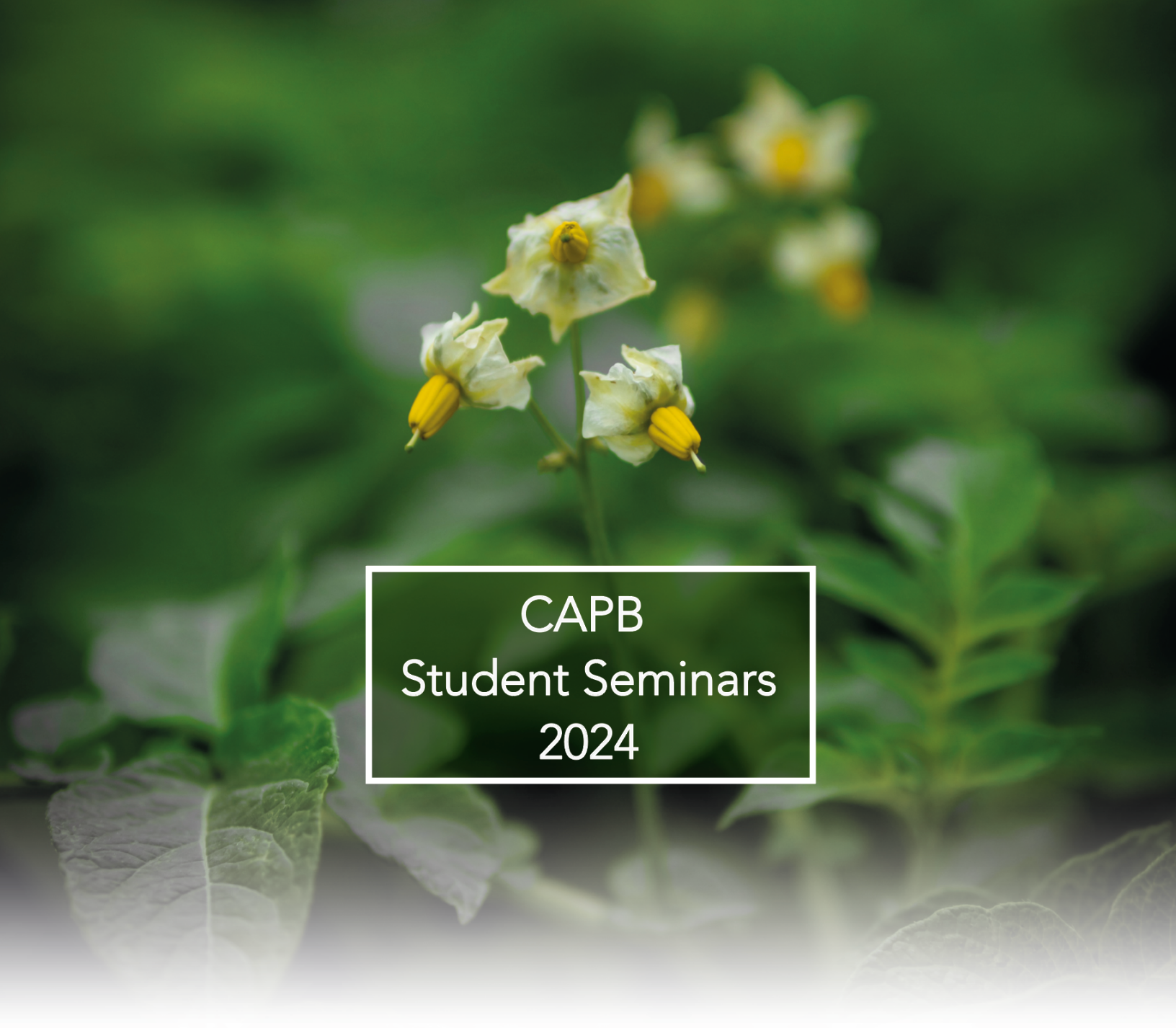

CAPB Student Seminars 2024

PROGRAM

March-April 2024

CANADIAN ASSOCIATION FOR PLANT BIOTECHNOLOGY
CAPB/ACBP

CAPB Student Seminars 2024

Dear Plant Biologists,

The 2024 Canadian Association of Plant Biotechnology (CAPB) Student Seminars is on its way! Presentations will be held on Thursdays at 3:00 PM EST/EDT (Montreal time), except for our last session that will take place on Wednesday, April 24th.

The talks will take place over 8 weeks on the Zoom platform. To attend, please follow this link:

https://ulaval.zoom.us/j/64743693623?pwd=aDZpQkpGOWYzWnNrbGhPbHJUbEtldz09

Prizes

CAPB Excellence Prizes will be awarded to the best speakers throughout the series! Be there to support your colleagues!

- 1st prize – 700$
- 2nd prize – 500$
- 3rd prize – 300$

How long?

Each talk will last around 20 minutes, followed by 5 minutes for questions.

Evaluation committee

Presentations will be evaluated by a committee composed of grad students, postdocs and researchers. The talks will be recorded to ensure consistent evaluations from one speaker to another when/if (a) committee member(s) were to be absent.

Researchers and professors: We believe that your presence and participation will enrich the discussions and interactions among attendees, and we invite you to attend the webinar series as observers. As leading researchers and educators in the field, your valuable insights and feedback will be greatly appreciated and will help foster a vibrant academic community. We count on you to boost your troops and encourage your lab members to attend our seminars!

We look forward seeing you at the CAPB Student Seminars 2024 and learning more about your research.

Thank you for your continued support to our association.

