for over one-third of total crop losses world-wide. This goal may be achieved by developing crops with broad spectrum disease resistance through ectopic expression of host defence peptides (HDPs). HDPs are small membrane-active peptides that are key components of the innate immune system of all living organisms; they act as the first line of defense against pathogenic microorganisms. The aim of our research is to test HDPs of plant origin for antimicrobial activity against major potato pathogens and to evaluate their cytotoxicity towards plant and mammalian cells, *in vitro*. This will enable us to identify the peptide(s) suitable for expression in plants. Four HDPs have been selected for this study: Skh-AMP1, from medicinal plant *Satureja khuzestanica*; Cr-ACP1, from *Cycas revoluta* (sago palm); Shepherin 2, from the roots of *Capsella bursa-pastoris* (shepherd's purse), and Cn-AMP1, from green coconut water of *Cocos nucifera*. The antimicrobial activities of these HDPs have been evaluated against *Phytophthora, Fusarium, Pectobacterium* and other phytopathogenic microorganisms. To ensure the desired pattern of transgene expression in plants, a truncated version of Douglas-fir *PmBiP Pro1* promoter (*PmBiP Pro1-3*) is being examined for tissue-specific activity using green fluorescent protein. The results of this study will pave the way for the development of plants with potent disease resistance.

The blind alga: The Antarctic green alga *Chlamydomonas* sp. UWO241 has a reduced repertoire of photoreceptor genes and an aberrant phototactic response

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The Antarctic green alga *Chlamydomonas* sp. UWO241 is an obligate cold extremophile (psychrophile) and an emerging model for photosynthetic adaptation to extreme conditions. UWO241 originates from the ice-covered Lake Bonney, an extreme but highly stable environment. In addition to the permanent cold (~5°C), this alga thrives under highly unusual light conditions with very low light levels (~15 µmol/m-2 s-1), a narrow spectrum enriched in blue-green light, and an extreme photoperiod. Genomic sequencing of UWO241 exposed an unusually large genome, hundreds of gene duplicates and expanded gene families, some of which could be aiding its survival in extreme conditions. In this work, we demonstrate that the gene encoding the blue-light photoreceptor phototrophin is duplicated in UWO241; however, the photoreceptor gene family is significantly reduced compared to the model mesophile *Chlamydomonas reinhardtii*. Mirroring this, the gene family encoding for light harvesting complex (LHC) proteins also contains gene duplicates, but UWO241 lacks some LHC homologs when compared to *C. reinhardtii*. UWO241 also possesses a very small eyespot and exhibits an aberrant phototactic response. We saw similar trends the genomic repertoire for photoreceptor and LHC genes in another Antarctic alga, the closely related *Chlamydomonas* sp. ICE. This suggests significant differences in the ability of low-light adapted polar algae to perceive and respond to light when compared to their mesophilic relatives, with implications on their evolution and survival under extreme conditions.

The water potential curve: A method to determine drought-induced changes in plant-water relations

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Canada’s agri-food sector is facing unprecedented challenges caused by drought due to ongoing climate change. Crop performance under drought depends on a successful coordination of processes at root, stem, and leaf level. This includes efficient stomatal regulation to avoid excessive water loss to the atmosphere. Recently, the water potential (WP) curve method was introduced by Knipfer et al. (2020, Plant Physiology) as a methodological advancement of the Scholander-type pressure chamber to predict stress thresholds of stomatal closure and turgor loss in a cost-effective way. In this presentation, I will discuss the conceptual basis of the WP curve method and provide an outlook about the future applications of this method for physiological phenotyping, selection of drought resistance genotypes, and evaluation of plant-water relations in natural and agricultural ecosystems.

Studies on the stability and interaction of the E3 ubiquitin Ligase HOS1 and proteins involved with cold acclimation and stomatal development in *Vitis riparia*

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ICE (inducer of CBF expression) transcription factors are involved in three essential steps of stomatal development by forming dimers with three other bHLH transcription factors SPCH, MUTE and FAMA. On the other hand, ICE proteins play an important role in preventing freezing injury through the ICE-CBF-COR pathway which activates cold acclimation. The ICE-CBF-COR pathway is negatively regulated by a RING-finger E3 ubiquitin ligase, HOS1 (high expression of osmotically responsive protein 1), homologs of which have been reported in *Arabidopsis thaliana* and *Vitis pseudoreticulata*. For both species, there are indications that HOS1 ubiquitinates ICE1, which leads to its degradation. However, it is not yet clear if HOS1 also interacts with and causes degradation of the stomata-specific proteins and any other *Vitis* ICE protein.
We successfully amplified and cloned the cDNA for the single homolog of *HOS1* from *Vitis riparia* (Vr), as well as for its 4 ICE proteins (VrICE1-4) and its stomata-specific proteins (VrSPCH, VrMUTE, VrFAMA). BiFC analyses after transient expression in tobacco leaves suggests that VrHOS1 can interact with all 4 VrICE proteins, VrSPCH and VrFAMA, but not with VrMUTE. Preliminary Western blot results showed that the presence of VrHOS1 does not lead to the degradation of VrMUTE but possibly does for VrICE1. Current experiments aim to analyze the degradation of all proteins due to VrHOS1 by Westerns, and to confirm interactions by pulldown assays.

L-Asparagine metabolism in *Phaseolus vulgaris* and *Glycine max*

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L-asparagine is an important source of nitrogen stored and transported in higher plants, favoured in legumes due to its advantageous 2N:4C ratio. The catabolism of asparagine in tissues occurs through two pathways: deamidation and transamination. Developmentally regulated in *Phaseolus vulgaris*, deamidation by asparaginase is predominant in seeds and young leaves, with activity decreasing rapidly as leaves mature. Asparaginases can be differentiated as K+-independent or K+-dependent, with legumes primarily utilizing K+-dependent asparaginases due to their higher catalytic activity and efficiency. As asparaginase activity declines, transamination by serine:glyoxylate aminotransferase in the peroxisome becomes the principal pathway for asparagine catabolism. Asparagine is transaminated into α-ketosuccinamate, which is further reduced into α-hydroxysuccinamate. Both metabolites are hydrolyzed by ω-amidase and used to synthesize other amino acids, ultimately incorporated into proteins. The objective of this investigation is to identify and characterize the dehydrogenase involved in the reduction of α-ketosuccinamate in the model legume *Glycine max*. Metabolite quantification in leaves confirmed significantly higher transamination activity in mature leaves. Enzyme assays with mature leaf tissue indicated dehydrogenase activity in the cytosol. Further protein purification will be carried out and mass spectrometry employed to identify the enzyme. Once identified, mutant strains in *Arabidopsis thaliana* will be utilized to determine the relevance of this pathway in various plant functions. This research will afford a better understanding of nitrogen metabolism in higher plants and hopefully contribute to advancements in crop nitrogen use efficiency.

CRISPR/Cas9 mediated knock-out of MsSPL8 alleles in alfalfa leads to super abiotic stress resiliency and distinct morphological alterations

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Alfalfa (*Medicago sativa* L.) is one of the world's most widely grown forage species with an estimated cropping area of ~30 million hectares worldwide. However, as a result of climate change, our ability to attain high levels of alfalfa productivity is likely to be progressively constrained by environmental challenges such as drought and salinity. As such, there is a critical need to develop alfalfa genotypes with enhanced resiliency to various types of abiotic stress. Previously, the RNAi-mediated down-regulation of the miRNA156 target gene, *SQUAMOSA PROMOTER-BINDING-LIKE 8* (*MsSPL8*), was found to enhance biomass production, as well as drought and salinity tolerance, in alfalfa. Unfortunately, due to negative public perception and regulatory constraints surrounding the use of transgenic crops, it remains a challenge to implement such a crop in growers' fields. CRISPR/Cas9-based genome editing is a recently developed alternative breeding tool that yields germplasm bearing a mutation that is identical in nature to many of those achieved using conventional breeding approaches, and the resulting plants can be made transgene-free in a straightforward manner. In this study, we successfully targeted *MsSPL8* alleles using this technology in tetraploid alfalfa, and isolated genotypes with mutations in approximately 25%, 50% and 75% of *MsSPL8* alleles, respectively. Furthermore, enhanced drought and salinity tolerance, along with distinct morphological alterations including early flowering and a significant reduction in internode length, were noted in the first generation of edited genotypes, which suggests that CRISPR/Cas9 can provide an effective breeding tool in alfalfa.

Manipulating carbon flow in *Arabidopsis* seeds and assembling a genetic characterization platform for protein-related genes to accelerate canola breeding.

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Canola (*Brassica napus* L.) is a major crop valued for its high seed oil content, while the meal by-product is rich in protein and is mainly used as animal feed. The presence of carbohydrates in the seed meal hinders animal digestion, which reduces its economic value. Canola meal protein also has great potential for the plant-protein food industry. Therefore, there is interest in breeding high protein, high oil canola by partially reallocating carbon flow from seed cellulose biosynthesis towards storage proteins. Because characterization of protein-related genes in canola is time and labor intensive, we propose creating a rapid screening platform using *Arabidopsis thaliana.* We have metabolically engineered *A. thaliana* with reduced seed cellulose through seed-specific RNAi-downregulation of *CELLULOSE SYNTHASE 1* (At*CESA1*) and increased seed oil through overexpression of *DIACYLGLYCEROL ACYLTRANSFERASE 1* (Bn*DGAT1*). Our preliminary studies on At*CESA1*-RNAi/Bn*DGAT1*-overexpression in T2 *A. thaliana* has confirmed relative increases in seed oil content by 4-8%. These lines have been advanced to the T3 generation and seed oil, protein, and carbohydrate analysis are underway. Once we have acquired a population of homozygous T3 lines with high seed oil and reduced seed cellulose, it will be used for characterizing candidate genes involved in increasing canola seed protein. This platform enables the acceleration of fundamental genetic characterization research needed for breeders to develop value-added canola with high seed protein.

Transcription factors controlling monoterpene metabolism in lavender

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Numerous plant terpene synthase (TPS) genes have been described over the past few decades. However, regulation of expression of these genes is poorly understood. The main objective of the studies reported here was to identify transcription factors (TF) that control the expression of two monoterpenes synthase genes, including linalool synthase (*LinS*) and 1,8-cineole synthase (*CinS*) genes, which control essential oil yield and composition in *Lavandula x intermedia*. The 5' upstream genomic DNA (promoter) sequences for *L. x intermedia LinS* and *CinS* genes were cloned and used in a Yeast One-Hybrid assay in order to identify TFs that interact with these regulatory regions. The assay identified over 90 proteins that bind to one or both promoters. In order to investigate the nature of this interaction, each promoter fragment was fused to the *E. coli gusA* (GUS) reporter gene, and transformed separately into *Nicotiana benthamiana* plants. These plants were then transformed to co-express individual TF proteins. While six of the tested TFs induced expression from both promoters, two specifically activated CinS promoter, and two did not induce either promoter.

Transcriptional down-regulation of various genes in alfalfa leads to superior tolerance to abiotic stresses and distinct morphology

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Two different water stress extremes, drought and flooding, pose a severe threat to alfalfa (*Medicago sativa* L.), which is a popular forage legume that is highly esteemed for its relatively high yield and superior nutritional quality. In this study, two gene homologs (*TAC1* and *HB2*) found previously to negatively regulate responses to various types of abiotic stress in other plant species were identified in alfalfa, and RNAi genotypes exhibiting down-regulation of each gene, respectively, were generated. The resulting RNAi genotypes were subjected to drought and flooding treatments to assess their resilience to these stresses. Alfalfa genotypes with reduced expression of *MsTAC1* exhibited distinct morphology, such as increased plant height, late flowering and decreased dry root biomass compared to empty vector controls. Furthermore, these genotypes displayed enhanced tolerance to drought, which may be due, at least in part, to a reduction in water loss through transpiration. The down-regulation of *MsHB2* in alfalfa also led to morphological changes, including decreased plant height and increased root length when compared to empty vector controls. In addition, these genotypes also exhibited enhanced tolerance to flooding, which was evidenced by significant increases in leaf chlorophyll content under stress and survival compared to empty vector controls. Further experiments are underway to unravel the mechanisms driving increased abiotic stress tolerance in these genotypes. Our aim is to use the knowledge gained in this study to produce highly adaptable transgene-free alfalfa germplasm using CRISPR/Cas, which could reduce production losses by enhancing biomass production under extreme weather conditions.

Embryo tissue culture of five Strychnos species

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Strychnos are the widely varied genus from the *Loganiaceae* family with over 200 identified species worldwide. Seven of these species have been identified in Botswana. They have been used in folk medicine as remedies for many ailments such as snake bites, low libido, gonorrhea, malaria, diabetes mellitus, diarrhea, amenorrhea and a source of nourishment. Five *Strychnos* species found in Botswana also edible. These are *S. cocculoides, S. spinosa, S. pungens, S. innocua* and *S. madagascariensis*. Two among the above species have been listed as endangered species (*S. cocculoides* and *S. spinosa*). This study was designed to develop a fast and sustainable micropropagation protocol of the five S*trychnos* species in order to overcome seed dormancy, for rapid multiplication of species and preservation of the individuals with desirable characteristics. Produced plants were also to be sterile to allow for downstream processes including genetic transformation. Sterile *Strychnos* species plants (and a standard protocol) were produced using embryo culture. The protocol produced sterile seedlings with a germination rate between 92 and 100 % and germination time of 7-9 days. This contrasted with the conventional propagation through seeds which did not produce sterile plants, had a germination percentage of 76.19 ± 1.14 % and a germination time of 58.65 ± 1.67 days. The present study details a method to produce sterile *Strychnos* seedlings from the embryos of the unripe fruits. This will help to produce in vitro culture shoots necessary for other genetic and metabolomic studies.

Guardians of the apoplast: investigation of the stress-responsive leucine-rich repeat receptor kinases in plants

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Plants cannot see danger – instead, they use a large network of receptor kinases to recognize external signals. The leucine-rich repeat receptor-like kinases (LRR-RLKs) are the largest family of plant cell-surface receptors. In *Arabidopsis thaliana*, the LRR-RLK I subfamily is defined by the presence of leucine-rich repeats and a malectin-like domain in the extracellular region, a single-pass transmembrane domain, and an intracellular kinase domain. Members of this family, including *FRK1* (flg22-induced receptor-like kinase) and *IOS1* (impaired oomycete susceptibility), have been implicated in immune signaling, yet this family remains relatively unstudied.

