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Scientific Program

December 3rd, 2021

All times are in North America Mountain Time (Edmonton / Denver time)

9:00-10:15	**Welcoming Remarks and Plenary Talk** **Chair: R. Glen Uhrig** *(University of Alberta)*
9:00–9:15	**Welcoming Remarks:** **R. Glen Uhrig** *(University of Alberta)* **Marcus Samuel** *(University of Calgary)* – Vice-President, CSPB
9:15–10:15	**Plenary speaker: Prof. Dr. Iris Finkemeier** *(University of Münster, Germany)* Elucidating the roles of lysine acetylation in the regulation of plant metabolism
10:15–10:30	**[short break]**

10:30-11:30	**Concurrent session 1: Plant–environment interactions** **Chair: Stacy Singer** *(AAFC Lethbridge Research and Development Centre)*
10:30–10:45	**Stacy Singer** *(AAFC Lethbridge Research and Development Centre)* CRISPR/Cas9-mediated mutation of *MsSPL8* alleles in alfalfa leads to morphological changes and enhanced drought tolerance
10:45–11:00	**Devang Mehta** *(University of Alberta)* Quantitative proteomics identifies common and distinct responses to nitrogen, phosphorus, potassium, and sulphur deficiency in *Brassica napus*
11:00–11:15	**Kamrun Nahar** *(Sher-e-Bangla Agricultural University, Bangladesh)* Melatonin ameliorates salt-induced damage in rice improving the antioxidant defense and methyglyoxal detoxification systems
11:15-11:30	**Samson Osadolor** *(University of Alberta)* Comparative analyses of molecular responses in pines to infection by *Cronartium harknessii*

10:30-11:30	**Concurrent session 2: *Brassica* systems** **Chair: Amr Kataya** (*University of Calgary*)
10:30–10:45	**Wendy Lyzenga** *(University of Saskatchewan)* Characterization of a phloem-associated E3 ligase which functions in long-distance mineral nutrient homeostasis
10:45–11:00	**Kallum McDonald** *(University of Alberta)* Developing a rapid genetic screening platform from *Arabidopsis* to accelerate breeding for increased seed protein in canola
11:00–11:15	**Sabine Scandola** *(University of Alberta)* Systems-level phenotypic and molecular profiling of kale cultivars grown under LED lights
11:15-11:30	**Kumar Abhinandan** *(University of Calgary)* Rest in peace: The disputable role of ARC1 during self-incompatibility in *Brassica napus*

11:30-12:30	**Poster session 1**
12:30–13:15	**[lunch break]**

13:15-1:15	**Plenary talk** **Chair: Thu-Thuy Dang** *(University of British Columbia, Canada)*
13:15-14:15	**Prof. Dr. Pamela Soltis** *(University of Florida, USA)* Polyploidy and plant diversification
14:15-14:30	**[short break]**

14:30-15:30	**Concurrent session 3: Biochemistry and genetics** **Chair: Devang Mehta** *(University of Alberta)*
14:30–14:45	**Amr Kataya** *(University of Calgary)* Identifying the substrates of the peroxisomal PP2A holoenzyme
14:45–15:00	**Anh Nguyen** *(University of British Columbia)* Discovering and harnessing hydroxylase enzymes for chemo-enzymatic synthesis of anticancer compounds
15:00–15:15	**Matthew McConnachie** *(University of British Columbia)* O-methyltransferases in camptothecin biosynthesis
15:15-15:30	**Harleen Kaur** *(University of Alberta)* Gibberellin regulates starch metabolism in the developing seeds of *Pisum sativum* L.

14:30-15:30	**Concurrent session 4: Transcripts and technologies** **Chair: Lauren Erland** *(University of British Columbia)*
14:30–14:45	**Sean Robertson** *(University of Manitoba)* AutoSeq: An R Shiny app for fast, automated RNA-seq data processing
14:45–15:00	**Gamalat Allam** *(University of Western Ontario)* miR156/SPL network negatively regulates aluminum stress tolerance in *Medicago sativa* by targeting SPL13
15:00–15:15	**Udaya Subedi** *(AAFC Lethbridge Research and Development Centre)* Transcriptional down-regulation of *MsHB2* in alfalfa leads to distinct morphological changes and superior tolerance to waterlogging
15:15-15:30	**Dilini Atugala** *(University of Calgary)* Establishing an *in vivo* approach to identify interactions between plant RNA-binding proteins and their mRNA targets
15:30-16:30	**Poster session 2**
16:30–16:45	**[short break]**

16:45-17:00	**Student awards and closing remarks** **Douglas Muench** (*University of Calgary*) **– Western Region Director, CSPB**

ORAL PRESENTATION ABSTRACTS

9:00-10:15	Welcoming Remarks and Plenary Talk
9:15–10:15	**Plenary speaker:** **Elucidating the roles of lysine acetylation in the regulation of plant metabolism** Iris Finkemeier University of Münster, Münster, Nordrhein-Westfalen, Germany Chemical modifications on proteins enable cells to regulate protein functions in response to environmental cues by altering protein localization, molecular interactions or enzymatic activities. The lysine side chains within proteins is subject to several chemical modifications, such as the reversible acetylation, which removes the positive charge of the lysine residue, and which occurs in different sub-cellular compartments. Lysine acetylation is regulated by the antagonistic action of lysine acetyltransferases and deacetylases. While the function of lysine acetylation on nuclear histone proteins has been extensively studied, the role of non-nuclear acetylation has only started to be uncovered. Here I will present our latest research on this protein modification and its modifiers in plants
10:15–10:30	**[short break]**