Sincerely yours,

Justin Boissinot, CAPB Student and Postdoc Representative
On behalf of the CAPB Student Seminars Committee (Justin B., C. Charron, J. VanderBurgt)

Schedule

All weekly sessions will start at 3:00 PM EST/EDT (Montreal time)

March 7

3:00 PM EST : Omar Abdelwahab, Université Laval

March 14

3:00 PM EDT : Alistar Moy, Laurentian University

March 21

3:00 PM EDT : Fanfan Li, McGill University
3:30 PM EDT : Hodan Halane, Western University / AAFC (LRDC)
4:00 PM EDT : Sameena Alam, University of Alberta

March 28

3:00 PM EDT : Ladan Ajdanian, Université Laval
3:30 PM EDT : Sean Robertson, University of Manitoba
4:00 PM EDT : Ritesh Kumar Yadav, University of Manitoba

April 4

3:00 PM EDT : Adrian Monthony, Université Laval
3:30 PM EDT : Louie Cris Lopos, University of Lethbridge/AAFC Morden R&D Center
4:00 PM EDT : Madeline Lehmann, University of Alberta / AAFC Lethbridge R&D Center
4:30 PM EDT : Janani Radhakrishnan, University of Alberta

April 11

3:00 PM EDT : Anirup Sengupta, University of Manitoba
3:30 PM EDT : Udaya Subedi, University of Alberta
4:00 PM EDT : Sajjad Sobhanverdi, Université du Québec à Trois-Rivières

April 18

3:00 PM EDT : Amir Danesh, Université du Québec à Trois-Rivières
3:30 PM EDT : Emediong Etukudo, University of Saskatchewan
4:00 PM EDT : Mohammed Musthafa Mukthar, University of Alberta

April 24

(exceptionally on a Wednesday)

3:00 PM EDT : Jared Bento, University of Manitoba
3:30 PM EDT : Jessica Samson-Tshimbalanga, Université Laval
4:00 PM EDT : Rajbir Kaur, University of Manitoba

ABSTRACTS

Performance analysis of conventional and AI-based variant callers using short and long reads

Omar Abdelwahab, Université Laval

March 7th, 3:00 PM EST

The accurate detection of variants is essential for genomics-based studies. Currently, there are various tools designed to detect genomic variants, however, it has always been a challenge to decide which tool to use, especially when various major genome projects have chosen to use different tools. Thus far, most of the existing tools were mainly developed to work on short-read data (i.e., Illumina); however, other sequencing technologies (e.g. PacBio, and Oxford Nanopore) have recently shown that they can also be used for variant calling. In addition, with the emergence of artificial intelligence (AI)-based variant calling tools, there is a pressing need to compare these tools in terms of efficiency, accuracy, computational power, and ease of use. In this study, we evaluated five of the most widely used conventional and AI-based variant calling tools (BCFTools, GATK4, Platypus, DNAscope, and DeepVariant) in terms of accuracy and computational cost using both short-read and long-read data derived from three different sequencing technologies (Illumina, PacBio HiFi, and ONT) for the same set of samples from the Genome In A Bottle project. The analysis showed that AI-based variant calling tools supersede conventional ones for calling SNVs and INDELs using both long and short reads in most aspects. In addition, we demonstrate the advantages and drawbacks of each tool while ranking them in each aspect of these comparisons. This study provides best practices for variant calling using AI-based and conventional variant callers with different types of sequencing data.

Decrypting molecular mechanisms involved in counteracting copper and nickel toxicity in Jack Pine (*Pinus banksiana*) based on transcriptomic analysis

Alistar Moy, Laurentian University

March 14th, 3:00 PM EDT

The remediation of copper and nickel afflicted sites is challenged by the different physiological effects imposed by each metal on a given plant system. *Pinus banksiana* is resilient against copper and nickel, providing an opportunity to build a valuable resource to investigate the gene expression responding to each metal. The objectives of this study were to 1) Characterize the transcriptome of *P. banksiana* exposed to nickel and copper and 2) Assess the differential gene expression of in nickel resistant in comparison to copper resistant genotype to identify mechanisms specific to each metal. The Illumina platform was used to sequence RNA that was extracted from seedlings treated with each of the metals. The transcriptome analysis of *P. banksiana* seedlings exposed to nickel or copper allowed us to gain a deeper insight into the distinct genetic response of this species to excess copper and nickel. There were 449 differentially expressed genes (DEGs) between copper RG and nickel RG at a high stringency cutoff, indicating a distinct pattern of gene expression toward each metal. Annotation of the top upregulated genes in copper RG compared to nickel RG identified genes and mechanisms that were specific to copper and not to nickel. NtPDR, AtHIPP10 and YSL1 were identified as genes associated with copper resistance. Various genes related to cell wall metabolism and photodamage mitigation were identified and included genes encoding for HCT, MPG, polygalacturonase and a blue copper protein. Annotation of the top downregulated genes in copper RG compared to nickel RG revealed genes and mechanisms that were specific to nickel and not copper. Various regulatory and signaling related genes associated with the stress response were identified and included UGT, TIFY, ACC, Dirigent protein, Glyoxyalase I. Additional research is needed to determine the specific functions of signaling and stress response mechanisms in nickel resistant plants.