In this work, we infer the phylogeny of LRR-RLK I across 113 species of plants. We find support for 10 subclades, most of which contain sequences from a range of species. However, we find that subclade I.1 shows a large recent Brassicaceae-specific expansion driven by tandem duplication. This finding suggests that the LRR-RLK I.1 plays an important role in stress adaptation in Brassicaceae and unveils a new avenue of research.

Mapping of quantitative trait linkage (QTL) in *Brassica napus* L. for tolerance to excess moisture stress

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Among different forms of abiotic stress, excess moisture impacts growth and development and causes yield losses in the *Brassica napus* L. ranging from 30-45%. The consequences of excess moisture stress include oxygen limitation, as well as reduction in photosynthesis, and nutrient uptake. Limited photosynthesis rate is ascribed mainly to dysfunctions in the electron transport chain in chloroplasts as a result of oxygen depletion. Plants experiencing excess moisture also exhibit lower germination rate, stunted growth, decreased yield and in extreme cases, death of the plant. The aim of this project is to study excess moisture tolerance in *B. napus* and map the genes responsible for tolerance using quantitative trait linkage (QTL) techniques. My hypothesis is that quantitative trait loci will be discovered at the sites of genes encoding secondary metabolites, as well as metabolites related to reactive oxygen species scavenging, oxidation reduction, and regulation of transcription and translation. The objective will be achieved by developing a doubled haploid (DH) population from two crosses between a sensitive and tolerant parent and then phenotyping the progeny to determine each genotype's tolerance or sensitivity to excess moisture. The individuals will then be genotyped using the 60k SNP BeadChip Array and then analyzed for QTL. Results from this project will assist researchers in understanding *B. napus* tolerance in excess moisture conditions and provides a key opportunity to identify candidate genes through high-coverage analysis.

Automatic Root Segmentation using an ImageJ/Fiji based approach

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The ability to analyze root growth is important to understand the function of genes involved in nutrient and water uptake, and root growth responses to environmental conditions. Studying root growth can also provide information on the regulation of cell wall biosynthesis. Here we describe a simple approach for automatic root segmentation for *Arabidopsis thaliana*. Our approach relies on the open-source image analysis tool ImageJ/Fiji to provide reliable and reproducible root growth data. We grew *A. thaliana* seedlings on plates containing plant growth media and agar. Digital images of these seedlings including roots and cotyledons were taken and analysed using an in-house developed tool for automated Fast Identification and Determination of Root Length (Fast IDR). This tool is written in the ImageJ/Fiji Macro language. Fast IDR performs batch processing of the images using a series of processing steps including segmenting, skeletonizing, and estimating root length. In the final step, root length data are exported in CSV file format. While manual analysis of root length following standard protocols requires about 40-45 minutes per image, or 400-450 minutes for 10 images, the Fast IDR protocol requires only about 10 minutes for the batch analysis of 10 images, which includes both control and mutant roots. The Fast IDR tool is a viable option *for A. thaliana* root growth analysis. To enhance the user-friendly capabilities of our tool the next steps are to implement the methods into a plug-in for the ImageJ/Fiji tool.

Green synthesis of silver nanoparticles from strychnos species and their photocatalytic and antimicrobial activities

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Nanoparticles are chemical molecules characterized by their small size and big surface area. Due to these properties, nanoparticles have recently been gaining a lot of attention in different fields of science, agriculture, medicine and engineering. Different types of nanoparticles with different characteristics have been synthesized and analyzed. However, green synthesis among physical and chemical synthesis approaches, is a very good approach as it reduces toxicity by using non-toxic reductants derived from biological extracts and it produces less waste. Indeed, silver nanoparticles successfully synthesized from Strychnos plant species have been characterized and tested for their activities. Few techniques such as X-ray diffraction, Fourier transfourier infrared spectroscopy and Raman spectroscopy were used to study and confirmed the synthesized silver nanoparticles characteristics. Interestingly, silver nanoparticles have showed antibacterial activities against gram-negative and gram-positive bacteria. Additionally, Photocatalytic activity of the nanoparticles was showcased by the ability of silver nanoparticles to degrade methylene blue.

Cultivating *Salix viminalis* in agricultural-riparian transition areas to produce biomass and mitigate N2O emissions from potato cropping systems on Prince Edward Island.

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Cultivating shrub willow (*Salix viminalis*) in agricultural-riparian transition areas has been proposed as a strategy for mitigating agriculturally derived riparian nitrous oxide (N2O) emissions while producing biomass, with minimal encroachment on existing agricultural land. Our study investigated the biomass potential of willow buffers receiving no direct fertilization other than what was transported from upslope agricultural fields and the willow's impact on greenhouse gas emissions. Plant assimilation of carbon, nitrogen, and phosphorus were measured in the stem and root biomass. Soil cores were taken from the rooting zone at 0-30 and 30-50 cm. N2O, CO2 and CH4 flux at the soil-atmosphere interface was measured using non-steady-state static chambers, and NO3- exposure and other environmental parameters were measured. Agricultural-riparian willow accrued 7.4 oven-dry tonnes of biomass ha-1 (7.4 dry tonnes of carbon, 99.8 kg nitrogen, and 14.0 kg phosphorous ha-1), representing 27.9 tonnes CO2 ha-1 sequestered. They also significantly mitigated fertilizer induced N2O emissions even when high NO3- inputs occurred from upslope agricultural fields and following precipitation events when the potential for nutrient saturation was more likely. Mean cumulative seasonal reductions of 1.32 kg N ha-1 were observed in willow relative to cultivated fields. The mean cumulative average global warming potential of willow was 613 kg CO2e ha-1 lower than cultivated fields, with reductions of up to 2918 kg CO2e ha-1 observed. This represented a 90% decrease (up to 98%). These results indicate that strategic agricultural-riparian Salix cultivation is effective for mitigating riparian N2O emissions on PEI.

Cold stress response in *Arabidopsis* root is regulated by specific actin isovariant, ACTIN8

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Low temperature stress is a significant threat for crop productivity world-wide. Previously, we demonstrated that cold-induced alteration of cellular homeostasis of auxin is the primary cause for plant growth inhibition. It was also showed that cold stress affects the intracellular cycling of several proteins, including auxin efflux carriers, PINs. Actin cytoskeleton, which assists the movement of protein through providing a cellular track plays an important role in regulating the intracellular protein trafficking. However, whether actin is involved in the cold temperature response remains elusive. In the present study, we tried to elucidate the role of actin in regulating cold stress response using vegetative class isovariant specific actin mutants. The root growth recovery assay after cold treatment revealed distinct differences in the actin mutants response to cold stress. Although ACT8 and ACT2 belong to the same clad, *act8-2* shows a hypersensitive response to cold stress, while *act2-1* responds like wild-type. The double mutant of ACT8 and ACT2 (*act8-2 act2-1*) shows *act8* like phenotype, indicating that ACT8 is functionally dominant. In addition, cellular auxin homeostasis is drastically altered in *act8-2* mutant root both after cold stress and during recovery stage as judged by the auxin marker line *IAA2-GUS*. The altered redistribution of auxin in *act8-2* mutant under cold stress was found to be directly linked to the altered trafficking of PIN2 which serves as auxin efflux carrier. Collectively, these results suggest that the cold stress-induced alteration in cellular auxin homeostasis and response is regulated by actin isovariant ACT8.

The 4Ms (Making, Maintaining, Managing and Mastering Meristems) of plant developmental plasticity from a founder cell perspective.

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A cornerstone of plant developmental plasticity is their iterative growth over time that is developmentally timed and fine tuned by the inputs from the environment. This iterative growth is coordinated by the meristems that are formed during embryogenesis. The continued maintenance of the apical meristems and their activity; the formation of the axillary meristems in the shoot; lateral and adventitious meristems in the root; and the wound healing capability, determines the architecture and the adaptation of the growth habit of the plants to their habitat. From an evolutionary perspective, the gradual adaptation of plants to diverse terrestrial habitats is illustrated in the different groups of land plants, i.e., bryophytes, lycophytes, pteridophytes, gymnosperms and angiosperms. One of the key evolutionary features is the gradual reduction of gametophytic phase coupled with the elaboration of the sporophytic phase during alternation of generations. The rudimentary meristematic program originated in the gametophytic phase of bryophytes which provided sustained growth of the gametophytic body and was eventually co-opted and consolidated into stem cell niches in the sporophytic phase of the later formed land plants. The first shoot meristem arose from the shoot founder cells and followed the evolutionary trajectory to form phytomeric units from the shoot meristem and the first root meristems emerged from phytomeric units. Here, we present the evolutionary trajectory of the meristems and embryogenesis to facilitate the development of a universal model of embryo development in land plants to better understand the mechanisms that underly plant adaptation to the diverse and changing environments.

Protein levels of several *Arabidopsis* auxin response factors are regulated by multiple factors and ABA promotes ARF6 protein ubiquitination

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The auxin response factor (ARF) transcription factors are a key component in auxin signaling and play diverse functions in plant growth, development and stress response. ARFs are regulated at the transcript level and posttranslationally by protein modifications. However, relatively little is known regarding the control of ARF protein levels. We expressed five different ARFs with an HA (hemagglutinin) tag and observed that their protein levels under the same promoter varied considerably. Interestingly, their protein levels were affected by several hormonal and environmental conditions, but not by the auxin treatment. ABA (abscisic acid) as well as 4 °C and salt treatments decreased the levels of HA-ARF5, HA-ARF6 and HA-ARF10, but not that of HA-ARF19, while 37 °C treatment increased the levels of the four HA-ARFs, suggesting that the ARF protein levels are regulated by multiple factors. Further, MG132 inhibited the reduction of HA-ARF6 level by ABA and 4 °C treatments, suggesting that these treatments decrease HA-ARF6 level through 26S proteasome-mediated protein degradation. Further, ABA treatment drastically increased HA-ARF6 ubiquitination, without strongly affecting the ubiquitination profile of the total proteins. Together, these results reveal another layer of control on ARFs, which could serve to integrate multiple hormonal and environmental signals into the ARF-regulated gene expression.

How does Propyzamide mediate microtubule destabilizing effect?

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In this study, we explored the involvement of Microtubule Organization 1 (MOR1) in the microtubule-destabilizing effects of the herbicide Propyzamide (PPM). PPM is used as a herbicide but the precise mechanism by which it works is unknown. MOR1 is a plus-end tracking protein that catalyzes microtubule polymerization. We identified a mutant allele of MOR1, *mor1-11*, that displays an altered response to PPM. Whereas PPM at low concentrations causes left-handed root twisting in the wild type, it generates right-handed twisting in *mor1-11*. To understand the basis for this chirality-reversal phenotype, we hypothesized that microtubule polymer levels and /or dynamicity would differ between *mor1-11* and wild-type under PPM treatment. We used a live-cell imaging approach to quantify microtubules visualized in hypocotyls expressing a 35S promoter-driven GFP-tubulin 6 reporter. Microtubule polymer levels were reduced to a greater extent after treatment with PPM in the wild type compared to *mor1-11*, suggesting that the *mor1-11* point mutation alters the effects of PPM. We next used Fluorescence Recovery After Photobleaching (FRAP) to measure the relative turnover of tubulin subunits as a proxy for microtubule dynamics. In wild type, a 3h treatment with 20 µM propyzamide resulted in no significant difference in tubulin subunit turnover relative to the control treatment. In *mor1-11*, however, the same treatment generated a statistically significant increase in tubulin subunit turnover. Our results suggest that MOR1 could be a target of PPM. Our FRAP findings also provide mechanistic insight into the relationship between microtubule dynamicity and the handedness of organ twisting.

Expression Profiling Of The Tomato CBP60g Gene Family During Bacterial Infection And Elevated Temperature

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In the model organism *Arabidopsis thaliana*, the transcription factors CBP60g and SARD1 are involved in the biosynthesis of a temperature-vulnerable defense hormone, salicylic acid (SA). The objective was to investigate SA production in relation to immune elicitors, 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 (MgCl2), or *Pseudomonas syringae pv. tomato* (*Pst*) DC3000, incubated at 23°C (control temperature) or 32°C (elevated temperature) and then extracted 1-3 days post-inoculation (dpi). Immune elicitors such as the bacterial flg22 peptide and SA were also infiltrated into tissues and were harvested 1 dpi at 23°C. RNA was extracted from the infiltrated tissues and used as a template for RT-PCR. After immune elicitation with flg22 and SA, tomato *CBP60g-1,9,* and *10* genes demonstrated constitutive expression. *CBP60g-2,3* and *6* were mostly expressed for all treatments, while *CBP60g-7* was only expressed in replicate A. *CBP60g-4,5,8*, and *11* were never expressed. In comparison, after pathogen infection, tomato *CBP60g-1,2,3,9* and *10* were always expressed, while *CBP60g-4,5* and *7* were not. *CBP60g-6,8* and *11* exhibited variable expression amongst replicates and treatments. After combined pathogen infection and elevated temperature, *CBP60g-1,2,3,* and *9* were constitutively expressed. *CBP60g-4* was likely contaminated and thus not expressed, like *CBP60g-5,6,7,10,* and *11*. *CBP60g-8* was expressed variably under different treatments. These insights improve our molecular understanding of defensive mechanisms and will potentially help optimize the growth and resistance of economically valuable crops in our dynamically changing world.

Investigating the functions of in vivo hyperphosphorylation of the glutamate decarboxylase AtGAD1 in phosphate-resupplied *Arabidopsis thaliana*

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Inorganic phosphate (Pi) has a crucial role in plant development, yet is often the most limiting macronutrient of many soils. Pi starved (-Pi) plants elicit a Pi starvation response altering gene expression and metabolism to enhance efficiency of Pi acquisition and use. Our recent collaborative study assessed the impact of Pi nutrition on the intracellular phosphoproteome of suspension cell cultures of *Arabidopsis thaliana.* The glutamate decarboxylase isozyme AtGAD1 was identified as the most hyperphosphorylated protein 48 h following resupply of Pi to -Pi cells. GAD catalyzes the first committed step of the 4-aminobutyrate (GABA) shunt by decarboxylating glutamate into GABA, an important yet enigmatic ‘stress’ metabolite and potential signal molecule in plants. GAD is of particular interest since it is the only enzyme of central plant metabolism known to be activated by Ca2+/calmodulin-binding. Phosphorylation regulation of plant GADs has not been studied although similar hyperphosphorylation of human HsGAD2 mediates its association with membranes of neurotransmitter-releasing vesicles. Our work in parsing the function of AtGAD1 phosphorylation is focused on two hypotheses: (i) phosphorylation has an inhibitory effect on GAD activity and/or (ii) phosphorylation affects GAD subcellular localization. This involves biochemical approaches conducting *in vitro* kinetic assays of purified phospho- and dephospho-AtGAD1, and molecular cloning to generate mCherry-AtGAD1 fusion constructs to visualize subcellular partitioning in response to Pi status. Assessing the interplay between Pi nutrition and AtGAD1 phosphorylation will contribute to elucidating the physiological roles of GABA and the GABA shunt during Pi deprivation, and potentially other stresses such as salinity.