10:30-11:30	Concurrent session 1: Plant–environment interactions
10:30–10:45	**CRISPR/Cas9-mediated mutation of MsSPL8 alleles in alfalfa leads to morphological changes and enhanced drought tolerance** Stacy Singer[1], Udaya Subedi[1], Kimberley Burton Hughes[1], Abdelali Hannoufa[2], Gaganpreet Kaur Dhariwal[1], Kazi Kader[1], Guanqun Chen[3], Surya Acharya[1] [1]Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, Alberta, Canada T1J 4B1, [2]Agriculture and Agri-Food Canada, London Research and Development Centre, London, Ontario, Canada N5V 4T3, [3]University of Alberta, Department of Agricultural, Food and Nutritional Science, Edmonton, Alberta, Canada T6G 2P5 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, alfalfa productivity is likely to be progressively constrained by drought in coming years due to climate change, and as such, there is a need to develop alfalfa germplasm with enhanced resiliency to water deficit conditions. 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 previously. However, due to negative public perception and regulatory constraints surrounding the use of transgenic crops, it is challenging to implement such a crop in the field. CRISPR/Cas9-based genome editing is an alternative breeding tool that yields germplasm bearing mutations that are indistinguishable from those achieved using conventional breeding approaches, and the resulting plants can be made transgene-free through segregation. In this study, we successfully targeted *MsSPL8* alleles using CRISPR/Cas9 in tetraploid alfalfa, and isolated genotypes with mutations in approximately 25%, 50% and 75% of *MsSPL8* alleles, respectively, in the first generation. Morphological alterations, such as early flowering, a reduction in leaf size and a decrease in internode length, were observed in edited genotypes, and their severity was often dependent on the number of alleles mutated. In addition, edited genotypes displayed enhanced resilience to drought conditions, and further research is underway to unravel the mechanisms driving this phenomenon in these plants.
10:45–11:00	**Quantitative proteomics identifies common and distinct responses to nitrogen, phosphorus, potassium, and sulphur deficiency in *Brassica napus*** Devang Mehta[1], Sabine Scandola[1], Ibrahim Khodabocus[1], R. Glen Uhrig[1] [1]University of Alberta, Edmonton, Alberta, Canada Appropriate soil nutrient management is critical for modern Canola (*Brassica napus L.*) varieties and hybrids to meet their yield potentials. Canola fields in Western Canada generally require the application of nitrogen, phosphorus, sulphur, and to a lesser extent, potassium fertilizers and deficiency in these

key macronutrients can result in severe growth phenotypes leading to significant yield losses. However, the molecular impact of nutrient deficiency, and especially, the overlaps between responses to various nutrient deficiencies is not yet well understood. Here we performed a comparative quantitative proteomics analysis of both shoot and root tissue harvested from soil-grown Canola plants experiencing either nitrogen, phosphorus, potassium, or sulphur deficiency. Our results show intriguing similarities in plant responses to deficiency in multiple nutrients. We also find very distinct proteome-level changes between shoot and root tissue of plants experiencing nutrient stress, suggesting the presence of highly organ-specific responses to nutrient deficiency. Our results pave the way for a more comprehensive understanding of the shared and distinct response mechanisms of plants to multiple essential nutrients.

11:00–11:15 **Melatonin ameliorates salt-induced damage in rice improving the antioxidant defense and methyglyoxal detoxification systems**

Kamrun Nahar[1], Kamal Uddin Ahamed[1], Masayuki Fujita[2], Mirza Hasanuzzaman[3]

[1]Department of Agricultural Botany, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh, [2]Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Miki-cho, Kita-gun, Kagawa 761-0795, Japan, [3]Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh

Melatonin having pleiotropic functions in regulating plant physiological functions can be an effective agent for mitigating the salt stress effects in rice plant. Present study investigated the role of melatonin (M_1= 50 µM Melatonin, M_2= 100 µM Melatonin) in improving the physiology of salt affected (S_1= 4 dS m^{-1} NaCl, S_2= 6 dS m^{-1} NaCl) rice plant (*Oryza sativa* L. cv. BRRI dhan67). Salt stress resulted in oxidative damage in rice plants in a dose-dependent manner which is indicated by high rise of H_2O_2 and malondialdehyde which occurred due to interrupted antioxidant defense system. Salt stress also resulted in the reduction of growth, impaired osmoregulation and K/Na homeostasis. High rise of methylglyoxal (MG) and altered activity of glyoxalase system enzymes pointed toward MG toxicity in salt distressed rice plants. On the other hand, exogenous melatonin application improved superoxide dismutase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase, catalase and glutathione peroxidase activity of salt affected plants, compared to salt affected plants without melatonin. Adjusting the antioxidant defense system and glyoxalase system melatonin lessened oxidative stress and MG toxicity, respectively in salt affected plants. Exogenous melatonin improved leaf relative water content and modulated proline level, reduced Na and improved K level of salt affected rice plants. According to the result of the present study, improved plant physiology was the outcome of exogenous melatonin application in salt affected rice plant which was due to the adjustment of osmoregulation, ion homeostasis, antioxidant defense and glyoxalase system.

11:15-11:30 **Comparative analyses of molecular responses in pines to infection by *Cronartium harknessii***

Samson Osadolor[1], Marion Mayerhofer[1], Rhiannon Peery[1], Janice Cooke[1], Chandra McAllister[2]

[1]Department of Biological Sciences, University of Alberta, [2]Department of Oncology, University of Alberta, Edmonton

Western gall rust (WGR) is a fungal disease affecting pines across North America. Attack by the causative agent, *Cronartium harknessii*, leads to the production of galls on stems and branches of plants. Gall development can lead to reduction and distortion in growth of plants, including mortality of juvenile trees. *C. harknessii* affects particularly lodgepole (*Pinus contorta*) and jack pine (*Pinus banksiana*). Quantitative resistance has been documented in both species, with jack pine being more resistant to *C. harknessii* than lodgepole pine.

Understanding the molecular mechanism conferring interspecies and intraspecies host differences in quantitative resistance to *C. harknessii* is of utmost importance for effective resistance breeding. To achieve this, high-performance liquid chromatography along with transcriptomic resources were used to examine differences in defense hormone and gene responses to *C. harknessii* between lodgepole and Jack pine, between more resistant (MR) and more susceptible (MS) families of lodgepole pine, and also between MR and MS families of jack pine.

Generally, rust fungi including *C. harknessii* are considered obligate biotrophs, hence we predicted that *C. harknessii* inoculation will trigger the biotroph-associated salicylate (SA)-mediated signalling network rather than the necrotroph-associated jasmonate (JA)-mediated signalling network in pines. Both hormone and gene expression profiling indicated that SA is not involved in the defense response to WGR and JA is induced by *C. harknessii* to suppress the host defense.

Our findings represent a significant step for genomic selection and genome-wide association studies to identify the genetic mechanism for resistance against *C. harknessii* in pines.