Mutant medicine: a gene discovery pipeline for Madagascar periwinkle

Fanfan Li, McGill University

March 21st, 3:00 PM EDT

Catharanthus roseus, commonly known as Madagascar periwinkle, is a medicinal plant that produces an abundance of monoterpenoid indole alkaloids (MIAs), notably the anticancer compounds vinblastine and vincristine. While the canonical pathway leading to these drugs has been resolved, the regulatory and catalytic mechanisms controlling many lateral branches of MIA biosynthesis remain largely unknown. Here, we describe an ethyl methanesulfonate (EMS) *C. roseus* mutant (M2-117523) that accumulates high levels of MIAs. The mutant exhibited stunted growth, chlorotic leaves, and a lesion-mimic phenotype. Metabolic profiling revealed that lesions are enriched with over 25 MIAs, including several lateral branch compounds, which are absent or in low abundance in the wild type. Notable among the upregulated MIAs was akuammicine, a promising medicinal target and a weak opioid agonist with a preference for the non-addictive **κ**-opioid receptor. This unique metabolic shift was associated with a higher metabolic flux at the base of the pathway and a significant upregulation of biosynthetic and regulatory genes. Prominently upregulated genes include the transcription factors WRKY1, CrMYC2, and ORCA2, and the biosynthetic genes STR, GO, and Redox1. The cellular morphology of the lesions was also analyzed using confocal microscopy, revealing cell death at the mesophyll level and progressive degradation of chloroplasts in the mutant. Furthermore, dark treatment induces lesion formation in our mutant, linking the LMM phenotype with a possible mutation in the light-harvesting complex. A comparative transcriptomics study is underway to identify candidate genes involved in lesion formation and the associated metabolic shift. Co-expression and network analyses are being used to unravel further the link between a potential mutation in the light-harvesting complex and the shift in MIA biosynthesis, specifically akuammicine. Further study of this mutant will contribute to understanding akuammicine biosynthesis and regulation, with applications in guiding plant and heterologous engineering for medicinal uses.

Development of *in situ* passive sampling of pea root exudate chemical signals: insight into their role in response to Aphanomyces root rot disease

Hodan Halane, Western University / AAFC (LRDC)

March 21st, 3:30 PM EDT

The yield and quality of pea is facing drastic reduction globally due to Aphanomyces root rot (ARR) disease caused by the oomycete, *Aphanomyces euteiches*. ARR disease results in an estimated $100 million in annual pea yield loss worldwide, and $20 million just within the Canadian prairies. Current management options for the disease are very limited, making the release of cultivars with optimal resistance high in priority. Root exudates are involved in multitude of functions including plant-microbial signal exchange and release of defense-related compounds, known as specialized metabolites. Effective sampling of root exudates is crucial for understanding plant-microbe interactions. However, traditional sampling methods are often invasive, whereby a significant interference with the plant roots occur. In this study, we present the novel implementation of coated blade spray (CBS), a solid-phase microextraction-based technology, for in situ passive sampling root exudate, that is more ecologically relevant. The metabolite profiles of pea root exudates were investigated using CBS and compared to traditional methods using Liquid Chromatography-High Resolution Mass Spectrometry. Our results showed CBS method to have comparatively similar isoflavonoid abundances as traditional methods. To assess the effectiveness of CBS as root exudate passive sampling method, temporal targeted metabolomics was performed using roots exposed to *A. euteiches* zoospores. The phytoalexin, pisatin, was detected and relatively quantified. The levels of pisatin were found to be significantly impacted by the exposure of the roots to *A. euteiches*. Correlation between the days post-inoculation and pisatin was assessed giving insight into the effectiveness of the plant's innate immune response over time. CBS demonstrated to be effective in temporal passive sampling with minimal disruption to the plant interface by reducing the removal of matrix. The implementation of the proposed sampling method offers valuable tool for studying plant-pathogen interactions mediated by root exudate, facilitating the advancement of sustainable disease management strategies.

Over-expressing miR408 to enhance photosynthetic efficiency and climate change resilience in alfalfa

Sameena Alam, University of Alberta

March 21st, 4:00 PM EDT

The global population is projected to reach nearly 9.8 billion by 2050, which will pose a challenge for food security. Meeting this demand will not only require efforts to reduce food waste and establish equitable food distribution, but will also necessitate increased agricultural productivity. Unfortunately, yield gains across many crop species have stagnated in recent years, and improving photosynthetic efficiency could be pivotal for enhancing their yield potential. MicroRNA408 (miR408) has been shown previously to act as an important positive regulator of photosynthesis in several plant species, largely through the down-regulation of various genes encoding copper-containing proteins. In this project, my aim is to elucidate the function of miR408 in the context of photosynthesis in alfalfa, which is one of Canada's most valuable forage crops. MiR408 over-expression vectors were generated and successfully introduced into alfalfa using Agrobacterium-mediated transformation. To confirm the over-expression of miR408 in transgenic plants, both conventional and stem-loop quantitative reverse transcription PCRs (qRT-PCRs) were performed. The cleavage of putative miR408 target genes identified using in silico analyses will be validated using 5' RLM-RACE, and RNA-Seq will also be carried out to further our understanding of the transcriptional changes incurred through the modulation of miR408. Ultimately, the long-term goal of this research is to identify genes regulated by miR408 in alfalfa, which when knocked out using CRISPR/Cas9 could enhance photosynthetic efficiency and biomass production, thus contributing to global food security.

Optimizing ex-vitro one-step RUBY-equipped hairy root transformation in drug- and hemp-type cannabis