Multigenerational heat-stressed progeny of *Arabidopsis* displays notable phenotypic, genotypic, and epigenotypic variations

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Plants are sedentary organisms that constantly sense changes in their environment and react to various environmental cues. On a short-time scale, plants respond through alterations in their physiology, and on a long-timescale, plants alter their development and pass on the memory of stress to the progeny. The latter is controlled genetically and epigenetically and allows the progeny to be primed for future stress encounters, thus increasing the likelihood of survival. The current study intended to explore the effects of multigenerational heat stress in *Arabidopsis thaliana.* Comparison of the stressed lineages F2H (the 2nd generation of the stressed progeny) and F25H (the 25th generation of the stressed progeny) to controls (F2C and F25C) exhibited a higher tolerance. The F25H stressed progeny showed
a 7-fold higher number of non-synonymous mutations than the parental non-stress line which might lead to biological variations subjected to natural selection at the microevolution level. Methylome analysis revealed that the F25H stressed progeny showed a lower global methylation level in the CHH context than the control progeny which might be a part of adaptation strategies to heat stress. F25H stressed progeny displayed higher methylation changes in the gene body and lower in the body of Transposable Elements (TEs). Gene Ontology analysis revealed that CG-DMRs were enriched in processes such as response to abiotic and biotic stimulus, cell organizations and biogenesis, and DNA or RNA metabolism. Hierarchical clustering of these epimutations separated the heat-stressed and control parental progenies into distinct groups which revealed the non-random nature of epimutations.

Preliminary Evaluation of the Effect of Environmental Factors (Salt Spray) on the Production of Squamatic Acid in Lichen-Fungus *Cladonia uncialis*

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Cladonia uncialis is a terricolous lichen, growing on soil or moss mainly in sun-exposed areas. Previous collections from MB always produced one (usnic acid) and sometimes two major secondary metabolites (usnic acid and squamatic acid), but coastal collections in NL and NS predominantly produced both usnic and squamatic acids. The objective of the present study was to test two factors that may trigger the production of squamatic acid. *C. uncialis* samples were collected within three locations in Newfoundland (Birchy Lake (inland); Northern Peninsula and Port aux Basques (coastal)) using a strip transect method. Lichen species composition growing near *C. uncialis* was recorded and percentage of similarity between sites was identified using hierarchical cluster analysis with Sorensen's distance measure and the group average linkage method. Effect of salt spray on squamatic acid was examined in fifteen *C. uncilais* samples under laboratory conditions (Control, 35g/L, 40g/L) for three months. Thin Layer Chromatography (TLC) was conducted to confirm the presence of squamatic acid. Squamatic acid was present in *C. uncialis* samples collected from coastal locations (Northern Peninsula and Port aux Basque) but absent from thalli collected inland (Birchy Lake). None of the samples sprayed with salt produced squamatic acid. The Birchy Lake transects form clusters with 88% similarity in species composition. The Northern Peninsula and Port aux Basques transects form clusters showing 60% and 68% similarity. These preliminary findings suggest that sea salt is not the environmental factor triggering squamatic acid production. Species composition may influence environmental conditions and will be

discussed.

Salinity and CLASP: Role for the Microtubule-Associated Protein CLASP under high salinity in *Arabidopsis*

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Salinity adversely affects plant growth phases and alters development. In this study, we investigated how the Cytoplasmic Linker Associated Protein (CLASP) is involved in *Arabidopsis thaliana* (Col-0) seed germination and seedling growth under high salinity. Germination rates of seeds lacking CLASP expression (*clasp-1*) were similar to the wild-type (98%) under control conditions but decreased to a much greater extent under salinity (*clasp-1* 61% vs WT 90% at 100mM NaCl). To elucidate the basis for salinity-induced germination failure in *clasp-1* mutants, we applied a range of chemical and hormone treatments that have previously been shown to either enhance or reduce germination under high salinity. Applying the nitric oxide donor sodium nitroprusside, the active antioxidant reduced glutathione, Gibberellic acid, Indole-3-acetic acid or Jasmonic acid mitigated the salinity-induced seed dormancy in *clasp-1* under 100mM NaCl. In contrast, the nitric oxide scavenger CPTIO, a ROS producing enzyme inhibitor DPI, the antioxidant ascorbic acid, high CaCl2, the auxin transport inhibitor N -1-naphthylphthalamic acid, and complete darkness further reduced germination of *clasp-1* seeds under 100mM NaCl. These suggest that *clasp-1* seeds have ROS, nitric oxide, and hormone imbalances during germination under high salinity. Furthermore, seedlings of *clasp-1* showed greater sensitivity to low NaCl concentrations (50, 100 mM) resulting in reduced seedling survival, relative rosette area, relative fresh weight, and relative root length. We further observed that plants engineered to overproduce CLASP were also hypersensitive to salt stress. We conclude that CLASP at moderate levels contributes to maintaining proper seed germination and seedling growth during salt stress.

Foliar glycoalkaloids of potato and the herbivorous pest Colorado potato beetle

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Colorado potato beetle (CPB) infestations can result in defoliation of entire potato fields in one week. The major foliar secondary metabolites of potato are glycoalkaloids, solanine and chaconine. These glycoalkaloids have well-characterized cellular and biochemical toxicity. The *GAME4* gene in the glycoalkaloid biosynthetic pathway was knocked down in potato using RNAi. *GAME4* RNAi plants had reduced production of solanine and chaconine. Comparison of CPB feeding on *GAME4* RNAi plants and wild-type plants showed that the rate of feeding was similar, though, there was accelerated development in CPB feeding on low glycoalkaloid *GAME4* RNAi plants. In a previous study, CPB avoided feeding on wild *Solanum* relatives of potato with low levels of solanine and chaconine. Together these studies showed that solanine and chaconine were not a CPB feeding deterrent. However, other biological effects on CPB from feeding on glycoalkaloids were likely and these were examined in the current study through gene expression analysis of CPB feeding on wild-type and *GAME4* RNAi plants using RNA-seq. Results show that CPB feeding on low glycoalkaloids had reduced expression of genes encoding ABC transporters, which function in detoxification. This result suggests that ABC transporters function in adapting CPB to feeding on toxic glycoalkaloids. CPB biological processes affected by feeding on low glycoalkaloids include lipid biosynthesis, Golgi function, cell division and development. The results suggest that CPB is highly adapted to feeding on high glycoalkaloids and changes to feeding on foliage with low glycoalkaloids affected their physiology and development.

The ubiquitin ligase XBAT35.2 regulates plant response to biotic and abiotic stresses.

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Ubiquitin ligases are central components of the ubiquitination pathway that selects and facilitates the attachment of ubiquitin, a small regulatory protein, to target substrates. The covalent attachment of ubiquitin to selected targets has varying consequences; most notable is the degradation of modified proteins by the 26S proteasome. The ubiquitin-proteasome system (UPS) plays an integral role in all aspects of plant growth and development and response to environmental stress. The Golgi-localized RING-type ubiquitin ligase, XBAT35.2, mediates the proteasome-dependent degradation of Accelerated Cell Death 11 (ACD11) to induce cell death and promote pathogen defense (1). Here we described a novel role for ACD11, a lipid transfer protein, in promoting abiotic stress tolerance (2). ACD11 is shown to enhance salt and drought tolerance, while stress-stabilized XBAT35.2 targets ACD11 for degradation to attenuate abiotic stress responses. An E3 is capable of regulating multiple processes via the ubiquitination of different substrates. However, this work shows that a single E3-substrate pairing can regulate multiple processes; in this case, defending against pathogens and coping with abiotic stresses. XBAT35.2 is characterized as an ubiquitin ligase with multiple and opposing roles in plant responses to biotic and abiotic stresses.

(1) Liu et al., (2017) The RING-Type E3 Ligase XBAT35.2 Is Involved in Cell Death Induction and Pathogen Response. Plant Phys., 175 (3) 1469-1483; https://doi.org/10.1104/pp.17.01071

(2) Li, et al., (2020), Arabidopsis RING-type E3 ubiquitin ligase XBAT35.2 promotes proteasome-dependent degradation of ACD11 to attenuate abiotic stress tolerance. Plant J., 104:1712-1723; https://doi.org/10.1111/tpj.15032

Characterizing roles of the *Arabidopsis thaliana* kinase PXK4 in flowering:

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Phosphorylation and ubiquitination are two of the most abundant post-translational modifications in plants. They each play essential roles in by modulating proteins such as mediating protein-protein interactions or triggering changes in protein abundance. Protein kinases, catalyzing phosphorylation events on target proteins, are extensively associated with plant development and stress responses. Of interest is the *Arabidopsis thaliana* protein kinase PXK4. Utilizing *pxk4-2* mutants, we observed early flowering and a significant reduction in FLC expression. Phosphoproteomic analysis of *pxk4-2* mutants showed a significantly decreased phosphorylation of HUB2. HUB2 as a RING-type E3 promotes monoubiquitination of H2B and the active transcription of a major flowering repressor, FLC. Comparative Tandem Affinity Purification (TAP) analysis of the HUB2 protein interactome using HUB2-TAP expressing *pxk4* mutant and wild-type plants, suggests that HUB2 phosphorylation may be required for the formation of protein complexes involved in flowering regulation. These results suggest that PXK4 might phosphorylate HUB2 to promote the formation of protein complexes that ultimately impact FLC transcription and flowering.

Examining the effects of altered levels of Phytoglobin 2 during seed oil accumulation in Brassica napus

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Canola (*Brassica napus* L.) is the most commonly grown Brassica species and is the third largest source of edible oil. Seed oil biosynthesis is influenced by the transcription factors *LEAFY COTYLEDONS 1* and *2* (*LEC1* and *2*) and *WRINKLED 1* (*WRI1*). *LEC1* affects the early stages of embryogenesis by modifying sugar levels and overexpression of *LEC1* induces genes encoding enzymes of the fatty acid biosynthetic pathway. *WRI1* increases the production of triacyglycerols and elevates seed oil content up to 40%. Both *LEC1* and *WRI1* are regulated by nitric oxide (NO) and auxin, important components in seed development and oil synthesis. Phytoglobins (Pgbs) are heme-containing proteins able to scavenge NO and alter auxin synthesis and signaling. Here we examine the effects of constitutive (with a 35S promoter) and seed-specific (with a cruciferin promoter) over-expression of *BnPgb2* on seed oil level and fatty acid compositions in *Brassica napus*. To assess the link between *BnPgb2* and auxin during seed oil accumulation, the same genotypes will be used to measure the transcript abundance of *LEC1*, *LEC2*, *WRI1*, auxin transporters and auxin biosynthetic genes during seed development. Reporter lines visualizing auxin, PIN1 and PIN7, and pharmacological experiments interfering with auxin flow and level will also be employed. Overall, this study will determine a possible function of Pgbs during seed oil synthesis.

3D Architecture of Plant Mesophyll Tissues

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The anatomical features of leaves are crucial determinants for the efficiency of fundamental biophysical processes. The development as well as arrangement of mesophyll cell layers and network of intercellular spaces define the internal architecture of the leaves. Alteration in the distribution of mesophyll cells and airspaces affects the gaseous transport and thus photosynthesis. Manipulating leaf architecture therefore represents a tool for engineering improved plant cultivars with altered photosynthetic rate. However, the precise extent to which the anatomical features affect biophysical processes is still unknown as earlier studies were based on simplified assumptions of the 3D architecture of leaves. To produce a more detailed understanding of the effect of geometrical traits of the mesophyll tissue and the developmental processes generating these, their characteristics and effects on transport processes must be modelled and measured. State-of-the-art microscopy and imaging techniques have now rendered it possible to compute realistic 3D geometries of leaves at high resolution and allow researchers to propose precise models for biophysical processes. In this project two such state-of-the-art techniques are used—confocal laser scanning microscopy and X-ray microcomputed tomography (microCT). We integrate modern microscopic techniques and computational tools to analyze the architecture of mesophyll layers and intercellular spaces in the complex 3D leaf anatomy.

Reduced pollination extends floral longevity or sex-phase duration at different points in the season in a native wildflower

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Declines in pollinator species richness and population density can reduce the number and quality of pollinator visits to plants. Plants can respond to these declines in pollination services by plastically adjusting the longevity or sex-phase duration of individual flowers. Any plasticity in these flower-level traits could then affect the expression of inflorescence-level traits, including daily floral display size and phenotypic gender. We studied the effects of experimentally reducing pollination on floral and inflorescence traits of female and hermaphroditic *Lobelia siphilitica* plants. We compared the longevity and male- and female-phase duration of *L*. *siphilitica* flowers exposed to ambient and reduced pollination treatments. We then tested whether plasticity in floral longevity and sex-phase duration affected daily display size and phenotypic gender.
We found that experimentally reducing pollination extended the male-phase duration of early-season flowers and the longevity of late-season flowers. However, an extended male phase was not associated with a more male-biased phenotypic gender, and extended floral longevity was not associated with a larger daily display.
Our results suggest that *L*. *siphilitica* plants can respond to pollinator declines by plastically adjusting both the longevity and sex-phase duration of individual flowers at different points in the season. Consequently, plasticity in flower-level traits could be one mechanism by which plants respond to decreases in pollination services caused by pollinator declines.