10:30-11:30	Concurrent session 2: *Brassica* systems

10:30–10:45 **Characterization of a phloem-associated E3 ligase which functions in long-distance mineral nutrient homeostasis**

Wendy Lyzenga[1], Leon Kochian[1], Byung-Kook Ham[2]

[1]Global Institute for Food Security, [2]University of Saskatchewan

Plant growth and development is impacted by many abiotic factors from both above the ground and below the ground. The plant vascular system, composed of xylem and phloem, mediates the long-distance movement of water, nutrients and signaling molecules. The phloem sieve tube system allows for the transport of photoassimilates from source to sink. However, analysis of phloem exudate with mass spectrometry has revealed the full complexity of phloem sieve tube system and its role as a major signalling highway for pathogenesis-related, hormones, and developmental signals. Proteomic analysis of phloem exudate from cucumber, pumpkin, and watermelon have revealed that 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 response to environmental change. We are characterizing the biological role of an E3 ligase identified in phloem exudate. Using Arabidopsis knockout lines, and over-expression lines, we have identified overlapping roles for this E3 ligase in phosphorus and iron homeostasis. In addition, we have investigated the impact of this E3 ligase on sucrose-mediated root phenotypes. These results suggest that this phloem-associated E3 ligase may integrate carbon distribution with phosphorous deprivation signals. This analysis provides insights into understanding the molecular determinants of phosphate-limited stress signaling and extends our knowledge to improve nutrient use efficiency in crops

10:45–11:00 **Developing a rapid genetic screening platform from *Arabidopsis* to accelerate breeding for increased seed protein in canola**

Kethmi Jayawardhane[1], Kallum McDonald[1], Gavin Chen[1]

[1]University of Alberta, Edmonton, Alberta, Canada

Canola (*Brassica napus* L.) is a major oilseed crop in the Canadian Prairies. After oil extraction, the protein- and cellulose-rich seed meal is mainly used as animal feed. There is research interest in increasing the digestibility and nutritional value of canola meal by partial diversion of carbon flow from cellulose to storage protein biosynthesis without compromising the seed oil content. However, the genetic characterization research needed to achieve this is time and labor intensive when done in canola directly. We propose creating a rapid screening platform using *Arabidopsis thaliana* L.*,* a fast growing relative of canola, to accelerate the genetic worked needed to reallocate seed carbon. We are using seed-specific RNAi-downregulation of Arabidopsis *CELLULOSE SYNTHASE 1* (At*CESA1*)*,* overexpression of *B. napus DIACYLGLYCEROL ACYLTRANSFERASE 1* (Bn*DGAT1*), and overexpression of several protein biosynthesis-related genes from Arabidopsis (At*AAP1*, At*UmamiT18*, At*AAT1*, and At*ASN1*) to test the suitability of seed carbon reallocation for increasing protein content. We have successfully obtained At*CESA1*-RNAi/Bn*DGAT1*-overexpressing lines with moderately reduced seed cellulose, maintained seed oil, and maintained or increased seed protein. These findings confirm the efficacy of the carbon reallocation strategy and transformation of these lines with protein gene overexpression vectors is underway. Once the lines protein overexpressing lines have been analyzed, this platform can be used for rapidly characterizing candidate genes involved in increasing canola protein. This platform will accelerate the fundamental genetic characterization research needed for breeders to develop canola with high seed protein and improved digestibility, creating value for farmers, animal agriculture, and canola export market.

11:00–11:15 **Systems-level phenotypic and molecular profiling of kale cultivars grown under LED lights**

Sabine Scandola[1], Richard Glen Uhrig[1]

[1]University of Alberta, Edmonton, Alberta, Canada

Kale (*Brassica oleracea* convar. acephala) are nutritious leafy greens that are consumed for their abundance of vitamins and micronutrients. Typified by curly, serrated or wavy leaves, there are a number of kale varieties, which are defined based on their leaf morphology and origin. Due to its nutritious nature, kale is an excellent candidate for vertical farming and controlled environment growth, however harvesting of kale for nutritional content demonstrated time-of-day sensitivities. Knowing that 1/3 of plant genes are impacted by the circadian clock, new studies on Brassicas show that knowledge of diel fluctuations can have a major impact on cultivation practices. Here, we selected nine kale

varieties for growth under standard LED light conditions and analyzed them using our real-time phenomics platform and a newly developed mass spectrometry acquisition workflow. Showing dynamic fluctuations in both the diel proteome and metabolome, this study aims to establish diel differences and systems-level connections between Kale species grown under precision LED lighting systems.

11:15-11:30 **Rest in peace: The disputable role of ARC1 during self-incompatibility in *Brassica napus***

Kumar Abhinandan[1, 2], Xingguo Lan[3], Neil Hickerson[1], Sara Far[1], Marcus Samuel[1]

[1]Department of Biological Sciences, University of Calgary, Calgary, Alberta, [2]2020 Seed Labs Inc, Nisku, Alberta, [3] Northeast Forestry University, College of Life Sciences, Harbin, China

Flowering plants utilize self-incompatibility (SI) as a genetic mechanism to prevent inbreeding and to promote outcrossing. In Brassicaceae stigmas, the S-locus regulates haplotype-specific receptor-ligand interaction that converges on an E3 ligase, ARC1, that is responsible for degradation of compatibility factors leading to a haplotype-specific pollen rejection response. However, the role of ARC1 during SI response has remained contentious within the scientific community for over 20 years. Through in-depth analysis, we have been able to show that highest expression of *ARC1* coincides with *SRK/SP11* expression during anthesis in Brassicaceae. We were also able to create *Brassica napus* ARC1 knockout plants using the CRISPR-Cas9 gene-editing platform. Multiple CRISPR edited ARC1 loss-of-function transgenic Westar canola lines were characterized for the edits and tested for their ability to reject incompatible pollen. In all these ARC1 knockout plants, SI was compromised as they readily accepted haplotype-specific self-incompatible *B. rapa S47* pollen. In contrast, the non-transgenic wild-type Westar stigmas strongly rejected the *B. rapa S47* pollen. To further validate the requirement of ARC1 for SI, we transferred the ARC1-KO allele by crossing the compatible Westar (SRK/SP11-47) to an incompatible W1 (SRK/SP11-910) cultivar. The F1 hybrids were further analyzed for compromised incompatibility, and CRISPR edits were confirmed through Sanger sequencing. The plants with traits homozygous for *SRK910* and ARC1 edits were tested for phenotypic stability of compromised incompatibility through multiple generations (F4) with no escapes. These observations strongly indicate that the presence of ARC1 is an absolute requirement for the manifestation of SI in Brassicaceae.

11:30-12:30 **Poster session 1**

12:30–13:15 **[lunch break]**

13:15-14:15 **Plenary talk**

13:15-14:15 **Polyploidy and plant diversification**

Pamela Soltis

Florida Museum of Natural History, University of Florida, Gainesville, Florida, USA

Polyploidy, or whole-genome duplication (WGD), has long been recognized as an important speciation mechanism in plants. However, WGD has biological effects that extend far beyond the generation of new species. WGD is a key integrator across levels of biological organization, with effects that range from the molecular and subcellular levels to those of the ecosystem and Tree of Life. The immediate impact of WGD is duplication of all nuclear genetic material, but over time, the component subgenomes become fractionated to yield a composite of duplicated and unduplicated loci. This loss of duplicate genes can begin to occur surprisingly quickly, in perhaps only a few generations. Through gene loss and shifts in gene expression, polyploid individuals originating from a single polyploidization event may become genetically and phenotypically unique, together forming a morphologically, physiologically, and/or ecologically polymorphic population, in contrast to classical views of allopolyploids as genetically identical and chromosomally fixed F1 hybrids. This array of genetic and phenotypic novelty may provide new variants that can potentially drive evolution in new directions, with consequences for the tempo of diversification at macroevolutionary scales. Case studies in Tragopogon (Compositae) will illustrate patterns of duplicate gene loss and shifts in gene expression in synthetic and natural allopolyploids of recent origin. On longer timescales, signatures of ancient WGDs in Compositae and across angiosperms are often associated with accelerated rates of species diversification, suggesting a causal role of WGD in the diversification of these clades. Although statistical support for co-localized WGD events and diversification rate shifts is low across all angiosperms, many individual WGDs appear to be associated with the origins of novel features and increased diversification, suggesting that features that arise via microevolutionary processes may translate into key innovations on macroevolutionary timescales.