Ladan Ajdanian, Université Laval

March 28th, 3:00 PM EDT

Cannabis (*Cannabis sativa* L.), once concealed by the veil of prohibition, is now emerging as a versatile and promising plant species, riding the wave of recent legalization. This transformation has unlocked unprecedented opportunities for both medical research and industry growth, positioning cannabis on a trajectory to reach a projected market size of USD 444.34 Billion by 2030. Despite the plant's capability to produce more than 545 potentially bioactive secondary metabolites, its legal categorization in Canada, the USA, and Europe hinges on the concentration of a solitary cannabinoid, Δ9-tetrahydrocannabinol (THC), found in female flowers. Beyond the major cannabinoids (THC and cannabidiol (CBD)), cannabis synthesizes around 150 additional cannabinoids referred to as minor and/or rare cannabinoids. In recent decades, hairy root (HR) culture, an established method facilitated by *Agrobacterium rhizogenes*-mediated transformation techniques, has gained significant attention by academic research teams, biotechnology companies and pharmaceutical industries as a convenient and viable approach to produce target metabolites due to its rapid growth and stability in terms of both biochemistry and genetics. Here, we optimized an ex-vitro one-step HR transformation of the RUBY system in cannabis, shedding light on its potential applications in secondary metabolite production. Drug-type seedlings exhibited the highest hairy root induction, increasing by 58.8% compared to hemp-type seedlings. Also, among 3 different strains (A4, K599, ARqual) that we used, the A4 strain consistently demonstrated the highest transformation efficiency (75%) irrespective of genotype, while the ARqual strain yielded the lowest one (8.33%). In conclusion, even though in vitro HR transformation of hemp-type cannabis using *A. rhizogenes* has been documented previously, our study presents the first ex vitro one-step transformation in cannabis. Compared to the in vitro method, our ex-vitro method offers simplicity, speed, and reduced contamination risk, making it an optimal choice for the efficient production of secondary metabolites using CRISPR/Cas system in cannabis.

Exploring the drought response of developing rice leaves at single cell resolution

Sean Robertson, University of Manitoba

March 28th, 3:30 PM EDT

Single-cell RNA-sequencing has allowed for the in-depth characterization of transcriptome differences between plant cell types and states and during the events of cell fate specification. However, there are currently few studies investigating the effects of abiotic stressors on the transcriptomes of developing cells. Here, we used single nuclei RNA-sequencing to characterize the transcriptomes of rice leaves developing in long-term drought conditions. Through this analysis, we captured the transcriptomes of a developmental gradient of cell lineages for each of the major tissue systems, from their origin as dividing cells in the shoot apical meristem, through differentiation, to mature cells in epidermal, mesophyll, and vascular tissues. We also identified drought responsive genes within different cell types and states. We show that there is extensive heterogeneity with respect to the number of drought responsive transcripts, and the magnitude of response in the transcriptomes of different cells. This knowledge can guide rational engineering of crops for enhancing drought tolerance by highlighting transcripts of low abundance or in small subpopulations of cells.

Genome-Wide Association Studies for winter hardiness and Fusarium head blight resistance in winter durum wheat

Ritesh Kumar Yadav, University of Manitoba

March 28th, 4:00 PM EDT

Canadian winter wheat breeding programs prioritize Fusarium head blight (FHB) disease resistance and winter hardiness in their efforts to mitigate yield and quality losses due to FHB epidemics and winterkill. The joint Genome-Wide Association Study-Genomic Selection (GWAS-GS) approach has shown great potential for deciphering the genetic basis of complex traits, facilitating more accurate prediction of breeding values and enhancing genetic gains for difficult-to-phenotype traits and complex traits such as FHB resistance and winter hardiness. There was only a single winter durum cultivar (OAC Amber, 2010) registered in Eastern Canada. At present, no winter durum wheat candidates are available for testing in Canadian prairies. The broad objective of our study is to foster the development of durum wheat germplasm with enhanced winter hardiness and FHB resistance utilizing cutting-edge genomic tools and approaches and ultimately facilitate the development of field-ready winter durum cultivars for Canada in future. In order to identify valuable genetic variants for FHB resistance and winter hardiness, several GWAS models such as mixed linear model (MLM), compressed MLM, enriched compressed MLM, and, fixed and random model circulating probability unification (FarmCPU) will be employed. Our project uses an assembled panel of ~250 winter durum germplasm including pure lines from Canada, Europe, and USA and winter hexaploid wheat x durum cross derivatives. The assembled winter durum panel was phenotyped in 2021-22 and 2022-23 and is being phenotyped at multi-location in 2023-24 (Winnipeg, Carman, and Ottawa) for FHB resistance and winter hardiness. Further, the accessions were genotyped for genome-wide markers using Genotyping-by-sequencing (GBS). We will provide an update about the progress of this ongoing study, to identify quantitative trait loci (QTL) for FHB resistance and winter hardiness.

Gas and Grass: Molecular Interplay of Ethylene and Sexual Plasticity in Cannabis

Adrian Monthony, Université Laval

April 4th, 3:00 PM EDT

Cannabis (*Cannabis sativa* L.) is a dioecious plant species, meaning it has separate male (XY) and female (XX) individuals. The factors affecting sex determination in cannabis have not been widely explored, however ethylene has been shown to play a crucial role in this process. Despite being well understood in many plants, the molecular control of ethylene biosynthesis and signaling has yet to be deciphered in cannabis. The present research delves into the putative biosynthesis and signaling mechanisms that underlie the influence of ethylene on sex expression in cannabis. Additionally, a straightforward assay designed to detect the triple response, a phenotype sensitive to ethylene, in seedlings is presented. By leveraging ortholog analyses and available transcriptomic data, a putative compilation of ethylene-related genes (ERGs) is assembled, serving as the foundation to model an ethylene biosynthesis and signaling pathway within cannabis. Transcriptome analyses revealed patterns of differential gene expression, which were identified as putative sexual plasticity ERGs. Finally, an ethylene producing solution (ethephon) successfully induced phenotypic changes in hemp and drug-type cannabis seedlings associated with ethylene sensitivity, providing a rapid assay for future ethylene insensitive mutant studies. Taken together these findings lay the groundwork for future studies aimed at better understanding the effect of specific ERGs on sex plasticity in cannabis.