Investigating the role of PUBs in the self-incompatibility pathway of transgenic SI A. thaliana of the C24 accession

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In order to promote outcrossing and to increase genetic diversity, members of the Brassicaceae family possess an intraspecific mating barrier, known as the self-incompatibility system, that allow the pistil to recognize and reject self-pollen. The SI system is initiated by the *S*--haplotype specific interactions between the highly polymorphic *S* locus genes: *SCR/SP11*, the male determinant gene, and *SRK,* the female determinant gene. This interaction is followed by the activation of *ARC1*, a member of the PLANT-U-BOX family of E3 ubiquitin ligases, which targets its substrates for degradation by ubiquitination. One of the identified downstream targets of *ARC1* is the exocyst complex and this event disrupts the delivery of water and resources required by the pollen to hydrate and germinate. Species have evolved to be self-compatible due to the pseudogenization of *SCR* and *SRK*, and the deletion of *ARC1*. Interestingly, when the self-compatible *Arabidopsis thaliana* of the C24 accession was transformed with *SCRb* and *SRKb* from the self-incompatible *Arabidopsis lyrata*, the transgenic lines exhibited strong SI phenotypes despite lacking *ARC1*. We hypothesize that there is an unidentified factor in the transgenic SI *A. thaliana* C24 that performs a similar biological function as *ARC1*. The two candidates for this study are *PUB16* and *PUB17* as they are the two most closely related PUBs to *ARC1*. Our experimental approach uses the CRISPR/Cas9 system to generate single knockout mutants and the double knockouts mutants in the SI *A. thaliana* C24 background, which will then be assessed for pollen-pistil interactions.

Phytoglobin Expression as a Potential Marker for Waterlogging Tolerance in Barley

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Barley (*Hordeum vulgare* L.) is one of the most susceptible cereal species to excess moisture stress. In Western and Central Canada, the flat topography and heavy snowfall accumulation caused it to be a leading reason of barley yield loss for decades. Excess moisture brings about hypoxic stress which reduces basic physiological functions in both root and shoot cells. One of the most common symptoms of excess moisture is the reduction in photosynthetic rate and damage of tissues following accumulation of reactive oxygen species. Phytoglobins are heme-containing proteins induced by hypoxia that exercise protective roles through their ability to scavenge cellular nitric oxide (NO). The role of the barley Phytoblobin 1 gene (*HvPgb1*) during conditions of waterlogging was examined in transgenic plants over-expressing *HvPgb1*, and wild type plants. Relative to these, the barley plants over-expressing *HvPgb1* were more tolerant to waterlogging: retention of dry shoot and root biomass were higher and exhibited fewer chlorotic symptoms. The same plants were also able to better retain gas exchange parameters including photosynthetic and transpiration rates, and stomatal conductance. RNAseq analyses are being conducted, at 24 and 48 hours after the imposition of the stress, to identify transcripts regulated by *HvPgb1*. Expression level of *HvPgb1* was also measured in commercial varieties subjected to waterlogging, and a strong positive correlation (R2 = 0.9248) between *HvPgb1* expression level and tolerance to waterlogging was observed at 20h. These results reveal the promise HvPgb1 has as a marker for breeding programs aiming to enhance excess moisture tolerance in barley.

Identification and characterization of ubiquitination components which function as long-distance signaling agents through the cucumber phloem in phosphorus homeostasis.

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The plant vascular system, composed of xylem and phloem, plays a foundational role in plant physiology by mediating the long-distance movement of water, nutrients and signaling molecules. The vascular system impacts many agronomic properties of crops such as mineral nutrient use, flowering time, plant architecture, and carbon allocation. Proteomic analysis of phloem exudate from cucumber, pumpkin, and watermelon have revealed a wide range of proteins, including the components of the ubiquitin proteasome system, are present in the phloem sieve tube system. Ubiquitination is a post-translational modification and represents a theme in plant signaling responsive to environmental change. We are characterizing the role of a cucumber phloem E3 ligase in phosphorus and iron homeostasis. In addition, we are using tandem ubiquitin binding entities (TUBES) to immunoprecipitate ubiquitinated proteins under phosphate-limited and phosphate-sufficient conditions from cucumber phloem exudate. Immunoprecipitated proteins were subject to LC-MS/MS analysis. Gene ontology analysis predicted that the identified ubiquitinated proteins are involved in a wide range of biological processes and a number of ubiquitinated phloem proteins include functions in stress and metabolic processes. This analysis provides insights into understanding the molecular determinants of phosphate-limited stress signaling and extending our knowledge to improve nutrient use efficiency in crop species.

An affinity-tagged version of the cob-1 point mutant phenocopies the high sucrose phenotype: a key step to understanding how COBRA works

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COBRA (COB) is a membrane-anchored, cell wall-associated protein that was discovered in a mutant screen in *Arabidopsis thaliana* that aimed to identify genes controlling root expansion. The *cob-1* mutant, which has a single amino acid substitution, has a conditional root swelling phenotype; when grown on 4.5% sucrose, which stimulates rapid root expansion, cellulose levels drop and root tips undergo radial swelling. COBRA is therefore an ideal candidate regulator of cellulose synthesis but as a low abundance protein, it is difficult to purify in sufficient quantity for biochemical analysis. To overcome this limitation, we engineered a 6xHIS-tagged version of COB for purification on Ni columns. Immunoblotting analysis indicated that after secretion to the cell wall COBRA undergoes cleavage, and that one of the fragments is returned to the cytoplasm via endocytosis. The degree to which COBRA is cleaved is hypothesized to have a regulatory effect on cellulose synthesis. If this hypothesis is correct, we predict that the degree of cleavage will be altered in the *cob-1* mutant when grown on high sucrose. To test this prediction we used site-directed mutagenesis to recreate the *cob-1* point mutation in the *6xHis-COB* construct. The mutant construct was then introduced into heterozygous *cob-4* null alleles (*cob-4* is homozygous-lethal) by Agrobacterium-mediated transformation, and transgenic lines homozygous for both *cob-4* and *HIS-cob-1* were selected. The T3 generation *HIS-cob-1 cob-4* plants phenocopy the *cob-1* high-sucrose radial swelling phenotype, thus adding a powerful new tool for investigating the mechanism by which COBRA controls cellulose biosynthesis in plants.

Relying on light in a light-limited environment: Chlorophyll biosynthesis in the Antarctic psychrophile *Chlamydomonas* sp. UWO241

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You would not expect to find photosynthetic life 17 meters below the surface of a permanently ice-covered Antarctic lake, where there is only low blue light half the year and darkness the other half. Yet this is exactly where the green alga *Clamydomonas* sp. UWO241 thrives. Algae and plants produce chlorophyll (Chl) through a complex and well-regulated pathway, allowing them to absorb light from the sun to power photosynthetic reactions and carbon fixation. Despite its low light environment, UWO241 has lost the genes required for light independent Chl biosynthesis and instead relies on a light dependent enzyme. Curiously, a related green alga isolated from the same lake, *Chlamydomonas* sp. ICE-MDV, has retained the genes for Chl biosynthesis in the dark. Growth of both species under varied light quantities and qualities highlighted the effects of this loss on Chl biosynthesis. Under varied light conditions UWO241 has a reduced ability to alter Chl levels compared to ICE-MDV, and instead relies on alteration of Chl *a:b* ratios to acclimate to the different light conditions. Despite the ability of ICE-MDV to alter Chl levels to a greater degree than UWO241, its growth rates were significantly decreased in some light conditions. This suggests that the ability to synthesize Chl is not the single limiting factor in survival under varied light conditions. Future work aims to untangle the survival strategies used by these unique low-light thriving psychrophiles, providing insight into photosynthetic adaption under extreme shading.

Live-cell imaging of mesophyll morphogenesis in *Arabidopsis* leaves

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Leaf spongy mesophyll cells form an interconnected network of branched cells and intercellular spaces to maximize the surface area available for light capture and photosynthetic gas exchange. In spite of their importance for photosynthetic activities, the morphogenesis processes of spongy mesophyll are still poorly understood largely due to the difficulties of internal tissues imaging.
To investigate the morphogenetic events leading to cell separation and branching in Arabidopsis thaliana, I used mesophyll-specific promoters to facilitate imaging of mesophyll cell shape and microtubule (MT) organization over multiple spatiotemporal scales. By means of live-cell imaging and quantitative image analysis, I show that cells enlarge by selective expansion of cell wall regions in contact with intercellular spaces. Meanwhile, intercellular spaces provide a strong positional cue for the stable MT bundles, which in turn promote efficient dilation of intercellular spaces and cell branching. My data provide insights into mesophyll morphogenesis and MT organization and lay the groundwork for future investigations.
Distinct from its overlying epidermis layer, spongy mesophyll cells not only undergo division and expansion, also separate from their neighbor cells to form the labyrinth of intercellular spaces. Seeking to understand the coordination between cell division, expansion and separation during mesophyll morphogenesis, I carefully examined mesophyll's cell division pattern. My results showed that the unique CLASP-dependent alternating cell division pattern programs the distribution pattern of air spaces, which in return affect the subsequent cell division orientations, thus forming a dynamic feedback loop, which is crucial for the normal mesophyll morphogenesis.

In-vitro adventitious root formation is influenced by expression of Phytoglobins

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Phytoglobins (Pgbs) are heme-containing proteins, which through their ability to scavenge nitric oxide (NO), interfere with NO mediated responses many of which are required for the development of tissues and organs during plant development. It has been previously reported that Pgbs alter auxin synthesis and signalling and, through NO, control the behaviour of the root system during conditions of stress. Auxin flow is required for the formation of roots, and NO regulates this flow through PINs. This work examines how altered expression of Pgbs influences the *de novo* formation of adventitious roots using an established *in vitro* system, employing Arabidopsis cotyledons as explants. Preliminary results reveal that relative to wild type, over-expression of *Pgbs* enhances the formation of adventitious roots, while suppression of the same genes reduces the number of roots produced. The link between Pgbs, NO and auxin during the ontogenesis of adventitious roots will be further examined by utilizing reporter lines for localization of PINs, as well as pharmacological treatments altering the levels of NO in the tissue and the amount and flow of auxin. Collectively, these i*n vitro* studies will enhance our understanding on the established protective role of Pgbs on root cells during conditions of stress.

Gibberellin regulation of protein accumulation in developing pea (*Pisum sativum* L.) seeds)

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Many field pea varieties have a mutation in the *PsGA3ox1* gene which causes a decrease in bioactive gibberellins (GAs), a plant hormone that regulates growth and development. This mutation in field pea leads to lower GA levels, producing shorter stemmed plants useful for cultivation; however, its effects on seed composition are not well understood. This study tests the hypothesis that part of GAs effect on seed development is through modulation of protein accumulation in the developing seeds. Using GA overproducing and isogenic null control lines, changes in seed tissue free amino acid and total nitrogen content were determined to identify potential GA-induced effects on processes that affect protein accumulation during seed development. Cotyledon nitrogen content per seed and estimated protein content were elevated in the GA-overproducing line during development and at maturity compared to the null line, suggesting that seed storage protein accumulation is influenced by GA. Developmental variation in the profiles of key free amino acids involved in seed nitrogen transport and storage in seed coat, endosperm, and cotyledon seed tissues indicate that GA could potentially regulate amino acid transport and metabolism dynamics within developing seeds. These modifications, in turn, could influence the rate of storage protein synthesis in the cotyledons with possible implications on final seed protein content. The knowledge gained on GA regulation of storage protein production during seed development can be used to improve protein content in conventional field pea varieties, which could address issues faced by global agriculture and the plant-protein industry.

Characterization of norbelladine synthase and noroxomaritidine reductase, catalyzing the first key steps in Amaryllidaceae alkaloid metabolism

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Amaryllidaceae alkaloids (AAs) are a large group of plant specialized metabolites with diverse pharmacological properties. Norbelladine is the entry compound in AAs biosynthesis and is produced from the condensation of tyramine and 3,4-dihydroxybenzaldehyde (3,4-DHBA). There are two reported enzymes capable of catalyzing this reaction both with low yield. The first one, norbelladine synthase (NBS), condenses tyramine and 3,4-DHBA. The second one, noroxomaritidine reductase (NR), catalyzes a reduction reaction to produce norbelladine. To confirm the enzyme(s) catalyzing the first committed step of AAs biosynthesis, both NBS and NR were identified from the transcriptome databases of two Amaryllidaceae plant species *Narcissus papyraceus* and *Leucojum aestivum*. Both the genes were cloned, expressed in *Escherichia coli* and enzymatic assays were. The assays included each enzyme separately and combined in one reaction. The production of norbelladine was detected using high-performance-liquid-chromatography–tandem-mass-spectrometry. Our results suggest that both NBS and NR function together in a sequential manner for the condensation of tyramine and 3,4-DHBA into norcraugsodine followed by a reduction into norbelladine. Moreover, using transient expression of yellow fluorescent protein (YFP) fusions in *Nicotiana benthamiana* leaves, NBS and NR both localized to the cell cytoplasm confirming their colocalization to work together in the same cellular compartment. In addition, the protein homology modeling and molecular docking studies predicts the binding of tyramine and 3,4-DHBA to NBS and norcraugsodine to NR. Together, our study establishes that both NBS and NR participates in biosynthesis of norbelladine and will further strengthen the establishment of synthetic biology platforms to produce AAs.

Reduced viral accumulation under dark growth condition in *A. thaliana* and *Nicotiana benthamiana*: The uninviting dark side of plant-virus interaction.

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Plant growth is strongly influenced by their photoperiod, where light receptor or circadian clock-related genes will react upon the presence or absence of light and regulate the expression of the plant's genes accordingly. These light-related genes might be involved in viral proliferation during an infection. Our investigation was linked to either the plant's circadian clock or though photo-receptor related genes. We also verified if this phenomenon was unique to At or if it also happened in *Nicotiana benthamiana* (Nb) by infecting it with a *Plantago asiatica mosaic virus* (PIAMV) tagged with GFP. Circadian clock mutant and photo-receptor gene mutants used were toc1, and blus1 and pifq, respectively. Wild-type (Col-0), *toc1*, *blus1* and *pifq* mutant plants were infected with both PIAMV and *Cucumber mosaic virus* (CMV). Observations were made in our lab that virus accumulation in infected *Arabidopsis thaliana* (At) plants is lower when the plant is left in dark growth conditions than those grown in the regular 12h/12h light/dark photoperiod or during a 24h light photoperiod. Interestingly, the results of our on-going experiment with At mutants as well as our results on Nb show that PIAMV accumulates in Nb leaf tissue under regular photoperiod but not under dark conditions in our western blots experiments, suggesting that this phenomenon is not an isolated case from At but rather the cause of an essential pathway for viral infection that require light.