14:15-14:30 **[short break]**

14:30-15:30	Concurrent session 3: Biochemistry and genetics

14:30–14:45 **Identifying the substrates of the peroxisomal PP2A holoenzyme**

Amr Kataya[1, 2], Sierra Mitchell[1], Marcus Samuel[1], Greg Moorhead[1]

[1]Department of Biological Sciences, University of Calgary, Calgary, T2N 1N4, Canada, [2]Department of Chemistry, Bioscience, and Environmental Engineering, University of Stavanger, Stavanger, 4036, Norway

Protein phosphatase 2A (PP2A) is a heterotrimeric complex comprising catalytic, scaffolding, and regulatory (B) subunits. In Arabidopsis, 17 regulatory subunits are present, which can lead to 255 possible PP2A holoenzyme combinations with the five catalytic and three scaffolding subunits. The regulatory subunits are essential for substrate specificity and localization of the complex and are classified into non-related B, B', and B'' families in higher plants. The PP2A holoenzyme is imported into plant peroxisomes by piggybacking on the B'θ subunit. Plants with the B'θ subunit knocked out are impaired in fatty acids mobilization after germination, underpinning the involvement of PP2A in maintaining fatty acid degradation. To understand how the peroxisomal PP2A holoenzyme functions, we identified its substrates using a yeast two-hybrid screen of an Arabidopsis soluble cDNA library and BiFC with fourteen selected phosphoproteins involved in fatty acid β-oxidation and peroxisome biogenesis. As a result, we identified ketoacyl-CoA thiolase, enoyl-CoA isomerase, PEX14, and E3 ubiquitin-protein ligase SINA-like as putative substrates of PP2A-B'θ. E3 ubiquitin-protein ligase SINA-like was revealed as a single target seven times in the Y2H and obtains a novel motif for PP2A binding. In-vitro protein-protein interactions, Y1H, and BiFC studies of multiple mutagenized E3 ubiquitin-protein ligase with B'θ confirm binding with independence of the novel motif. Future studies exploring the regulation of these substrates will reveal how the peroxisomal PP2A holoenzyme functions.

14:45–15:00 **Discovering and harnessing hydroxylase enzymes for chemo-enzymatic synthesis of anticancer compounds**

Anh Nguyen[1], Thu Thuy T. Dang[1]

[1]University of British Columbia, Kelowna, BC, Canada V1V 1V7

Some of the most effective chemotherapeutic agents come from nature. New and diverse therapeutic options for cancer treatments are always in high demand, and plant-derived natural products provide us with a vast treasure trove to this end. My project aims to develop a biotransformation approach for production of clinically important anticancer compounds as well as new-to-nature derivatives by exploring and discovering new biocatalysts from plants. Among biocatalysts with substantial implications in plant natural product metabolism, cytochrome P450 enzymes (CYPs) stand out with their ability to activate and derivatize substrates via oxidation with striking chemo-, regio- and stereo-selectivities. Such properties can be explored and exploited for the biosynthesis of bioactive compounds, expanding the much-desired chemical and bioactive space of pharmaceuticals. Multidisciplinary techniques, including bioinformatics, molecular cloning, and organic synthesis, were implemented to discover and characterize two new cytochrome P450 monooxygenases. This discovery enabled heterologous production of anticancer precursors in the baker yeast *Saccharomyces cerevisiae*. Up to 13 quinoline-type derivatives have been successfully synthesized by combining bio- and chemo-catalysts sequentially in the chemoenzymatic process. This research is a step forward in quinoline alkaloid biochemistry and leading to the economical and sustainable production of more active and water-soluble anticancer derivatives.

15:00–15:15 ***O*-methyltransferases in Camptothecin biosynthesis**

Matthew McConnachie[1], Thu Thuy T. Dang[1]

[1]University of British Columbia, Kelowna, BC, Canada V1V 1V7

10-Hydroxycamptothecin (10HCPT) and 10-methoxycamptothecin are 2 of the main bioactive compounds found in the medicinal plant Camptotheca accuminata (Decne). These compounds are precursors for the semi synthesis of the leading anticancer drugs Irinotecan and Topotecan as well as many other drugs in the trial phases. 10-Hydroxycamptothecin O-methyltransferase (10HCPT OMT) is an enzyme which is responsible for the methylation of 10HCPT. Previous work has shown that this enzyme is coopted from flavonoid metabolism and can methylate a wide range of flavonoids. We hypothesized this enzyme could methylate other hydroxylated camptothecin derivatives as well as other alkaloids. To further investigate methylation of camptothecin derivatives additional OMTs were identified using bioinformatics tools and screened. This presentation will discuss the identification and characterization of two additional OMTs against a variety of alkaloids

15:15-15:30 **Gibberellin regulates starch metabolism in the developing seeds of *Pisum sativum* L.**

Harleen Kaur[1], Jocelyn A. Ozga[1], Kosala D. Wadhuthanthri[1], Dennis M. Reinecke[1]

[1]Plant BioSystems Group, Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, AB, Canada T6G 2P5

Gibberellins (GAs) are an important class of plant hormone that control seed growth and development in pea. *PsGA3ox1* overexpression in the semi-dwarf cultivar 'Carneval' (TG1 line) increased bioactive GA_1 concentration and produced 10 to 23% larger seeds (particularly embryo) during the seed-filing development period (8 to 20 days after anthesis, DAA) and at maturity compared to the control C1 plants. In TG1 seeds from 14-20 DAA, the embryo showed greater growth and enhanced starch levels than did the C1 seeds. ADP-glucosepyrophosphorylase (AGPase) is a key rate-limiting enzyme that regulates starch biosynthesis in seeds. To understand the role of GAs in carbohydrate partitioning into the developing pea embryo, the enzyme activity and expression profile of AGPase was studied in C1 and TG1 seed coat and cotyledon tissues during the seed-filling stage. Starch begins to accumulate in the rapidly developing cotyledons at 12 DAA and increases with further development. Higher AGPase enzyme activity was associated with higher transcript abundance of *PsAGPS1* (the most abundant small subunit of AGPase from 12-16 DAA) and *PsAGPS2* (at 12 DAA) in the cotyledons of TG1 compared to C1. These data suggest that GAs can enhance starch accumulation in the cotyledons by upregulating AGPase activity at least partially through increasing *AGPase* expression.