Gene-editing for the Accelerated Improvement of Grain Yield and Leaf Rust Resistance in Wheat (*Triticum aestivum* cv. Fielder)

Louie Cris Lopos, University of Lethbridge; AAFC Morden R&D Center

April 4th, 3:30 PM EDT

Wheat is a staple crop that faces the challenges of plateauing yields and susceptibility to rapidly evolving pests and diseases, which threaten to compromise its ability to meet the growing human demand. Wheat improvement programs utilize genetic diversity from available germplasms to confer beneficial traits into elite wheat cultivars; unfortunately, the process is complex and lengthy, the genetic mechanisms of the desired traits are not yet fully understood, and oftentimes, the introgression of beneficial genes from wild relatives lead to linkage drag. CRISPR-Cas9 and Prime-editing are RNA-guided gene-editing technologies that can introduce mutations into a targeted part of the genome for the purposes of gene functional discovery, genetic diversity expansion, and crop improvement. In this ongoing study, we aim to improve the grain yield and leaf rust resistance of wheat cv. Fielder using CRISPR-Cas9 and Prime-editing technologies, respectively. In order to improve grain yield, we are knocking-out the orthologs of previously identified rice microRNA408 targets: OsUCL8 and OsUCL30, using CRISPR-Cas9. It was previously reported that the post-transcriptional regulator, microRNA408, downregulates the expression of genes that negatively affects photosynthetic efficiency and grain yield; its overexpression was shown to improve these traits in *O. sativa*, *A. thaliana*, and *N. benthamiana*. We generated plants with diverse insertion/deletion mutations that introduce premature stop codon in TaeUCL8 and TaeUCL30 coding sequence. These plants will be evaluated in terms of grain yield, expression of photosynthetic genes, and other agronomic traits such as heading time, productive tiller number, and number of heads. On the other hand, we intend to use an optimized prime-editing strategy to fix the susceptible alleles of the leaf rust resistance genes Lr21 and Lr34 which encode truncated, ineffective proteins in cv. Fielder. We will evaluate these prime-edited plants by characterizing their responses to the indoor inoculation of leaf rust causative pathogen, *Puccinia triticinia*.

Knockdown of Galacturonosyltransferase 4 (GAUT4) and Suppressor of Overexpression of CONSTANS 1 (SOC1) in Alfalfa as a Potential Means to Improve Biomass Yield and Digestibility

Madeline Lehmann, University of Alberta / AAFC Lethbridge R&D Center

April 4th, 4:00 PM EDT

Alfalfa (Medicago sativa) is the most widely cultivated perennial forage legume in the world. Its great economic importance in the beef and dairy industries is owed to its many favourable traits, including relatively high quality and yield, and symbiotic nitrogen-fixing capabilities. However, the projected increase in global demand for beef and dairy products and the loss of arable land to urbanization necessitate further improvement to productivity. Moreover, the inefficient conversion of plant biomass into animal products during rumen fermentation results in both economic losses and negative environmental impacts. Thus, digestibility provides another important trait for enhancement in this species. Previously, it was demonstrated that knocking down the expression of the pectin biosynthetic gene, GALACTURONOSYLTRANSFERASE 4 (GAUT4), in several plant species led to improvements in dry matter yields and extractability of cell wall sugars, possibly through alterations in cell wall linkages. Other studies have demonstrated that knocking down the expression of flowering time genes, such as SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), results in prolonged activity of vegetative meristems, leading to delayed flowering and improved biomass yield in several plant species. As such, our overall aim is to determine the effects of down-regulating these two candidate genes in alfalfa. Therefore, RNA interference (RNAi) vectors were designed to individually down-regulate expression of GAUT4 and SOC1. These vectors were introduced into alfalfa by Agrobacterium-mediated transformation, and transgenic GAUT4 and SOC1 knockdown genotypes were regenerated. These genotypes are currently being assessed for morphological alterations, including growth characteristics, vegetative biomass, and flowering time. Downstream, knockdown genotypes will be assessed for metabolomic and cell wall characteristics, as well as in vitro digestibility. If successful, these genes could serve as targets for the future molecular breeding of alfalfa to improve not only our ability to meet demand, but also the environmental sustainability of livestock production.

The potential role of dehydrins in response of *Arabidopsis thaliana* to clubroot pathogen (*Plasmodiophora brassicae*)

Janani Radhakrishnan, University of Alberta

April 4th, 4:30 PM EDT

Canola (*Brassica napus* L.) being one of the important Canadian innovations, contributed about CAD $14.4 billion in exports in the year 2022. One of the major biotic threats affecting canola production is the clubroot disease caused by an obligate parasitic protist, *Plasmodiophora brassicae* Woronin. It is a soil-borne pathogen consisting of a complex life-cycle of primary and secondary infection stages in the root hair and root cortex respectively resulting in gall formation and obstruction of water uptake and nutrient translocation. Although several clubroot resistance (CR) genes have been identified from different *Brassica* species, the rapidly evolving pathotypes warrant the identification of new sources of resistance. Dehydrins are genes belonging to the class II of Late Embryogenesis Abundant (LEA) protein family that are expressed during dehydration stress. Their intrinsically disordered nature enables membrane protection, enzyme cryoprotection and reactive oxygen species scavenging. There are limited studies conducted on the antimicrobial properties of dehydrins conferred by the lysine-rich conserved motif called K-segment. With this background, the long-term objective of this project is to determine if dehydrin genes could be viable candidates for improving clubroot resistance in canola. Ten dehydrin genes identified in the model plant, *Arabidopsis thaliana* would be used for the study. The differentially expressed Arabidopsis dehydrin genes due to clubroot infection would be identified using quantitative reverse transcription -polymerase chain reaction (qRT-PCR). The identified genes would be cloned into the pBinGlyRed3 binary vector containing a kanamycin-resistant selectable marker. Agrobacterium-mediated transformation of Arabidopsis (Col-0) would be used to obtain the overexpression lines of the selected genes. The transformed plants would be evaluated for tolerance to drought induced by 20% polyethylene glycol (PEG-8000) and stress caused by clubroot pathogen (in terms of disease index). The findings from this *A. thaliana* study might offer novel targets for increasing clubroot resistance in canola.