Resistance to viroids can be transferred between plant species using natural variation in the AGO2 gene

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Viroids are the smallest plant pathogens, consisting of a small (240-375 nt) RNA genome that does not encode for any known protein. Among the many protection mechanisms deployed by plants against pathogens, RNA silencing recognizes foreign double-stranded RNA (dsRNA) through the action of Dicer-like (DCL) endoribonucleases. This leads to the cleavage of dsRNA into small RNAs (sRNAs) of 21-24 nucleotides. The latter are then loaded onto Argonaute (AGO) proteins, which uses the sRNAs to target homologous single-stranded RNA. We have shown previously that the AGO2 proteins from Arabidopsis thaliana (At) and Nicotiana benthamiana (Nb) have differential activity against viruses. We have tested if this differential activity could be transferred between species and if this might extend to anti-viroid defense. Transgenic tomatoes expressing either NbAGO2 or AtAGO2, were generated. As expected, plants expressing AtAGO2, were markedly less susceptible to potato virus X. At the same time, after infection with potato spindle tuber viroid (PSTVd), no viroid symptoms were observed in tomato lines expressing NbAGO2, and no viroid accumulation was detected in NbAGO2 lines. These results suggest that RNA silencing can be an effective defense against viroids and that this phenomenon may determine host range in part.

Understanding phloem loading mechanisms through single-cell transcriptomics
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Phloem loading is a starting point of long-distance transport of photoassimilates. Despite the importance of the phloem loading process as a key determinant of crop yield, neither the cell types involved nor the precise transport mechanism are fully understood. Here we used single-cell RNA sequencing to identify the mRNA outfit of cells involved in phloem loading in Arabidopsis and maize. We provide evidence that Arabidopsis and maize use a different path for phloem loading of sucrose and demonstrate potential roles of the phloem parenchyma (in Arabidopsis) and bundle sheath (in maize) in the efflux of not only sucrose but amino acids. Our results provide valuable resources to build hypotheses and develop strategies to identify yet known factors involved in the transport of nutrients.

In-vitro adventitious root formation is influenced by expression of Phytoglobins
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Phytoglobins (Pgbs) are heme-containing proteins, which through their ability to scavenge nitric oxide (NO), interfere with NO mediated responses many of which are required for the development of tissues and organs during plant development. It has been previously reported that Pgbs alter auxin synthesis and signalling and, through NO, control the behaviour of the root system during conditions of stress. Auxin flow is required for the formation of roots, and NO regulates this flow through PINs. This work examines how altered expression of Pgbs influences the *de novo*formation of adventitious roots using an established *in vitro*system, employing Arabidopsis cotyledons as explants. Preliminary results reveal that relative to wild type, over-expression of *Pgbs* enhances the formation of adventitious roots, while suppression of the same genes reduces the number of roots produced. The link between Pgbs, NO and auxin during the ontogenesis of adventitious roots will be further examined by utilizing reporter lines for localization of PINs, as well as pharmacological treatments altering the levels of NO in the tissue and the amount and flow of auxin. Collectively, these i*n vitro*studies will enhance our understanding on the established protective role of Pgbs on root cells during conditions of stress.

Structural and functional leaf diversity lead to differences in photosynthetic capacity across *Juglans regia* accessions

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Leaf structural and functional characteristics drive inherent and stress-induced differences in net assimilation rate (*A*n). Germplasm collections hold potential for improving productivity and stress tolerance, yet few *Juglans regia* cultivars are used widely as scion in commercial walnut production. In this study we explored the anatomical and biochemical bases of photosynthetic capacity using X-ray microcomputed tomography, gas exchange and leaf chemical analysis in 11 *J. regia* accessions collected from the National Clonal Germplasm Repository (Winters, California). These accessions have distinct genetic divergence and originate from different latitudes with varying temperature and precipitation gradients. *A*n differed significantly among accessions and was greater in those from lower latitudes coincident with increases in stomatal and mesophyll conductance (*g*s, *g*m), leaf thickness, mesophyll porosity and gas-phase diffusion, leaf mass and nitrogen, and stomatal density. CO2-saturated assimilation rates were consistent with patterns in *A*n. Greater *A*n was found in accessions native to climates with more frost-free days and precipitation seasonality but lower temperature seasonality in lower latitudes. Even under dehydration, *A*n, *g*m and porosity remained higher for low-latitude accessions. *J. regia* accessions originating from lower latitudes, which have leaf structural traits and biochemistry that enhance photosynthesis, could be used as commercial scions or breeding parents for greater productivity and improved stress tolerance.

Effect of mining disturbance on the diversity and structure of bacterial communities associated with lodgepole pine trees naturally regenerating at unreclaimed gravel pit

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Anthropogenic disturbance events are common across the Canadian boreal and sub-boreal forests but their impact on the microbial community structure of forest trees is poorly understood. In this study, we analyzed how the relative abundance, diversity and taxonomic composition of endophytic and rhizospheric bacterial communities associated with lodgepole pine (*Pinus contorta* var. *latifolia*) change after gravel mining operations, by sequencing the v4 region of the 16S rRNA gene using the Illumina Miseq platform. Lodgepole pine trees were collected from the Skulow gravel mining pit (nutrient-poor site lacking topsoil) and the nearby undisturbed forest stand, located in central British Columbia, Canada. *Proteobacteria* was the most abundant phylum at both gravel (65%) and forest (60%) sites (exclusively represented by *Alpha* and *Gammaproteobacteria*), followed by *Actinobacteria* which was more abundant in the pine rhizosphere (22%) at gravel pit as compared to the undisturbed forest (10%). Bacterial alpha-diversity was significantly lower in the above-ground (needle-stem) communities in contrast to the below-ground (root-rhizosphere) communities at both sites. Beta-diversity was significantly affected by the mining activity with distinct bacterial communities observed in different tree niches at the gravel pit and forest site. To conclude, our findings indicate that lodgepole pine trees, naturally regenerating at the gravel mining pit, support a distinct bacterial microbiome to potentially sustain their growth in such a nutrient-poor ecosystem.

Dynamic mRNA-sRNA interactions coordinate gene expression during meiosis in wheat

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Plant sexual reproductive success hinges on the precise coordination of meiotic events, yet data on the transcriptional landscape and regulatory controls that operate during meiosis in plants are limited. Here, the high-quality coverage of wheat male meiocyte mRNA and sRNA profiles achieved with Illumina and Oxford Nanopore sequencing platforms enabled in-depth analysis of transcriptional dynamics throughout meiosis. Amidst a meiotic landscape of repressed transcription, active transcription of a subset of genes was uncovered. Detection of an enriched population of small and long non-coding RNAs in meiocytes included an inverse relationship between mRNA and sRNA levels, supporting spatio-temporal regulation of gene expression by regulatory non-coding RNAs during meiosis. Machine learning algorithms identified meiosis essential genes and confirmed the candidacy of the *Pairing homoeologues 1,* which controls orderly pairing of chromosomes in wheat. These findings challenge conventional understanding of meiotic transcriptional dynamics and regulatory controls, providing novel information on the complexity of this crucial biological process.

Biochemical Characterization of O-Methyltransferases Involved in Benzylisoquinoline Alkaloids Biosynthesis in Sacred Lotus

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Benzylisoquinoline alkaloids (BIAs) constitute a major class of plant specialized metabolites, including the narcotic analgesic morphine and related compounds used as raw materials for the semi-synthesis of pain-relievers (oxycodone and hydrocodone) and drugs administered to treat opioid addiction (naltrexone) or overdose (naloxone). Due to their complex stereochemistry, which challenges alternatives such as chemical synthesis and synthetic biology, traditional farming of opium poppy remains the sole commercial source of BIA-derived pharmaceuticals. The ancient aquatic plant known as sacred lotus (*Nelumbo nucifera*) produces both enantiomers of numerous BIAs, in contrasts with the predominantly *S* stereochemistry found in opium poppy and related species. For this reason, *N. nucifera* is a promising new model system to investigate BIA metabolism with a focus on better understanding the molecular basis of enzyme stereospecificity that could be further applied to improve synthetic biology platforms. Herein we report the identification and characterization of *O*-methyltransferases (OMTs) involved in the early steps of BIA biosynthesis in sacred lotus. Using the available draft genome of *N. nucifera* and a combination of phylogenetic analysis, gene amplification, cDNA cloning, protein heterologous expression, kinetic assays, and HPLC-MS/MS analysis, we showed that NnOMT1 is a regiospecific 6-*O*-methyltransferase (6OMT) accepting both *R* and *S* substrates, whereas NnOMT5 is mainly a stereospecific 7-*O*-methyltransferase (7OMT). Interesting, NnMT5 catalytic activity, despite the enzyme low similarity to functionally characterized 7OMTs, suggests a possible independent recruitment in sacred lotus. Organ-specific correlational studies on alkaloid content, enzymatic activity in plant crude extracts and transcript levels supported NnOMTs physiological role in the plant.

Rhizosphere *Pseudomonas fluorescens* protect *Arabidopsis thaliana* from an opportunistic pathogen through a colonization-dependent mechanism.

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Plants can form commensal interactions with soil microorganisms, creating a root microbiome that may provide benefits to the plant host, such as protection from pathogens. While members of the microbiome can produce antimicrobial compounds *in vitro*, it is largely unknown how microbiota contribute to pathogen protection *in planta*. In this study, we used *Arabidopsis thaliana* and root-associated *Pseudomonas fluorescens* as a model to investigate plant-microbiome interactions in the rhizosphere. *P. fluorescens* N2C3 is an opportunistic pathogen within the *P. fluorescens* species complex, and causes detrimental effects on *A. thaliana* in the absence of beneficial members of the root microbiome. 30 *Pseudomonas* strains were screened for their ability to protect against N2C3, but in most cases, only strains closely related to N2C3 were found to protect against N2C3 pathogenesis. To uncover the genetic mechanisms that allow some beneficial *P. fluorescens* strains, but not others, to protect *Arabidopsis* from N2C3, we tested deletions in 16 candidate genes and performed comparative genomics to identify additional genes that are unique to protective strains. Using a reverse genetics approach, we found that a response regulator required for rhizosphere colonization and bacterial membrane permeability, ColR, is necessary for protection, indicating that competitive exclusion may contribute to pathogen protection. Uncovering genes that contribute to pathogen protection can help identify members of the root microbiome that improve plant resistance to opportunistic pathogens. This can have agricultural applications, such as the use of beneficial strains for microbiome engineering in dysbiotic soil systems.

Quantitative proteome and PTMome analysis of *Arabidopsis thaliana* root responses to persistent osmotic and salinity stress

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Studies investigating plant responses to osmotic and/or salt stress have largely focused on short-term systemic responses, leaving our understanding of intermediate to longer-term adaptation (24 h - days), less understood. In addition to protein abundance and phosphorylation changes, evidence suggests reversible protein acetylation may also be important for abiotic stress responses. Therefore, to characterize protein-level effects of osmotic and salt stress, we undertook a label-free proteomic analysis of *Arabidopsis thaliana* roots exposed to 300 mM Mannitol and 150 mM NaCl for 24 h. We assessed protein phosphorylation, lysine acetylation and changes in abundance, detecting significant changes in the status of 302, 46 and 118 proteins, respectively. Comparison with available transcriptome data from analogous treatments, indicate that transcriptome- and proteome-level changes occur in parallel, while PTM changes do not. Furthermore, significant changes in abundance, phosphorylation and lysine acetylation involve different proteins from the same networks, indicating a concerted, multifaceted regulatory approach to prolonged osmotic and salt stress. Collectively, our findings indicate dynamic protein-level changes continue to occur in plant roots 24 h from the onset of osmotic and salt stress and that these changes differ across multiple levels of the proteome.

Turning the pep-tide: in vitro evaluation of linear host defense peptides for ectopic expression in plants

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The ectopic expression of host defence peptides (HDPs) in crops can significantly improve resistance to a wide range of pathogens, reducing crop losses to disease. HDPs are small, cationic peptides with antimicrobial and/or immunomodulatory activity. Compared to chemical pesticides, many HDPs have low cytotoxicity to plant and mammalian cells and hold negligible risks of inducing resistance in microorganisms. HDP-mediated resistance is also effective on a broad spectrum, unlike the protection awarded by *R*-gene expression. The aim of this study is to evaluate linear plant-derived HDPs Sm-985, BnPRP1, P4650, Ib-AMP1 analog, and Shepherin 1 *in vitro* and identify candidate HDPs suitable for expression in plants. Peptide antimicrobial activity and cytotoxicity have been evaluated *in vitro* by incubating various combinations and concentrations of HDPs with phytopathogenic bacteria, fungal conidia, plant cells, and human embryonic kidney cells. Under the control of the *BiPPro1-1* promoter, green fluorescent protein and stacked HDP genes will be introduced into model plants by *Agrobacterium*-mediated transformation. Tracking of fluorescent proteins will determine promoter activity in different plant tissues and organs. Disease resistance of transformed plants will be assessed by the severity of symptoms relative to their non-transformed counterparts. We predict that most tested HDPs will be non-cytotoxic, yet highly antimicrobial alone and in combinations. We also anticipate synergistic activity between at least three peptides and significantly enhanced disease resistance in plants expressing these combinations. This study will identify novel HDPs for engineering disease resistance in crops and contribute to global efforts of improving food security in an uncertain future.

Transcriptional dynamics during microspore reprogramming to embryogenesis in wheat

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Microspore embryogenesis (ME) is an efficient plant breeding tool for rapid fixation of genetic variants in a homozygous state. Due to significant inter- and intra-species variation in embryogenic potential, the true potential of this technology has not yet been fully realized. To understand the molecular mechanisms underlying microspore reprogramming to embryogenesis, we characterized the transcriptomes of microspores prior to and after 7 and 21 days of induction of embryogenesis in two wheat cultivars AC Nanda and AC Sadash with high and low embryogenic potential, respectively. Our results showed intricate differences between the two genotypes regarding the network of cell reprogramming, hormonal regulation, cellular protection, epigenetic mechanisms, sucrose and starch metabolism, and proteolysis. The low ME efficiency in AC Sadash was likely due to degradation of RNA and upregulation in ABC transporters and gametophyte development compared to AC Nanda. Our results shed light on molecular mechanisms that mediate developmental plasticity and cell reprogramming during embryogenesis in wheat, and allowed us to suggest treatments with specific modulators (such as hormones, antioxidants, and epigenetic chemicals) to improve ME.