14:30-15:30 Concurrent session 4: Transcripts and technologies

14:30–14:45 **AutoSeq: An R Shiny app for fast, automated RNA-seq data processing**

Sean Robertson[1], Olivia Wilkins[1]

[1]University of Manitoba, Winnipeg, Manitoba, Canada

Functional genomic assays like RNA-seq produce tens to hundreds of millions of reads. Preliminary processing of raw data, such as trimming adapters, aligning to a reference genome, or quantifying transcripts, is slow and computationally intensive. These processing steps, usually run on High Performance Computing clusters, require users to be familiar with shell/Bash scripting and job submission to workload schedulers. Here we present AutoSeq, an R Shiny app that allows users to build custom RNA-seq data processing workflows through a user-friendly web interface, allowing for quick pipeline setup without the need to manually download raw RNA-seq read files, reference, genomes, gene annotations, or tools. Users select alignment tools, parameters, and organisms, and AutoSeq exports Bash job scripts that can be run on Compute Canada clusters to produce a gene count matrix. Additionally, the AutoSeq scripts utilize highly parallelized job processing for optimal performance with high CPU efficiency. Notably, additional samples increase the run time incrementally, so the time saving improves as the experiment size increases. We ran 10 and 100 samples in series, which took ~4 and ~38 hours, respectively. In comparison, using AutoSeq scripts, the same samples were run in 12 and 30 minutes, respectively, emphasizing its usefulness for large sample sets. Finally, outputted gene count files can be uploaded for preliminary downstream analyses such as a principal component analysis or differential gene expression analysis directly within this app. AutoSeq is a powerful tool that allows beginner or advanced users to efficiently process large RNA-seq datasets with minimal manual work.

14:45–15:00 **miR156/SPL network negatively regulates aluminum stress tolerance in *Medicago sativa* by targeting SPL13**

Gamalat Allam[1], Yousef Papadopoulos[2], Mark Bernards[3], Abdelali Hannoufa[4]

University of Western Ontario, London, Ontario, Canada

Aluminum (Al) toxicity is a serious environmental stress facing global crop production in acidic soils. Al toxicity triggers oxidative damage, contributing to extensive losses in alfalfa, necessitating development of crops tolerant to this stress. 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. 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 then conducted qPCR analysis of alfalfa roots to identify *SPL* genes that are regulated in response to Al stress. Phenotypic analysis revealed that alfalfa plants with increased expression of miR156 had inhibited root growth, plant height, reduced number of branches, stem width, root and shoot biomass, internode length, and relative water

content 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, reduced number of branches, stem width, root and shoot biomass, internode length, and relative water content under Al stress by regulating SPL13.

15:00–15:15 **Transcriptional down-regulation of MsHB2 in alfalfa leads to distinct morphological changes and superior tolerance to waterlogging**

Udaya Subedi[1, 2], Gaganpreet Kaur Dhariwal[1], Kimberley Burton Hughes[1], Guanqun Chen[2], Surya Acharya[1], Stacy Singer[1]

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Drought poses a severe threat to alfalfa (*Medicago sativa* L.), which is a popular forage legume with relatively high yields and superior nutritional quality. As such, there is a critical need to develop alfalfa germplasm with improved tolerance to water deficit. *TELOMERASE ACTIVATOR 1* (*TAC1*), encoding a zinc finger transcription factor, was found previously to negatively regulate responses to various types of abiotic stress in Arabidopsis. In this study, a homolog of *AtTAC1* was identified in alfalfa, and RNAi genotypes were generated to examine its role in drought resilience. Under well-watered conditions, TAC1-RNAi genotypes with confirmed down-regulation of the target gene were found to exhibit higher fresh shoot weight, dry shoot weight, and root volume, as well as delayed flowering, compared to empty vector controls (EVs). TAC1-RNAi alfalfa genotypes also exhibited increased tolerance to drought, as evidenced by a significant reduction in the soil moisture content at which the plants wilted, as well as higher survivability and improved recovery after severe drought, compared to EVs. Moreover, TAC1-RNAi genotypes displayed a significant decrease in detached leaf water-loss compared to EVs, which corresponded with a significant reduction in stomatal density. Taken together, our results suggest that *MsTAC1* will be a useful target gene for inactivation using CRISPR/Cas as a means of producing drought tolerant, transgene-free alfalfa germplasm downstream.

15:15-15:30 **Establishing an in vivo approach to identify interactions between plant RNA-binding proteins and their mRNA targets**

Dilini Atugala, Douglas G. Muench
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RNA binding proteins (RBPs) have a central role in post-transcriptional regulation of gene expression. A recently developed "mRNA interactome" method has increased our confidence toward the identification of RNA targets of RBPs. This method involves *in vivo* UV crosslinking RBPs to their authentic mRNA targets, thereby resulting in covalently bound interactions that can be purified under denaturing conditions. Here we discuss the purification of the plant RNA-binding interactome from Arabidopsis roots. From *Arabidopsis thaliana* (At) roots, we captured 422 proteins, including 416 with high confidence that we have defined as the At-RBP set. Approximately 65% of these At-RBPs are bioinformatically linked with RNA biology, containing a diversity of canonical RNA-binding domains (RBDs). Similar to other model organisms, the plant RNA interactome consists of a large proportion of proteins with non-classical and unknown RBDs. We present validation data for these domain-categorized RBPs (classical, non-classical and unknown). This is followed by immunoprecipitation of atypical RBPs, followed by high-throughput sequencing of the bound RNAs. This research approach has provided us with a valuable tool to identify authentic RNA targets of RBPs and expands our understanding of the functional roles of RBPs in gene expression in plant cells.