Genome-Wide Association Study (GWAS) for leaf rust resistance in winter wheat

Anirup Sengupta, University of Manitoba

April 11th, 3:00 PM EDT

Leaf rust, caused by *Puccinia triticina*, is a common disease of wheat that affects both yield and quality. Leaf rust resistance is an important trait that is evaluated in the registration of wheat varieties in western Canada and is an effective strategy for sustainable disease management. Lr genes that confer resistance to wheat leaf rust can be divided into seedling resistance genes that are effective at all developmental stages and are typically race-specific, adult plant resistance (APR) genes that are effective only at the adult plant stage and are race-specific, and APR genes that are non-race-specific. However, the genetic basis of this resistance remains unclear, particularly in Canada Western Red Winter (CWRW) wheat. The objectives of this research are to identify the quantitative trait loci (QTL) controlling leaf rust resistance using genome-wide association study (GWAS) and develop genomic selection (GS) models for improving leaf rust resistance in winter wheat. The study involves a wheat population (GWAS panel) of approximately 300 western Canadian winter wheat lines and 100 winter wheat lines from the USA, eastern Canada, and Europe. The GWAS panel was evaluated for leaf rust resistance in seedling tests with multiple *P. triticina* races. The GWAS panel was also tested for resistance in inoculated field trials in Winnipeg and Morden, Manitoba. Genotyping was done using the 40K wheat barley Infinium array and the 25K wheat Infinium array. Both Infinium arrays are suitable for the imputation of additional SNPs. From the preliminary GWAS, some significant QTLs associated with leaf rust resistance have been identified. Furthermore, the leaf rust and SNP marker data will be used to develop GS models for estimating leaf rust resistance in CWRW breeding germplasm. Improved knowledge of the resistance genes and DNA markers for selecting these genes will improve the efficiency of wheat breeding programs.

Transcriptional down-regulation of MsWOX13-2 in alfalfa enhances tolerance to waterlogging stress

Udaya Subedi, University of Alberta

April 11th, 3:30 PM EDT

Soil waterlogging events are predicted to escalate globally as a result of climate change, posing a significant threat to the sustainability of alfalfa and livestock production in the coming years. Plants employ sophisticated adaptive mechanisms to cope with abiotic stresses by reprogramming transcriptional networks through the modulation of transcription factors (TFs). WUSCHEL-related homeobox (WOX) TFs are known for their involvement in various developmental processes and abiotic stress responses; however, their role in waterlogging resilience has yet to be examined. In this study, we characterized the alfalfa MsWOX13-2 gene, which we found to be expressed preferentially in roots and differentially under waterlogging stress. While the RNAi-mediated down-regulation of MsWOX13-2 in alfalfa had no significant effects on growth or morphological characteristics under control conditions, under waterlogged conditions, MsWOX13-2 RNAi plants exhibited enhanced performance, as evidenced by a reduced impact of stress on morphology and greater survivability compared to empty vector (EV) control genotypes. In addition, MsWOX13-2 RNAi genotypes exhibited an apparent reduction in leaf chlorosis under waterlogging, which correlated with higher chlorophyll retention and maximum quantum efficiency of photosystem II (Fv/Fm), compared to EV genotypes. This reduction in stress symptoms may be linked, at least in part, with the fact that MsWOX13-2 RNAi leaves accumulated less malondialdehyde (MDA), which is a marker for oxidative stress, and displayed higher superoxide dismutase (SOD) activity. RNA-Seq analysis confirmed alterations in transcript levels of genes (DEGs) related to photosynthesis, antioxidant activities, anaerobic respiration, cell wall modulation, hormones and transcription factors. Taken together, our results indicate that MsWOX13-2 functions as a negative regulator of waterlogging stress response in alfalfa, providing a novel putative target gene for downstream gene editing and/or breeding efforts in this species.

Candidate enzymes for C-C phenol coupling reactions involved in Amaryllidaceae alkaloid biosynthesis

Sajjad Sobhanverdi, Université du Québec à Trois-Rivières

April 11th, 4:00 PM EDT

Amaryllidaceae alkaloids (AAs) are well-known specialized metabolites with versatile antitumor, anti-microbial, and anti-acetylcholinesterase activities. Cytochrome p450 enzymes (CYPs) catalyze the para-para', para-ortho', and ortho-para' C-C couplings of 4'O-methylnorbelladine, a key cycling step required to produce the diversity of AAs complex structures. Little is known about the specificity of the enzymes that perform the different couplings. This study aimed to characterize specific CYPs for each type of coupling in AA's pathway of *Hippeastrum papilio*, using yeast as an expression system. To this end, coding sequences of CYPs previously characterized in relative species were used as bait to search for candidates in the transcriptome of *Hippeastrum sp*. Those with more than 50% similarity to the baits were transformed into yeast (INVSc1). Enzymatic assays uncovered one transcript (CYP96T2) with high similarity (87%) to CYP96T1 from *Narcissus sp. aff. pseudonarcissus*, which specifically cyclizes the central intermediate 4'O-methylnorbelladine to produce noroxomaritidine (para-para coupling), a building block for hameanthamine-type AAs. In addition, phylogenetic analysis demonstrated that this oxidoreductase has a different oxygen-binding motif, A(Q/G)X(NTQ), compared to C-C coupling CYPs from other alkaloid producing species. There was also a positive correlation both in the leaves and in the bulbs between AA accumulation profile and relative expression level of the target transcript. Hypothetically, all CYPs for three types of C-C coupling enzymes are highly similar in AAs pathway, and active site residues that govern the coupling specificity will be investigated. This study will pave the way for identification of target genes implicated in galanthamine biosynthesis, which could allow the increase in production of this Alzheimer's disease's medicine.