Abiotic stress response in *Brassica napus* and *Arabidopsis thaliana* with altered expression of phytoglobins.

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Plants exposed to excess moisture, such as waterlogging, experience hypoxic stress which compromises plant growth and development and reduces yield. Phytoglobins (Pgbs) are heme-containing proteins known to be induced by hypoxia and able to scavenge cellular nitric oxide (NO). Their overexpression has been linked to resilience to excess moisture. In the present studies, the behaviour of Arabidopsis *thaliana* and *Brassica napus* plants suppressing or over-expressing class one or class two Pgb (*Pgb1* or *Pgb2*) was examined during waterlogging. In both species, over-expression of Pgbs enhanced tolerance to waterlogging through retention of photosynthesis and root growth. In Arabidopsis, waterlogging impaired the PIN-mediated establishment of an auxin maximum in the root apex, required for the expression of WOX5 and the specification of the quiescent center. These abnormalities resulted in the abortion of root growth and substantial death of the root cells. These effects were exacerbated by suppression of Pgb and attenuated by the over-expression of the same gene. Arabidopsis plants over-expressing *Pgb1* were able to retain the expression and proper localization of *PIN1-4* and a strong auxin and *WOX5* signal in the root apex during waterlogging. These results, also confirmed by pharmacological treatments, retained the integrity of the root and prevented cell death. The role of auxin flow in waterlogging tolerance is currently being examined *in B. napus* plants. Collectively, this work suggests that Pgbs protect plants from waterlogging through the retention of the PIN-mediated auxin flow required for root function.

[1] These authors contributed equally to the work

Phytohormone-enhanced heavy metal responses in *Euglena gracilis*: Ni, Pb and Cd uptake and associated hormone and metabolomic dynamics

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Phytohormones, cytokinins (CKs) and abscisic acid (ABA) control plant growth and stress responses; but they also mediate various developmental processes in algae. Our previous studies suggested that exogenous applications of CKs and ABA induce growth promotion in *Euglena gracilis*. Yet, CK and ABA mode of action in algal adaptive strategies to heavy metals, and their involvement in phytohormone crosstalk remain largely unknown. Here, we highlighted phytohormone-specific responses of *Euglena* under heavy metal stress. Furthermore, we attempted to unravel the impact of exogenous hormone supplementation on metal uptake (nickel, lead, cadmium) and associated hormonal and metabolomics algal profiles. Endogenous hormone profiling by HPLC-(ESI)-HRMS/MS revealed that ABA- and CK-controlled response to metal stress relies on modifications in *Euglena* CK activity profiles. Hormonal crosstalk, which contributes to precise responses to metal stress, was investigated in this study by culture supplementation with trans-Zeatin (tZ) and ABA, and this revealed enhanced levels of gibberellins (GAs) during metal exposure. Metabolomics indicated activation of some key pathways underpinning metal accumulation, sequestration, and damage mitigation. These processes are related to the action of phytohormones under metal stress that affect the biosynthesis of thiol- compounds

(short metal binding peptides), lipid pathways, riboflavin metabolism, biosynthesis of cofactors/vitamin and carbohydrates metabolism. Phytohormones also stimulated production of bioactive secondary metabolites (e.g., terpenoids, alkaloids, flavonoids, carotenoids). Thus, our results provide insights into the biological roles of CKs and ABA in the enhanced metal uptake efficiency. These findings are essential to aid future microalga biotechnology for better bioremediation of heavy metals.

The role of autophagy in the *Arabidopsis* self-incompatibility response.

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Interactions between pollen and pistil are critical for controlling sexual reproduction in plants, both in maintaining genetic diversity and rejecting foreign pollen. The self-incompatibility (SI) trait is an additional layer of regulation within the pollen-pistil interaction, which allows for recognition and rejection of self-pollen, triggered by the interaction between the pollen S-locus protein11/*S*-locus cysteine-rich peptide and the pistil *S* Receptor Kinase. While this initial signalling event is well characterized, downstream players in the SI pathway are poorly understood. Previous studies have shown that autophagic bodies are present following self-pollination in *Arabidopsis lyrata*, although the exact role that autophagy was playing in the SI pathway was unclear. Recent developments in the generation of transgenic self-incompatible *Arabidopsis thaliana* have made it possible to more easily study the SI pathway, and any role that autophagy may play. Through combining two different accessions of transgenic SI-*A. thaliana* with autophagy deficient *AUTOPHAGY7* (*ATG7*) mutants, we were able to measure breakdowns in several key steps of self-pollen rejection. Additionally, fluorescent tagging of the autophagosome-marking ATG8a has allowed us to directly image autophagosomes forming upon SI pollination. Lastly, measuring ATG8a-GFP cleavage has allowed for quantification of autophagic activity during the SI response. These results give a role for autophagy within the SI pathway, adding to the understanding of the SI pathway as a whole.

A conserved module regulates receptor kinase signaling in immunity and development

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Ligand recognition by cell-surface receptors underlies development and immunity in both animals and plants. Modulating such receptor signaling is critical for appropriate cellular responses; however, the underlying mechanisms are still poorly understood. In plants, receptor kinases (RKs) are a major class of cell-surface receptors recognizing ligands to control diverse aspects of growth, development, and immunity. Here, we show that RKs that perceive pathogen-associated molecular patterns (PAMPs) in immunity and CLAVATA3/EMBRYO SURROUNDING REGION-RELATED peptides (CLEp) in development employ a similar regulatory signaling module. In the absence of ligand, signaling is dampened through association with specific type-2C protein phosphatases (PP2Cs) of the POLTERGEIST-LIKE (PLL) family. Upon activation, PAMP and CLEp receptors phosphorylate distinct cytosolic kinases of the RLCK-VII/PBL family, which, in turn, phosphorylate the PLLs, thereby promoting their release from the receptor complexes. Our work reveals a regulatory circuit shared between immune and developmental receptor signaling, which has broader and important implications for plant RK-mediated signaling in general.

Can Variability in Leaf %C be used as an Indicator of Plant Carbon Stress?

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Global warming is expected to increase mean annual temperatures and alter precipitation patterns globally, environmental changes that will lead to conditions causing plant carbon stress. An increase in the intensity and frequency of extreme heat or drought events will exacerbate the warming effects of various regions by increasing the terrestrial evaporative demand. These environmental conditions are likely to lead to an increase in the prevalence of carbon starvation, a phenomenon in which a plant exhausts its carbohydrate supply during a stress, by consuming carbohydrate pools faster than they can be generated by photosynthesis. Carbon starvation can be initiated by any environmental condition that limits photosynthesis for a prolonged period or forces a plant into a negative carbon balance. I investigated whether levels of leaf % carbon can be used as an indicator of plant carbon stress. To address this issue, I consulted the Web of Science database, to compile articles for a meta-analysis on the effects of low light, low CO2 concentration, high temperature drought, and high CO2 concentrations on leaf %C. In total, 22 articles (with a total of 135 data points) out of the initial 5015 papers were assessed using a random-effects meta-analysis. Compared to control treatments, the various experimental treatments had no statistically significant effects on leaf %C. I show that leaf %C is unlikely to be useful as an indicator of plant carbon stress across the relevant environmental conditions.

The proteins encoded by the Low expression of OSmotically responsive genes 2 locus in *Arabidopsis thaliana* exhibit redox-dependant modifications in response to oxidative conditions.

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The production of reactive oxygen species (ROS) is an inescapable consequence of aerobic respiration and photosynthesis. Plants have developed a robust redox-regulated proteome to manage the constant production of ROS by these processes. ROS play an essential signaling function through redox-dependent post-translational modifications. In particular, *S*-glutathionylation, the reversible modification of redox-sensitive protein thiols by the tripeptide glutathione, plays a key role in protecting thiols against irreversible oxidation. Growing evidence also indicates an implication of *S*-glutathionylation in the regulation of protein activity and function in plants. The locus *Low expression of OSmotically responsive 2* (*LOS2*) is quite peculiar. It encodes two proteins with very different functions that arise from differential translation. The first is the cytosolic isoform of the glycolytic enzyme responsible for most of the phospho*enol*pyruvate production, enolase 2. The other is LOS2, a transcription factor implicated in cold response regulation. Here we provide evidence that the two proteins, which share most of their sequence, are both sensitive to redox conditions. Recombinant versions of the two proteins were produced and purified. They were subjected to *in vitro* assays in the presence of glutathione in order to evaluate their sensibility to redox conditions. Our results support the occurrence of a covalent modification with glutathione for both proteins under oxidative conditions. This research could lead to a better understanding of redox regulation events in plant primary metabolism and in response to environmental stress.

High-Throughput Screening of Drought Tolerant Potatoes to Achieve "More Crop per Drop"

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Drought poses a major challenge to potato cultivation and productivity worldwide. Climate change is predicted to further aggravate this challenge by intensifying potato crop exposure to increased drought severity and frequency. Hence, there is an urgent need to adapt our potato production systems by developing drought tolerant cultivars that are appropriately engineered for the changing environment. This can be approached through the identification of drought-related physiological, biochemical and molecular traits and their deployment in new potato cultivars. However, our capacity to assess a large number of genotypes is constrained by the limited availability of high-throughput tools and technologies. The application of leaf and canopy level hyperspectral reflectance is a promising high-throughput field phenotyping tool for agricultural research. The reflectance data can be used to develop spectral indices to rapidly and efficiently screen germplasm under field conditions. Thus, the main objective of this work is to study the potential use of hyperspectral reflectance as a high throughput phenotyping tool to identify drought-related physiological, biochemical and morphological traits. We are currently evaluating 60 diverse potato genotypes subjected to drought stress. Our preliminary results revealed a wide difference across potato genotypes in their ability to tolerate drought stress, which was in turn associated with their capacity to maintain tuber yield, rates of photosynthesis, water use efficiency, leaf pigments, leaf protein and dry matter content. We will discuss whether these variations in drought-related characteristics are mirrored by differential spectral reflectance patterns across the potato genotypes.

Identifying new lipid droplet proteins in *Arabidopsis thaliana*: ERD7 localizes to lipid droplets via its senescence domain

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Lipid droplets (LDs) are neutral-lipid-containing organelles found in all kingdoms of life and are coated with proteins that carry out a vast array of functions. However, many of the molecular mechanisms underlying LD biogenesis, maintenance, and turnover are unknown, particularly in plants, where relatively few LD proteins have been studied. To address this, we analyzed proteomes of isolated *Arabidopsis thaliana* LDs and identified several novel LD proteins, including EARLY RESPONSIVE TO DEHYDRATION 7 (ERD7), a protein commonly associated with plant stress response. We show that ectopically-expressed ERD7 localizes to LDs in plant cells via its C-terminal senescence domain (SD). Phylogenetic analysis revealed that ERD7 belongs to a six-member family in Arabidopsis that, along with homologs in other plant species, is separated into two distinct subfamilies. Notably, the SDs of proteins from each subfamily conferred targeting to either LDs or mitochondria. Further, the SD from the ERD7 homolog in humans, spartin, localized to LDs in plants. Disruption of *ERD7* expression in Arabidopsis revealed no obvious changes in LD numbers or morphology in leaves and seeds under normal growth conditions, although this does not preclude a role for ERD7 in stress-induced LD dynamics. Consistent with this possibility, a yeast two-hybrid screen using ERD7 as bait identified numerous proteins involved in stress responses. Collectively, these observations provide new insight to ERD7 and the SD-containing family of proteins in plants and suggest that ERD7 is involved in functional aspects of plant stress response that also include localization to the LD surface.

Assessing the effect of drought stress on flag leaf epicuticular wax composition in old and new bread wheat cultivars

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Wheat (*Triticum aestivum* L.) is the most widely grown cereal crop in the world. With accelerated climate change, there has been an increase in drought events which has negatively impacted grain yield. One mechanism plants use to defend themselves from environmental stressors such as drought, is by sealing their surfaces with epicuticular waxes. Epicuticular waxes are a hydrophobic layer composed of lipophilic compounds which envelope plant aerial tissues and can help reduce non-stomatal water loss. Despite their role in drought tolerance, there is a lack of research on epicuticular waxes in Canadian bread wheat cultivars. To address this gap, the flag leaf wax composition of old and new bread wheat cultivars, with years of release ranging from 1969 to 2018, were analyzed. In addition to year-of-release, cultivars also differed in the ecozone they were bred for; Swift Current, Saskatchewan (Western ecozone), Brandon, Manitoba (Eastern ecozone). Eight bread wheat cultivars were subjected to a drought experiment in a greenhouse and flag leaf waxes were analyzed using gas chromatography and mass spectrometry. Significant differences were observed in total plant height, tiller number, head count and days-to-flowering. No significant differences were observed in total wax load between treatments, however, drought treated, Western and older cultivars had a significantly higher β-diketone content relative to control treated, Eastern and modern cultivars respectively. Further investigation into the genes responsible for this response, could help improve drought tolerance in future wheat lines.

Cells switch from stochastic to predictable behaviors during leaf development in *Arabidopsis*

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Precise coordination of growth, patterning, and differentiation is required for the acquisition of organ shapes in plants. However, it is still unclear how these key developmental processes are regulated at a cellular resolution, allowing for the robustness of plant organogenesis. Indeed, while growth is controlled by global positional information, cell-to-cell variability was also proposed to play a role in organ shaping. Here we use a confocal live-imaging pipeline that enables long-term observations of entire growing leaves to address the role of variability in organogenesis. Combining this method with quantitative image analysis allows for precise characterization of the leaf development at the global, regional, and cellular levels. Although the overall growth in leaves is variable, our findings suggest that after differentiation some cell type grow cell-autonomously while other rather follow positional information in the expanding leaf. Despite the growth variability exhibited by the entire organ, individual cells in the developing leaf follow more specific developmental trajectories. Our approach unravels complex interactions between cell behaviors in various tissues of the developing organ.

A shield against stress. Using cell wall structural modifications to overcome abiotic and biotic stress.