11:30-12:30 Poster session 1

P1. The transcriptional co-repressor SEED DORMANCY 4-LIKE (AtSDR4L) promotes embryonic-to-vegetative transition in *Arabidopsis thaliana*

Milad Alizadeh[1], Bailan Lu[1], Ting Wu[2], Ryan Hoy[1], Emma Laqua[1], Zhizhong Gong[2], Liang Song[1]

[1]University of British Columbia, [2]China Agricultural University

The phase transition from seeds to seedlings requires a sharp change in the transcriptional landscape. Specifically, the inactivation of embryonic programs during germination is critical for enabling the establishment of seedlings. How the master transcription factors (TFs) of embryo development, such as LEC1 and ABI3, are repressed during seed germination remains elusive. In *Arabidopsis thaliana*, we found a novel transcriptional co-repressor, AtSDR4L, whose expression is induced by LEC1 and ABI3 in late embryogenesis, while its protein in turn represses *LEC1* and *ABI3.* The negative feedback loop formed by LEC1, ABI3 and AtSDR4L may be a mechanism for the shutdown of embryonic programs during post-embryonic growth. AtSDR4L directly represses the expression of *LEC1* and multiple hormonal genes, likely through G-box, GGACC, and A-repeat motifs in the regulatory regions. The reduced expression of *LEC1* and the hormonal genes in turn negatively affects the embryonic programs of lipid storage (nutrient reserve accumulation) and primary dormancy. However, no direct binding of AtSDR4L was associated with the remaining LAFL master TFs, namely ABI3, FUS3 and LEC2. The mutants of *Atsdr4l* exhibited embryonic identities in the seedlings similar to *LEC1* over-expressors and germination deficiencies in the seeds. The mutant phenotypes are dependent on both intrinsic and extrinsic cues. Our data implies pathway-specific regulation of AtSDR4L on different embryonic programs, and reveals wide-ranging potential of AtSDR4L as a transcriptional regulator during the embryonic-to-vegetative phase transition

P2. Data acquisition approaches in proteomics: addressing technological limitations for plant systems biology

Devang Mehta[1], Sabine Scandola[1], R. Glen Uhrig[1]

[1]University of Alberta

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.

P3. Development of a designer RNA-binding protein to target and regulate the metabolism of endogenous mRNAs in plant cells.

Adam Fox[1], Douglas Muench[1]

[1]Department of Biological Sciences, University of Calgary

PUF proteins are a conserved group of sequence specific RNA-binding proteins that bind to RNA in a modular fashion. The RNA-binding homology domain of PUF proteins typically consists of eight clustered Puf repeats. Plant genomes code for large families of PUF proteins, and these show significant variability in their predicted Puf repeat number and amino acid sequence identity. We reported the identification of a novel RNA consensus sequence for an Arabidopsis PUF protein that contains an atypical and expanded RNA-binding domain. The Arabidopsis PUM23 (APUM23) consensus sequence was ten nucleotides in length, contained a centrally located UUGA core element, and preferred binding cytosine at nucleotide position 8. These RNA sequence characteristics differ from those of other PUF proteins, as all naturally occurring PUFs that have been studied to date bind to RNAs that contain a conserved UGU sequence at their 5' end and lack specificity for cytosine. We engineered APUM23 to alter its RNA target sequence and are using the expanded structural backbone of APUM23 to determine its RNA target specificity *in vivo*. This research will test whether this designer PUF can be used to alter the metabolism of target mRNAs by fusing it to effector domains, such as RNases and translational enhancers.

P4. TOR story: Investigating a rrole for CLASP in TOR-regulated growth transitions

Sean Ritter[1], Geoffrey Wasteneys[1]

[1]University of British Columbia

The ability of plants to adapt to changing light conditions is essential for their survival. TARGET OF RAPAMYCIN (TOR) is a protein kinase activated by growth factors and inactivated by energy deprivation, thereby acting as a master regulator of metabolic pathways in eukaryotes (Dobrenel et al., 2016). While targets of TOR have been identified, including the brassinosteroid (BR) responsive transcription factor BRASSINAZOLE RESTISTANT 1 (BZR1), downstream effectors are less established (Zhang et al., 2016). A candidate for coordination of TOR activity with hormonal signaling and microtubule organization is CLIP-ASSOCIATED PROTEIN (CLASP), a microtubule-associated protein that undergoes changes in abundance in response to altered light and nutrient levels (Halat et al., 2020). In addition to microtubule-organizing activity (Ambrose et al., 2007; Ambrose et al. 2011), CLASP has been demonstrated to undergo transcriptional regulation via BZR1, while sustaining the BR receptor BRASSINOSTEROID INSENSIVE 1 (BRI1) and auxin transporter PIN2, at the plasma membrane through an interaction with SORTING NEXIN 1 (SNX1) (Ambrose et al., 2013, Ruan et al., 2018). As TOR activity has been shown to alter BZR1 and PIN2 accumulation (Zhang et al., 2016, Yuan et al., 2020), I propose that light and nutrient-based control of CLASP acts as a crucial output of TOR signaling. Preliminary experiments indicated the *clasp-1* null mutant is less sensitive to pharmacological inhibition of TOR. Through characterization of the relationship between TOR and CLASP, this work will provide information on how the nutrient-sensing capabilities of TOR are translated into altered root development.

P5. Micropropagation and Transformation of *Cannabis sativa* L.

Gregory Robinson[1], Igor Kovalchuk[1]

[1]Department of Biological Sciences, University of Lethbridge, Lethbridge, AB

Cannabis sativa L. is a medicinal plant that has been used for thousands of years, however, due to decades of prohibition and stigmatization, little research has been performed on propagation and transformation techniques. Micropropagation is an alternative crop reproduction method where plants can be aseptically propagated for *Cannabis* plant multiplication and enables gene editing to be performed. Various tissue culture protocols for *Cannabis* have been reported, however, reports have low efficiency, are controversial, or are limited in tissue types, cultivars, and plant growth regulators (PGR) tested. This study examines the regeneration and transformation potential of *Cannabis* leaves, petioles, internodes, and

nodes across *Cannabis* cultivars for use in CRISPR-mediated gene editing. *Cannabis* explants were sterilized using 0.6% sodium hypochlorite & 3% hydrogen peroxide and initiated on Murashige and Skoog agar plates with different concentrations of common PGRs to induce callogenesis, shoot induction, and rooting. Transformation of *Cannabis* was performed via EHA105 strain of *Agrobacterium tumefaciens* carrying the pCAMBIA1301 construct with *uidA* gene to test the transformation efficiency by visual inspection of blue staining via GUS assay. Statistical analysis was performed using a Chi-square test or one-way ANOVA followed by Dunnett's post hoc test. Callogenesis, shooting, and rooting of Cannabis explants was shown to be tissue specific, cultivar specific, and PGR specific. Transformation efficiency was dependent upon explant tissue type. With well-developed micropropagation and transformation techniques, *Cannabis* can be propagated with higher multiplication rates and CRISPR/Cas9 gene editing can be performed to produce new cultivars with optimized or novel traits..