Investigation of the aromatic amino acids branch pathway in the marine diatom *Phaeodactylum tricornutum*

Amir Danesh, Université du Québec à Trois-Rivières

April 18th, 3:00 PM EDT

Aromatic amino acids (AAAs), including phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp), are essential building blocks for proteins and participate in various physiological processes in all living organisms. The biosynthesis of AAA in plants serves a more multifaceted purpose, as they are precursors for a diverse group of specialized metabolites (e.g., alkaloids, flavonoids, lignins, and aromatic antibiotics) that have a profound impact on growth and development, reproduction, and defense against biotic and abiotic stresses. In addition, AAAs are of particular interest due to their potential roles as intermediates in the synthesis of many industrially important products.

Diatoms are a diverse group of microalgae known for their ability to fix carbon dioxide and produce significant amounts of biomass. *Phaeodactylum tricornutum* is genetically engineered successfully and is particularly appealing due to its small genome size, quick generation time, and ease of genetic manipulation. Therefore, *P. tricornutum* is a promising candidate for metabolic engineering and the production of pharmaceutically relevant metabolites and as a platform for "nitrogen-containing compounds biofactory" for the development of an economically viable bio-based process. For this reason, we aim to characterize and genetic engineer the AAAs pathway in this diatom. We have identified two putative *P. tricornutum* Chorismate mutases (CM), Phatr3_draftJ417, one of the enzymes of the AAAs pathway. The coding sequence was amplified from cDNA, cloned under the constitutive promoter 40SRPS8, and fused to the yellow fluorescent protein gene in both C and N terminal to analyze its production, localization and enzymatic activity in *P. tricornutum*. Meanwhile, we are predicting the structural model of PtCM enzymes, to target the allosteric sites that are negatively regulated by Phe and Tyr and activated by Trp. Our perspective could provide new insights in *P. tricornutum* AAAs pathways to develop further tools in this marine microalgae.

Understanding and manipulating seed size in *Camelina sativa* using CRISPR/Cas9 technology

Emediong Etukudo, University of Saskatchewan

April 18th, 3:30 PM EDT

Camelina sativa (Camelina, False-flax) is a short season oilseed plant with great potential in the biofuel and oil industries due to its unique fatty acid profile, relatively short life cycle (85 – 100 days) and ability to thrive on marginal lands with low production inputs. However, large scale production of *Camelina sativa* is a challenge due to its tiny seed size (thousand seed weight (TSW) is < 2 g). This necessitates the development of genotypes with larger seeds with no negative impact on seed oil quality or quantity. To improve *Camelina sativa* seed size, we identified new variation in *Camelina sativa* seed germplasm by screening 218 lines for seed size polymorphism using the Marvin Seed Analyzer. We also introduced allelic variation by using CRISPR-Cas9 to create mutation at target sites of Enhancer of DA1 (EOD1) gene and Mediator of RNA polymerase II 25 (MED25) genes. Both genes are known to be negatively implicated in the regulation of final seed size in plants (*Arabidopsis thaliana*). A multi-parent advanced generation inter-cross (MAGIC) population of 1,125 recombinant inbred lines was also developed over a two-year period for mapping the quantitative trait loci (qtl) for seed size trait in *Camelina sativa*. Analysis of seed size parameters for EOD1 and MED25 mutant genotypes shows a significant increase in TSW and seed area. EOD1 mutant genotype also showed an increase in plant height while MED25 mutants had a negative effect on plant height. Fatty acid profiling of seeds from mutant genotypes showed no significant variation from wild type seed oil content. Our results shows that EOD1 and MED25 genes are negatively implicated in the regulation of final seed size in *Camelina sativa*, and loss of function of these genes could result in plants with larger seeds.

CRISPR/Cas9-mediated genome-editing for enhancing the total shoot lipid content in alfalfa

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April 18th, 4:00 PM EDT

Lack of statutory lipid content in vegetative tissues of forages leads to higher methane emissions primarily due to the reactions of the methanogens in the fermentation process and higher intake by ruminants. While this can be overcome through the supplementation of lipids in the ruminants' diet, this is costly and impractical. Alfalfa (*Medicago sativa* L.) is a notable forage crop in terms of nutritional quality and export value; however, it contains only 2-4% lipid content in its aboveground vegetative biomass on a dry matter basis. As such, the aim of this study is to enhance total shoot lipid content (TSLC) in alfalfa by disrupting PEROXISOMAL TRANSPORTER 1 (PXA1) and SUGAR DEPENDENT 1 (SDP1) genes using CRISPR/Cas9-mediated genome editing. Three guide RNAs (gRNAs) were designed for both genes separately, which were then inserted into a background vector and introduced into alfalfa using *Agrobacterium tumefaciens*-mediated transformation. Gene editing frequency droplet digital PCR (GEF-ddPCR) assays and Sanger sequencing were used to confirm the existence of mutations at the target sites. Eight unique PXA1-edited genotypes have been identified with GEF ranging from 25% to 75% through ddPCR. Similarly, 12 SDP-edited genotypes with GEF ranging from 25% to 75% have been confirmed using ddPCR and T7E1 endonuclease assay. No mosaics were identified in any of the above-identified genotypes. Preliminary shoot lipid analyses indicated that PXA1-edited genotypes possessed a significant 33.94% - 11.83% relative increase in stem lipid content compared to wild-type controls. The application of this gene editing method to develop new germplasm with high TSLC could contribute to a reduction in greenhouse gas emissions from ruminant production systems in the future.