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The Canadian agriculture industry is critical to food and economic security, however its future hangs in a delicate balance, threatened by climate change. Structural modifications in the cell wall through pectin cross linkages between calcium ions and boric acid, may be key to mitigating damage caused by stress. The objective of this research was to examine how changes in calcium and boron within various plant spp. may translate to increased resistance to multiple stresses. While calcium reduced ($p<0.05$) water loss in pure pectin, the efficacy of calcium in mitigating drought stress in *Allium* was inconsistent between species and lengths of dehydration. Nevertheless, confocal imaging showed localization of exogenously applied calcium to the apoplast in epidermal cells of *A. fistulosum*. *A. fistulosum* (freezing tolerant), was also more resistant ($p<0.05$) to dehydration compared to *A. cepa* (freezing susceptible). Mutants of BOR1, a boron transporter in *Arabidopsis*, also reduced dehydration tolerance. *Colletotrichum higginsianum*, a hemibiotrophic pathogen that uses physical pressure to circumvent the cell wall was observed in this system. *bor1* had a higher rate of water loss and percent electrolyte leakage following dehydration stress. Furthermore, the addition of boron to pure pectin reduced ($p<0.05$) water loss. In addition, *bor1* had the most rapid rate of *Colletotrichum higginsianum* infection. Our research demonstrates connections between dehydration stress, freezing stress and *Colletotrichum higginsianum* and is indicative that the cell wall and pectin likely play a critical role in defense. Nonetheless, response to stress is complex and pectin modifications alone are likely not a silver bullet.

Glutamine activates the Target of Rapamycin Pathway signalling pathway in Mature Leaves: Implications for plant nitrogen signalling

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Across eukaryotes, **t**arget **o**f **r**apamycin (TOR) is a master regulator of cellular nutritional status that coordinates biosynthesis with catabolism. We recently observed that plant TOR signalling is activated by elevated amino acid levels and this influences major metabolic fluxes. However, the genes involved in sensing amino acids upstream of TOR in animals and fungal are all absent in plants, and the molecular mechanisms by which plant cells sense cellular nitrogen levels have not been established. Therefore, we further characterized the amino acid-TOR kinase signalling nexus in plants. We established that glutamine (Gln) elicits the strongest amino acid activation of TOR in mature leaves. However, the TOR response to Gln is diurnally restricted, as it is not observed during daytime, where TOR instead responds strongly to light and sugars. We hypothesize that Gln acts to signal cellular nitrogen status via the TOR pathway in plants, consistent with Gln's signalling role in yeast and animals. To provide evidence for such signalling, we characterized the influence of the TOR inhibitor AZD8055 and Gln on protein synthesis and nitrogen assimilation in leaf discs. Metabolomic and proteomic isotopic labelling data established that AZD8055 inhibits protein synthesis and activates de novo nitrogen assimilation and protein degradation rates in mature leaves, leading to elevated amino acid levels. By contrast, Gln treatment inhibited uptake of external labelled compounds in a TOR dependent

manner, indicating a suppressive effect on nutrient uptake. The results are consistent with opposing effects for AXD8055 and Gln on nutrient signalling.

Genome-wide association study of transpiration rate in common bean (*Phaseolus vulgaris* L.) in drying soil

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Common bean is the most cultivated legume crop for direct human consumption due to its high content of protein, minerals, and vitamins. In the wake of climate change, drought is one of the main limiting factors to crop productivity and food security. Drought resistance is a major breeding objective that is affected by multiple factors such as transpiration rate. This study was aimed to investigate the genetic architecture of transpiration by analyzing the water deficit threshold eliciting stomatal closure. Three greenhouse experiments encompassing ninety-three genotypes from the Mesoamerican gene pool were conducted. To avoid evaporation, pots were sealed in the top and bottom, and transpiration rate (TR) was measured by weighing. TR of drought-stressed plants was normalized (NTR), and the daily soil water content was expressed as the fraction of transpirable soil water (FTSW). When NTR decreased linearly, the FTSW threshold (FTSWc) was estimated, and market-trait associations analyses were performed. NTR and FTSWc showed differences between genotypes, and in a genome-wide association study (GWAS), 38 significantly associated markers were identified. After false discovery rate correction (0.001) FTSWc had one significant peak on Pv01. Our results contribute to deciphering genetic elements associated with the control of transpiration and stomatal closure to identify and develop drought-resistant cultivars.

Investigating Enzymes Involved in Quinate and Chlorogenic Acid Metabolism

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Quinate is abundant in green plant tissues. It is used for the biosynthesis of chlorogenic acids (CGAs). CGAs are antioxidants, UV- light protectants and have antifungal properties. A lot is known about quinate and CGAs as metabolites, yet not all enzymes involved in their metabolism were characterized. In our work, we uncovered and characterized a family of quinate dehydrogenases, enzymes responsible for the metabolism of quinate. It was found that quinate dehydrogenases originated from closely related shikimate dehydrogenase paralog enzymes. Duplication of shikimate dehydrogenases allowed for neofunctionalization of a second copy. A single mutation of active site residue threonine to glycine allowed for the oxidation of quinate. It was found that quinate dehydrogenases arose from several independent events during evolution in land plants. Further, we are working on understanding *in vivo* roles of QDHs in plants.

Completion of the vindoline and catharanthine pathways in *Catharanthus roseus* facilitates characterization of MIA pathways in *Ochrosia elliptica*.

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Monoterpenoid indole alkaloids (MIAs) produced by members of the Apocynaceae family display valuable pharmacological properties. This includes the dimeric anticancer MIA vinblastine, which is produced from catharanthine and vindoline in *Catharanthus roseus*, and the antiarrhythmic MIA ajmaline from *Rauwolfia serpentina*, all of which are derived from strictosidine, the central intermediate in MIA biosynthesis. The recent completion of the catharanthine and vindoline biosynthetic pathways in *C. roseus*, as well as the characterization of most genes involved in ajmaline biosynthesis in *R. serpentina*, are facilitating discovery of homologous MIA biosynthetic genes in other species. *Ochrosia elliptica* produces a wide array of MIAs including ellipticine and apparicine, which possess anticancer and analgesic activities, respectively, and which may be derived from stemmadenine, an intermediate in catharanthine and vindoline biosynthesis. To identify potential gene candidates involved in MIA biosynthesis, the leaf transcriptome of *O. elliptica* was assembled *de novo* from 184 million trimmed Illumina reads into 365 339 predicted coding sequences using the Trinity pipeline on the European Galaxy server. Further clustering of similar (≥95%) peptide sequences with CD-HIT-PROTEIN and removal of peptides <200 amino acids yielded 25 102 sequences, averaging 439 amino acids in length. Querying the assembled transcriptome with known MIA biosynthetic genes indicated that *O. elliptica* possesses orthologues with ≥70% amino acid identity to 13 out of 15 genes involved in the assembly of secologanin, tryptamine, strictosidine, and stemmadenine. These promising results suggest that the transcriptome will be a vital resource for identifying gene candidates required for MIA biosynthesis in *O. elliptica*.

PvMATE8 is a Multidrug and Toxin Extrusion transporter involved in proanthocyanidin accumulation and postharvest seed coat darkening in common bean

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In common bean (*Phaseolus vulgaris* L.), postharvest seed coat darkening (PHD) is an undesirable trait that affects the crop value. PHD is caused by an increased accumulation of proanthocyanidins in the seed coat. The precursors of proanthocyanidins are synthesized in the cytoplasm, that subsequently get glycosylated and then transported to the vacuoles where polymerization occurs. Thus, vacuolar transporters play an important role in the accumulation of proanthocyanidins. In *Arabidopsis thaliana* and *Medicago truncatula*, vacuolar membrane-localized multidrug and toxic compound extrusion (MATE) like transporter named *TRANSPERANT TESTA 12 (TT12)* and *MtMATE1*, respectively, have been reported to transport epicatechin 3'-O-glucoside to the vacuole. Here, we report the identification of a vacuolar transporter PvMATE8 by comparing global gene expression profiles of two pinto bean cultivars CDC Pintium (regular darkening) and 1533-15 (slow darkening). PvMATE8 shows higher expression in CDC Pintium compared to 1533-15, localized in the vacuolar membrane and is able to rescue the *tt12* mutant phenotype in *Arabidopsis*. Identification of PvMATE8 will help better understand the mechanism of proanthocyanidin accumulation in common bean.

Cytosolic geraniol and citronellol biosynthesis mediated by a Nudix hydrolase in *Pelargonium graveolens*

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Geraniol, citronellol, and related esters are high-value acyclic monoterpenes used in food technology, perfumery, and cosmetics. Essential oil from rose-scented geranium (*Pelargonium graveolens*) is a major source of these 'citronelloids'. Although many species produce geraniol from geranyl diphosphate (GDP) in the plastid via a monoterpene synthase, a few make it in the cytosol through an alternative route involving a nudix hydrolase. We provide evidence that *Pelargonium* biosynthesizes citronelloids in the cytosol via geranyl monophosphate (GP) using a Nudix hydrolase and phosphatase. A Nudix hydrolase cDNA from *Pelargonium* glandular trichomes dubbed PgNdx1 encoded a cytosolic protein capable of hydrolyzing GDP to GP with a KM of ~750 nM but is only weakly active towards farnesyl diphosphate. Leaf protein preparations converted GDP to geraniol in *in vitro* assays, a process which could be blocked by phosphatase inhibitors, suggesting a two-step conversion of GDP to geraniol. *P. graveolens* chemotypes enriched in either geraniol or (-)-citronellol accumulate GP or citronellyl monophosphate (CP), respectively, the presumed precursors of their monoterpenoid end products. In contrast, (-)-isomenthone rich lines lack these prenyl monophosphates and monoterpene alcohols and instead feature high levels of cyclic *p*-menthane monoterpenes derived exclusively from the plastid. In citronellol rich lines, GDP, GP, and CP were readily detected, while citronellyl diphosphate was absent, suggesting that citronellol biosynthesis proceeds by reduction of GP to CP in this species. These findings highlight the cytosol as a compartment that supports monoterpene biosynthesis in *Pelargonium* and expands the role of Nudix hydrolases in plant volatile biosynthesis.

Characterization of guard cell-specific drought-responsive genes in *Arabidopsis thaliana*

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Drought, an abiotic stress factor, is projected to negatively impact crop growth in more than 50% of arable lands by 2050 consequently leading to a >10% reduction in crop production and posing a threat to the global food supply. Abscisic acid, a plant hormone, regulates plant stress response to various stressors including water deprivation, salt, and drought via stimulation of guard cell (GC) ion channels for stomatal closure. Since stomata regulate plant water content, many studies have investigated the potential of manipulating stomata to regulate transpiration and photosynthetic gas exchange. To this end, Anna van Weringh, a Ph.D. student in the Provart lab, profiled differentially expressed GC-specific drought-responsive genes using a nuclear RNA isolation assay. I aim to characterize LOF GC-specific drought-responsive mutant genes in *A. thaliana* using a reverse genetics approach. To identify phenotypically interesting candidates, initial screening will be performed for temperature and soil water content (SWC) followed by further experimentation to determine the carbon exchange rate and stomatal aperture width compared to the wild-type (WT) plants. The KO plants are hypothesized to have decreased GC regulation, resulting in cooler temperature, quicker SWC loss, increased carbon exchange rate, and increased stomatal aperture width compared to WT plants. The study of 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.

Data acquisition approaches in proteomics: addressing technological limitations for plant systems biology

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Mass-spectrometry based proteomics is the next frontier in experimental plant systems biology, from single-cell analysis to profiling dynamic post-translational modifications and protein complexes. However, it is essential to understand and systematically benchmark the limitations of existing proteomics technologies prior to integrating proteomics data into systems-level models. These constraints include an inability to accurately quantify proteomes with a large dynamic range, such as plant tissue, and the lack of reproducible measurement of the same proteins between replicates. In this talk I will use recent unpublished data from Arabidopsis cells to introduce these limitations to plant scientists interested in applying proteomics techniques to systems-level questions. I will also describe a new data acquisition method called BoxCarDIA that seeks to address these limitations. Our results demonstrate that BoxCarDIA can increase the quantitative depth of MS/MS analysis in plant cells by as much as 41%, improve the quantification of both low- and high-abundant proteins, and address the long-standing problem of missing values in plant proteomics. Crucially, these gains are realized with no change in instrumentation and no increase in analysis time. Our results establish BoxCarDIA as the new method of choice in label-free quantitative proteomics and point the way towards more reproducible measurements enabling large- scale comparative analyses.

FEELING THE HEAT: The Impact of Temperature on Systemic Immunity in *Arabidopsis thaliana*

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Elevated temperatures critically influence disease susceptibility by targeting vital components of the plant immune system. These include pattern-triggered immunity, effector-triggered immunity and basal resistance, which collectively occur at the local (primary) site of pathogen attack. We have shown that high temperature suppresses local defences by decreasing biosynthesis of the defence hormone salicylic acid (SA). SA is also intertwined with systemic defences (i.e. at distal sites from infection) through interplay with plant systemic acquired resistance (SAR) signals pipecolic acid (Pip) and *N*-hydroxy-pipecolic acid (NHP). However, it remains unclear how temperature impacts systemic immunity. Here we show that warm conditions effectively eliminate *Arabidopsis thaliana* SAR. Local infection with virulent bacteria at normal temperature expectedly led to SAR, enhancing disease resistance systemically. However, systemic immune priming from local bacterial infection was lost at elevated temperature. This was associated with abolished local and systemic expression of key Pip-NHP biosynthetic genes. Strikingly, a significant cluster of the SAR-regulated transcriptome is downregulated by high temperatures. Exogenous Pip treatment did not restore systemic immunity against virulent pathogens at elevated temperature, suggesting additional temperature-vulnerability of Pip-NHP signalling and response. This was supported by temperature-downregulation of Pip- and NHP-induced transcriptomes. Altogether, our research demonstrates that high temperatures broadly impact plant immunity locally and systemically. We propose a model reflecting how temperature regulates mutual amplification of SA and Pip-NHP during immunity, providing a comprehensive host defence landscape in a warming climate. This can serve as a molecular roadmap to repairing the immune system in an effort to develop climate-resilient plants.

Abscission in plants: Structural, chemical and transcriptomic analysis of protective surface layers

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Plants undergo a self-pruning mechanism called abscission to detach organs. Efforts to understand this process have been led by studies in a model plant species, *Arabidopsis thaliana*, in which the outer parts of the flower (sepals, petals, stamens) are shed to make way for the developing fruit. Abscission involves specialized cell layers called the abscission zone where separation takes place. When organs detach, newly exposed cells on the plant surface become sealed against water loss and pathogen entry through the formation of a protective layer. Plants in which the protective layer develops slowly or weakly are often susceptible to disease, making the study of this layer of importance to agriculture. The protective layer in Arabidopsis plants is suggested to be a lipid-based, cell wall-associated polymer such as cutin or suberin. To determine the identity of this polymer in Arabidopsis, abscission zones were harvested and subjected to chemical analysis using gas chromatography with flame ionization and mass spectroscopy methods of detection. This analysis indicates that a form of cutin is the primary component of the Arabidopsis protective layer. Light and electron microscopy will be used to examine the structure and deposition of this layer. Transcriptomic analysis will be carried out to test for the expression of key biosynthetic enzyme genes. Mutations in these genes will used to assess the impact of surface layer composition on the abscission process. These studies will shed light on the synthesis of protective surface layers in abscission zones.