P6. Effect of cannabidiol and cisplatin on colorectal cancer and normal colon cell viability

Viktoriia Cherkasova[1], Igor Kovalchuk[2], Olga Kovalchuk[2]

Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada T1K 7X8

Background. According to Canadian Cancer Statistics 2021 data, Colorectal cancer (CRC) is the third leading cause of cancer in Canada. The alarming statistics pushes scientists to search for more efficient therapeutic strategies against CRC with fewer adverse effects.

The research in our lab is concentrated on the effects of different breeds of *Cannabis sativa* extracts on cancer models, however, first we test single components to evaluate the substances that have highest levels of antitumor activity. Multiple preclinical studies showed that phytocannabinoid cannabidiol (CBD) had high cytotoxic effects on different CRC models, which was the topic of our recent experiments.

Objectives. In our study we tested changes of CRC and normal colon epithelial cell viability under cisplatin and CBD treatments.
Materials and Methods. The experiments were performed on three CRC cell lines (HT-29, HCT-116, and LS-174T) and human normal colon epithelial cells (HCEC). Cell viability changes were determined using MTT [3-(4, 5-dimethylthiazol-2-Yl)-2, 5-diphenyltetrazolium bromide] assay.

Results and Conclusions. All tested CRC cell lines showed significant decrease in cell proliferation capacity under CBD and cisplatin treatments. Interestingly, normal colon epithelial cells reacted to cisplatin (IC50 0.3mM) more drastically than to CBD (IC50 9mM). Our results suggested that normal colon cells may tolerate higher doses of CBD than cisplatin. Thus, CBD in therapeutic doses can have high cytotoxicity on CRC cells and almost no damaging effect on normal cells, which could potentially be implicated in the reduction of adverse effects of CRC therapy yet maintain the same levels of anti-tumor efficacy.

P7. Iron-deficiency associated metabolites in poplar as novel iron chelators for treating iron overload diseases in humans

Sarah Lane[1], Juergen Ehlting[1], Patrick Walter[1]

[1]University of Victoria

Iron is essential for plant growth, but is generally unavailable in many soils, especially alkaline soils. Plants have adapted strategies to increase iron uptake, including producing secondary metabolites in root tissues that can chelate iron directly. In humans, iron is typically limiting, but can accumulate to toxic levels in major organs as a consequence of diseases including cancer, thalassemia, and some forms of neurodegeneration, to devastating effect without treatment. Current treatments with chelators help to remove iron, but these have adverse effects with long term use. Novel chelators may be found among iron-associated secondary metabolites in plants.

Poplar plants were aeroponically grown under iron deficiency to stimulate iron-associated secondary metabolite production. Root tissue extracts. Extracts from iron-deficient plants produced more phenolic compounds and up to 4-

fold more iron-binding activity than those from iron-normal plants *in vitro*. Based on untargeted metabolomics, iron-deficiency responsive metabolites are diverse, and include a range of semi-polar, low molecular weight (140 – 500 m/z) compounds. Root extracts, deferoxamine (a clinical iron chelator), and chlorogenic acid (a phenolic plant chelator), are introduced to cultures of human monocytic cells grown under normal and chronic iron-overload conditions. Iron content was moderately reduced in iron-overloaded cells following treatment with root extracts from iron-deficient plants. Chlorogenic acid reduced iron equally well as deferoxamine, dropping to near normal iron levels with no appreciable impact on cell viability despite chronic iron exposure. These promising results warrant further investigation to include additional species with alkalinity tolerance.

P8. Effect Of phytocannabinoids on skin rejuvenation during aging and oxidative stress

Marta Gerasymchuk[1], Gregory Robinson[1], Olga Kovalchuk[1], Igor Kovalchuk[1]

[1]Department of Biological Sciences, University of Lethbridge, Lethbridge, AB

Background: *Cannabis Sativa* L. has been used over millennia for spiritual, medical, and industrial purposes. The *Cannabis* plant produces over 100 phytocannabinoids, including delta-9-tetrahydrocannabinol and cannabidiol. The therapeutic use of cannabinoids is effective in numerous pathological conditions such as fibrosis and skin disorders, however, there is a lack of scientific knowledge about the anti-aging and rejuvenation properties of cannabinoids.

Objective: to analyze the overarching patterns of replicative skin senescence and determine whether phytocannabinoids have anti-aging effects through the inhibition of inflammatory processes, activation of collagen production, preventing aging-related nuclear structure changes, and/or delaying the senescence of skin cells.

Methods: Human skin fibroblasts (CCD-1064Sk, ATCC) and foreskin fibroblasts (BJ-5ta, hTERT-immortalized cell line) were treated with hydrogen peroxide to reproduce cellular senescence, and subsequently treated with phytocannabinoids. Cellular and molecular mechanisms of aging were determined by β-galactosidase senescence assay, MTT colorimetric cell viability assay, western immunoblotting, reverse transcription-polymerase chain reaction, and nuclear DAPI staining.

Results: Fibroblasts exposed to hydrogen peroxide and treated with phytocannabinoids demonstrated significantly decreased molecular and cellular senescence markers than untreated cells. COL1A and COL3A, collagen genes associated with senescence, were downregulated. Nuclear staining showed significant structural changes of nuclei in aged fibroblasts compared to those treated with phytocannabinoids.

Conclusions: Phytocannabinoids exert an anti-aging effect on human skin fibroblasts and stimulate rejuvenation effects by increasing cell longevity, stimulating collagen production, and preserving nuclear structural integrity.

P9. Seed biopriming: A means to manipulate fatty acid composition in bajra (*Pennisetum glaucum* (L.)R.Br.)

Dr. Sunitha Parvathini[1], Prof. Aruna Lakshmi K[1]

[1]GITAM Deemed to be University

Abstract:
Seed priming with beneficial organisms assists in rapid germination, economic yields and tolerance to biotic and abiotic stresses in various crops. Experiments were carried out to verify seed biopriming influence on the ratio of ∑ UFA to ∑ SFA of bajra (*Pennisetum glaucum*). Our objectives were (i) to evaluate the influence of biopriming on the manipulation of fatty acid composition which reflects the quality of seedling or sprout and (ii) to assess whether seed priming process would improve or inhibit the rate and uniformity of germination.
Local and hybrid variety of bajra seeds were bioprimed with *Azospirillum* and *Azotobacter* species concentrations ranging from (2mg/ml to 50mg/ml) and the seedlings were evaluated for fatty acid profile. Priming increased unsaturated fatty acids and reduced saturated fatty acids. Palmitic acid reduced by 17% and 25.1% in hybrid and local varieties respectively. Conversely, priming increased linoleic acid by 20% and 43.2% and linolenic acid by 95.6% and

39.2% in hybrid and local varieties respectively. TU /TS ratio increased by 40.7% in hybrid and 54.1% in local varieties. Our results revealed that the parental fatty acid of the omega 6 series linoleic acid enhanced and was the most prominent component in both hybrid and local seed variety. Collectively, biopriming modified the n-3 fatty acid profile in bajra and also promoted speed and rate of germination. Linoleic and linolenic fatty acids are qualitative indicators of food. Thus, seed priming can be an effective option for producing high quality seeds leading to improved health.