Genome-Wide Association and Genomic Selection for Oil and Fatty Acid Profile in Rapeseed (*Brassica napus* L.)

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April 24th, 3:00 PM EDT

The improvement of yield- and seed-quality-related traits is the overarching goal of rapeseed (*Brassica napus* L.) breeding efforts. Genome-wide association studies (GWAS) and genomic selection (GS) are important biotechnological methods facilitating the accomplishment of breeding objectives and significant improvements in breeding cycle efficiency. There are three main objectives in this study: 1) GWAS to identify quantitative trait loci (QTL) for five seed quality traits (overall oil content, erucic, oleic, linoleic, and linolenic acids), 2) evaluating GS accuracy in predicting rapeseed hybrid fatty acid profile components, and 3) evaluating the ""GS + de novo GWAS"" method proposed to improve GS prediction accuracy. This project analyzes *454 Brassica napus* genotypes (92 parents, 362 hybrids) grown over 48 site-years. All genotypes were genotyped via Brassica 60K Illumina SNP array. Across 24 unique GWAS analyses, consensus QTL were compiled, revealing 161 unique QTL, including 22 QTL for erucic acid. Several QTL coincide with candidate genes identified in literature. Novel QTL have also been identified for all five traits, warranting further investigation. The accuracies of nine GS models were compared in their response to adjusting population size and composition (five model training/validation populations) and marker set density (high, intermediate, and low densities), producing 135 unique analyses. Prediction accuracies range from 25.8% (oil content) to as high as 89.1% (linoleic acid content), exhibiting positive correlation to the degree of training population/test population relatedness, negative correlation to trait complexity, and no significant correlation to marker set density or model choice. Results indicate that GS is highly accurate in certain populations and traits, especially for erucic, linoleic, and linolenic acids. Accuracy of the "GS + de novo GWAS" method will be compared to conventional GS models. The accuracy of GS in these experiments is promising for the implementation of GS technology in future rapeseed breeding programs.

Effects of water deficit on recombinant antiviral vaccine accumulation in *Nicotiana benthamiana* cultivated hydroponically in NFT gutters

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April 24th, 3:30 PM EDT

Plant molecular farming refers to the use of plants as bioreactors for synthesis of heterologous recombinant molecules. The gene vector *Agrobacterium tumefaciens* is commonly used for transient expression of recombinant proteins in combination with *Nicotiana benthamiana* (Nb), but few studies have examined the effects of environmental growth conditions on plant host performance in this expression system. While the use of NFT gutters for large-scale production of Nb plants is advantageous for automation and biosecurity considerations, such a cultivation system alters the root environment and impacts plant water relations compared to pots or trays, which can affect recombinant protein accumulation. Moreover, drought stress prior to a pathogenic infection has been shown to improve plant tolerance to the biotic challenge. To better understand the physiological impact of water deficit on protein yield and growth of the host, different intermittent fertigation frequencies were tested and the agroinfiltration process dissected (non-infiltrated, MES, P19 and HA). Characterization of the plants' leaf biomass as well as stable C isotope (13C), phytohormones, and RT-qPCR analyses were performed. Our results show that a reduction up to about 70% in fertigation volume to induce water deficit conditions during the growing phase resulted in as much as 60% higher hemagglutinin yields (HA·g-1 leaf fresh weight), pointing to phenotypic and physiological changes in Nb plants. As expected, water deficit limited plant growth but the resulting leaf biomass was more resistant to agroinfiltration and expression processes as it displayed less necrosis than that of well-watered plants. A better understanding of the interactions between water stress and the expression of recombinant proteins will emerge from this work, possibly representing a case of cross-tolerance between abiotic and biotic stress.

High throughput gene editing using CRISPR-Cas9 system in *B. napus* to improve shattering tolerance

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April 24th, 4:00 PM EDT

Brassica napus, commonly known as canola, is a significant oil-producing crop facing challenges in yield losses due to pod shattering. In the western regions of Canada, where canola is a major agricultural crop, an average loss of approximately 6% of the total yield during harvest occurs whereas this loss can escalate to over 20% in certain fields. This study aims to employ the CRISPR-Cas9 system, a precise genome editing tool to target the IND, SHP, and ALC genes, which are crucial in governing seed shattering in *B. napus*. Traditionally, many strategies have been employed to improve shattering tolerance including interspecific hybridization, genetic modification, and mutation techniques but they come along with negative agronomic traits or chromosomal irregularities. Hence, it becomes crucial to utilize advanced technologies like CRISPR-Cas9 to modify multiple copies of the target gene simultaneously without resorting to random mutagenesis or transgenes. The CRISPR-Cas9 genome editing system can efficiently induce double-stranded breaks in the targeted region and has been developed to accurately eliminate unwanted agronomic traits in *B. napus*. This system has already been efficiently applied in rapeseed to knock out all homologs of many genes. This study aims to establish a poly-cistronic CRISPR-Cas9 system in *B. napus* using a Rice pre-tRNA^{Gly} gene in a CRISPR vector. This will help to streamline gene editing by delivering multiple sgRNAs under a single promoter. Moving forward, the wild-type plants of rapeseed are infected with this vector via a novel agrobacterium-mediated transformation method. Then, the T1 generation is screened for Cas9 insertion and phenotypically characterized for shatter tolerance, with promising mutants subjected to Sanger sequencing for identification of key mutations generated in the targeted genes. The generation of shatter-tolerant *B. napus* hybrids is particularly essential for use in public breeding programs as they will be royalty-free and without any licensing restrictions.