Characterization of SPL4 role in drought stress and trichome development in alfalfa

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The impacts of climate change are expected to increase the demand for crops resistant to drought stress. Understanding how molecular mechanisms control plant response to stress is crucial to prevent losses in crop yield. Studies conducted in *Medicago sativa* (alfalfa) have shown that genetic modifications can induce plant mechanisms to increase stress tolerance. miR156 is a long non-codingRNA which negatively regulates gene expression at the post-transcriptional level. Trichomes are physical structures present on plant tissues that can aid plants in transpiration reduction. We studied the role of SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 4 (SPL4), a target of miR156, in alfalfa's response to drought and trichome development. We found that SPL4 regulates trichome development and drought stress response. Transgenic alfalfa plants with RNAi-silenced *SPL4* (SPL4-RNAi) have an increased trichome density under both control and drought conditions. In response to 14 days of withholding water, SPL4-RNAi plants had an increased root length, an increased water content in roots, shoots and leaves, and an increased water potential in the leaves when compared to wild-type plants. This study shows SPL4 has a role in trichome development and drought stress response, making it a potential target for modification to improve plant tolerance to drought stress.

Improving Tolerance to Environmental Stresses and Yield in Greenhouse-Grown Tomatoes by Engineering the Soil Microbiome

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Global food production is facing a number of challenges such as freshwater scarcity, shrinking arable land, and more frequent extreme weather events brought on by global warming. Some of these challenges could be alleviated by moving production of vegetables, such as tomatoes, from the field into greenhouses where environmental conditions can be controlled. However, greenhouse-grown tomatoes commonly suffer environmental stresses caused by temperature and humidity extremes, which lead to reduced plant growth and yields. Our project seeks to improve environmental stress tolerance and yield in tomato by introducing plant growth-promoting microbes (PGPM) to the plant microbiome. While a number of other studies have demonstrated the feasibility of this approach, they have predominately been performed under highly controlled conditions, so it is unknown if the effects of PGPMs are as robust under commercial greenhouse conditions. In this project, tolerance to environmental stresses and plant growth is being assessed in commercial tomato varieties grown in a pilot greenhouse under commercial greenhouse conditions following inoculation of a set of 86 different microbial species recently isolated by the Yergeau lab at INRS-CAFSB, which, based on preliminary data, improve growth in wheat seedlings. The mechanism(s) of action of the most promising microbes will be investigated through a combination of lab-based characterization and whole genome sequencing. In addition, the most promising microbes will be developed into commercial products by our industrial partner, Axter Agroscience Inc., a leading supplier of liquid fertilizers and biostimulants, and distributed nationwide for use by Canadian tomato growers.

Transcriptomic analysis identifies potential regulators involved in programmed cell death and remodelling of lace plant leaves (*Aponogeton madagascariensis*)

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The lace plant (*Aponogeton madagascariensis*) develops leaves with uniquely formed perforations using a developmentally regulated process called programmed cell death (PCD). The process of perforation formation in lace plant leaves is subdivided into several developmental stages: pre-perforation, window, perforation formation, perforation expansion and mature. The first three emerging “imperforate leaves” do not form perforations, while subsequent leaves form perforations via developmentally regulated PCD. PCD destined cells do not retain the antioxidant anthocyanin in spaces called areoles framed by veins of window stage leaves. Cells proximal to veins are called “NPCD cells” which retain red pigmentation from anthocyanin and do not undergo PCD. While the cellular changes that occur during PCD are well studied, the gene expression patterns driving PCD during leaf morphogenesis are unknown. We sought to characterize differentially expressed genes (DEGs) that mediate lace plant leaf remodelling and PCD. This was achieved using transcriptomics and comparing DEGs among different stages of leaf development, and between NPCD and PCD cells isolated by laser capture microdissection.

Cluster analysis of transcriptomes revealed pre-perforation and window leaves are characterized by higher expression of genes involved in anthocyanin biosynthesis. Mature and imperforate leaves upregulated genes associated with photosynthesis and negative regulation of PCD. PCD cells upregulated genes involved with hormonal transport, whereas NPCD cells possessed higher expression of anthocyanin biosynthesis. RNA-Seq revealed DEGs potentially involved in the unique

leaf development of the novel *A. madagascariensis* model. The data generated will be useful for functional experiments on lace plant leaf development and PCD in *planta*.

Determinants of substrate specificity in a catalytically diverse family of acyl-acyl carrier protein thioesterases from plants

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The catalytic diversity of the ACYL-LIPID THIOESTERASE (ALT) family of medium-chain acyl-acyl carrier protein thioesterases sets them apart from other, similar enzymes. ALTs can act on substrates of varied oxidation states, producing medium-chain (6-14 carbon) fatty acids, 3-hydroxy fatty acids, and methylketone precursors. While ALT enzymes have biotechnological potential as sources of these industrially valuable compounds, little understanding of what dictates their widespread substrate preferences currently limits their biotechnological use. Through targeted mutagenesis guided by computational modelling, we identified the first known determinants of ALT substrate specificity, including a six-amino-acid motif that dictates oxidation state preference, a positively charged surface patch that influences specific enzyme activity, and a region that can give rise to unnatural chain-length selectivity profiles when modified. These findings represent an advancement towards being able to engineer mutant ALTs with product profiles suited to specific biotechnological purposes, and also provide insight into the evolutionary origins and potential biological roles of ALTs.

Characterization of SPL12 role in regulating root architecture, nodulation and nitrogen fixation in *Medicago sativa*

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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. Similar to most other phenotypic traits, root system architecture is controlled at the molecular level by many genes, some of which were recently identified, including some coding for transcription factors from the SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL) family. We previously showed that transgenic *Medicago sativa* (alfalfa) plants overexpressing *microRNA156* (*miR156*) show increased nodulation, nitrogen fixation and longer roots. At least sixteen SPL genes including *SPL12* are targeted for silencing by microRNA156 in alfalfa. Thus, association of each target *SPL* gene to a trait or set of traits is essential for developing molecular markers for alfalfa breeding.

To determine the role of *SPL12* gene 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* silenced 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. The present findings suggest that SPL12 regulates root development and nodulation, as well as in nitrogen uptake and assimilation pathways.

miR156/SPL network negatively regulates aluminum stress tolerance in Medicago sativa

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Aluminum (Al) toxicity is a serious environmental stress facing global crop production in acidic soils. Alfalfa is the most extensively cultivated legume forage crop worldwide, necessitating development of crops tolerant to abiotic stress. Alfalfa improvement by conventional breeding is limited due to its polyploidy and allogamous reproduction, but new possibilities have arisen with the introduction of microRNAs as a breeding tool. microRNA156 (miR156), is highly conserved in plants and functions by downregulating SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (*SPL*) transcription factors. At least sixteen *SPL* genes are targeted for silencing by miR156 in alfalfa. SPLs further regulate the expression of a network of downstream genes that affect plant physiology and development through binding to gene promoters at a consensus DNA sequence known as the *SPL* Binding Domain (SBD). In this study, we determined the function of miR156 in regulating aluminum tolerance by investigating the phenotypic changes associated with altered expression of miR156. For this, we used three miR156 overexpression alfalfa plants to investigate miR156 function at the phenotypic level. We plan to conduct transcriptomic analysis of alfalfa roots to identify *SPL* genes and other downstream genes that are regulated in response to Al stress. Phenotypic analysis revealed that alfalfa plants with increased expression miR156 level had inhibited root growth and plant height under Al stress. Transcript analysis revealed that SPL13 is differentially regulated in response to Al stress. The current findings suggest that miR156 OE negatively regulates alfalfa's response to Al by inhibiting root growth and plant height, potentially by regulating SPL13.

Live Imaging of Leaf Spongy Mesophyll Morphogenesis and Microtubule Organization

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Leaf spongy mesophyll cells form an interconnected network of branched cells and intercellular spaces to maximize the surface area available for light capture and photosynthetic gas exchange. To investigate the morphogenetic events leading to cell separation and branching in *Arabidopsis thaliana*, we used mesophyll-specific promoters to facilitate imaging of mesophyll cell shape and microtubule (MT) organization over multiple spatiotemporal scales without interference from the overlying epidermal cells. We show that cells enlarge by selective expansion of cell wall regions in contact with intercellular spaces. Cell–cell contacts remain relatively fixed in size, forming the termini of interconnecting branches. Surprisingly, classic schizogeny (de-adhesion of neighboring cells) is relatively infrequent, being related to the local topology of cell junctions during early expansion. Intercellular spaces cue the position of stable MT bundles, which in turn promote efficient dilation of intercellular spaces and cell branching. Our data provide insights into mesophyll morphogenesis and MT organization and lay the groundwork for future investigations.

Constitutive defense potential (secondary metabolites) against the wide-spread herbivore *Lymantria dispar* L. across different conifer genera

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The Canadian boreal forest (25% of the world's intact forests), mostly composed of coniferous species, provides ecosystem services worth CDN$93 billion per year to Canada. However, conifers could increasingly become threatened by the gypsy moth, *Lymantria dispar L*., an invasive polyphagous defoliator feeding on more than 600 plant species. While oak (*Quercus* spp) is known as one of its preferred hosts, more recently, conifers such as Douglas-fir (*Pseudotsuga menziesii*) and lodgepole pine (*Pinus contorta*) were also found to represent appropriate hosts. Thus, it is urgent to gather in-depth information and expand screening to recently unsuspected hosts such as conifers to predict the potential damage to tree species at risk. In this study, we investigated and compared secondary metabolite profiles (phenolics and terpenes) for ten conifer species, encompassing multiple genera, economically important to Canada. Using needle-extracts analyzed on LC- and GC-MS and focusing on constitutively expressed metabolites, we found remarkable differences among conifers. Specific phenolic glucosides such as astringin (3-β-D-glucoside of piceatannol) and pungenin (3,4-dihydroxyacetophenone glucoside) were found in Norway spruce (*Picea abies*), white spruce (*Picea glauca*) and black spruce (*Picea mariana*) at significant quantities while in low concentration or absent among other studied conifer species. Further, in Norway spruce, white spruce, and Eastern hemlock (*Tsuga canadensis*), we found terpene compounds, such as caryophyllene, bornyl-acetate, and camphene as potential host defense metabolites of interest. These results, coupled with genomic information on gypsy moth's capability of detoxifying defensive secondary compounds of these conifers, can be used to predict future threat of invasiveness.

Defining Optimum: Growth Conditions Affect Heat Stress Resistance in the Antarctic Extremophile Chlamydomonas sp. UWO241

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Antarctica is home to many unique and endemic species that thrive under extreme conditions. Isolated 17 m below a permanent ice sheet at Lake Bonney (Antarctica) the psychrophilic green alga *Chlamydomonas* sp. UWO241 (hereafter UWO241) natural habitat is perennially stable with low temperatures (~5°C), low light irradiance (>11 µmol/m2s1), and high salinity (700 mM). Despite living at such extreme conditions, it has been shown that UWO241 grows faster and has greater photosynthetic efficiency at higher temperatures (10-15°C), salinities 70 times lower than it experiences in nature (10 mM), and light intensities a magnitude brighter (100 µmol/m2s1). This work challenges the classically held belief that organisms are at their optimum when they are experiencing the fastest growth rates. It proposes instead that an organism's ability to resist external stresses must also be included when determining optimal conditions. I examined UWO241's heat stress resistance and suggest that UWO241 is better adapted to low light and high salinity conditions. Algal cultures grown under 4°C conditions and subsequently transferred to 24°C (lethal temperature) survived the longest when grown under the combined conditions of low light (13 µmol/m2s1) and high salinity (700 mM) despite having very slow growth rates. The mechanism behind this unexpected response is still being investigated. Glycerol is known for its cryoprotectants an osmoregulant properties and this work shows that it accumulates in both light and salt stress conditions.

Mutagenesis of a plant P450 involved in MIA biosynthesis in *Catharanthus roseus* results in two distinct enzymatic functions.

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Catharanthus roseus produces over 100 monoterpenoid indole alkaloids (MIAs), which belong to a large class of nitrogen-containing secondary metabolites with diverse biological activities. The most abundant MIAs in roots belong to the aspidosperma class of MIAs and include hörhammericine, derived from (-)-tabersonine, and (+)-echitovenine, derived from (+)-vincadifformine. The cytochrome P450 (+)-vincadifformine 19-hydroxylase (V19H) converts (+)-vincadifformine to (+)-minovincinine that is *O*-acetylated to (+)-echitovenine, while (-)-tabersonine-19-hydroxylase (T19H) converts (-)-tabersonine and its derivatives, lochnericine and (-)-vincadifformine, to (-)-19-hydroxytabersonine, hörhammericine, and (-)-minovincinine, respectively. Since V19H displays 82 % amino acid identity with tabersonine 3-oxygenase (T3O) that converts (-)-tabersonine to (-)-tabersonine 2,3-epoxides in leaves, homology-based models of V19H were generated to understand why this P450 accepted (+)-vincadifformine rather than (-)-vincadifformine or (-)-tabersonine as substrates for hydroxylation. Docking experiments using the best V19H model showed that both (+)- and (-)-enantiomers of vincadifformine can occupy the V19H active site, which was proven experimentally by demonstrating that (-)-vincadifformine is a reversible competitive inhibitor of V19H. Models of T3O and T19H were created and superimposed on the model of V19H to investigate amino acid compositions in their binding sites and four amino acid residues were different in the conserved binding site of T3O and T19H. V19H mutants were generated with either single, double, or four-point mutations and all mutants retained their ability to convert (+)-vincadifformine to (+)-minovincinine. All single and double mutants were unable to convert (-)-tabersonine or (-)-vincadifformine to other products, while the four-point mutant gained T3O-activity enabling it to convert (-)-tabersonine to tabersonine 2,3-epoxide.

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