P10. The role of protein phosphorylation in mediating the interaction between Arabidopsis thaliana Shewanella-like phosphatase 1 and alpha-carboxyltransferase

Lana Wong[1], Gregory Moorhead[1]

[1]University of Calgary

Reversible protein phosphorylation, the most common post-translational modification (1), is essential in mediating most cellular functions in living organisms. With recent advances in omics-based technologies and its applications to studying the phosphoproteome of particular organisms, many previously uncharacterized phospho-proteins, protein kinases and protein phosphatases have been identified. *Shewanella*-like phosphatase 1 (SLP1), a novel *Arabidopsis thaliana* protein phosphatase localized to the chloroplast, is a protein of interest as it is predicted to play an antagonistic role to the constitutively active chloroplast localized kinase, casein kinase 2 (CK2). Through a quantitative mass spectrometry based phosphoproteomics study carried out by previous members of the Moorhead Lab, many putative substrates of SLP1 were identified, one of them being acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha (alpha-CT). Alpha-CT is a subunit of acetyl-CoA carboxylase (ACC), an enzyme that catalyzes the first committed step of *de novo* fatty acid biosynthesis (2). ACC catalyzes the ATP-dependant irreversible carboxylation of acetyl-CoA to produce malonyl-CoA, an intermediate that plays a vital role in the regulation of fatty acid metabolism (2). Antibodies were generated against two different phosphorylation sites of ACC, as well as the whole protein. Peptide blocking experiments indicated that the antibodies were phosphospecific, showing little preference for dephosphorylated peptides. Using this tool, the phospho-status of alpha-CT in light and dark conditions can be studied to help elucidate the role of protein phosphorylation in controlling the function of ACC, as well as its role in mediating the interaction between SLP1 and ACC.

P11. Elongation of lower internodes and lowest pod height in soybean are regulated by gibberellin

Ankita Thapar[1], Anh Tuan Pham[2], Belay T. Ayele[3]

[1]Master student, [2]Research Associate, [3]Professor

Soybean production is affected by several biotic and abiotic factors, and also specific morphological factors that result in yield and economic losses. Low pod height, which is associated with short lower internodes, is among the major factors that causes substantial harvest losses in the production of some soybean cultivars. This study examined the role of gibberellin (GA) in regulating these traits, and our results showed that treatment with GA can enhance the elongation of lower internodes/heights of the lowest pod bearing nodes in a cultivar that normally bears its first pod at lowest height. To gain insights into the molecular mechanisms for variations in the elongation of the lower internode or height of lowest pod bearing nodes, this study investigated the expression patterns of genes encoding GA biosynthesis and catabolism enzymes including *GA20ox* and *GA3ox* and *GA2ox* genes in the lower internodes of genotypes that are characterized by contrasting lowest pod height at different developmental stages. The results of this study showed that differences in the expression patterns of the GA biosynthetic and catabolic genes across the genotypes studied. The GA biosynthetic genes *GA20ox2*, *GA20ox3*, *GA3ox2* and *GA3ox3* exhibited higher expression levels in the lower internodes of the genotype with a higher lowest pod height than the genotype with shorter lowest pod height. On the other hand, the GA catabolic genes *GA2ox1*, *GA2ox2* and *GA2ox8* were found to have lower expression levels in the lower internodes of the cultivars with higher lowest pod heights.

P12. Decoding the genetic network for seed protein accumulation in oilseed crops

Sara Far[1]

[1]University of Calgary

Proteins derived from Canola (*Brassica napus*) meal are rising in popularity as a sustainable source of plant-based protein in human and livestock diet. However, the growth of the canola protein industry is restricted by the low crude protein content in canola seeds. Optimization of seed constituents in canola is exceptionally difficult considering canola's importance as an oilseed cash crop; efforts to increase protein content cannot compromise the oil content in seeds. Thus, optimization of protein content in canola requires a thorough understanding of the genetics that control seed protein accumulation. Through working with the related model organism, Arabidopsis thaliana, we have been able to identify that overexpression of a seed-specific transcription factor leads to increased seed protein content. However, since the current regulatory landscape favours gene-edited plants over the transgenics, there is an immediate need for gene-editing solutions for increasing the transcription factor expression in seeds. Consequently, the present work involves the identification and characterization of several negative regulators of the transcription factor. T-DNA knockout mutants of the candidate negative regulators were evaluated and crosses were generated to determine the genetic relationships between the mutants and the transcription factor of interest. These negative regulators are currently being targeted in canola through various gene-editing approaches.

P13. Mammalian melatonin agonist pharmaceuticals stimulate rhomboid receptors in plants

Lauren Erland[1], Jillian Forsyth[1], Liubov Frolova[1], Adam Yasunaga[1], Winnie Pun[1], Isaac Li[1], Susan Murch[1]

[1]Chemistry, UBC Okanagan

Melatonin is a human neurotransmitter and plant signaling metabolite that perceives and directs plant metabolism but the mechanisms of melatonin action in plants are undefined. We hypothesized that roots have a melatonin-specific receptor and/or transporter. To test this hypothesis *Arabidopsis* seedlings were grown with melatonin pharmaceutical receptor agonists: Ramelteon and Tasimelteon, and/or antagonists: luzindole and 4-P-PDOT. Ramelteon was found both to mimic and competitively inhibit melatonin in plants. Due to the higher selectively of Ramelteon for the MT1 receptor type, a sequence homology search for MT1 in *Arabidopsis* identified the rhomboid-like protein 7 (RBL7). In physiological studies, *Arabidopsis* RBL7 mutants were less responsive to both Ramelteon and melatonin. Quantum dot imaging studies of Ramelteon effects on melatonin transport in roots revealed distinct and competition localization of the compounds. We propose that RBL7 is a receptor for melatonin that directs root architecture and growth in a mechanism that is responsive to environmental factors.

15:30–16:30 **Poster session 2**

P14. Control of Leaf Vein Patterning by Regulated Plasmodesma Aperture

Enrico Scarpella[1], Linh Nguyen[1]

[1]University of Alberta

How vascular networks form in multicellular organisms is a central question in biology. In animals, the formation of vascular networks requires cell migration and direct cell-cell interaction, both of which are precluded in plants by a cell wall that keeps plant cells apart and in place; therefore, vascular networks form differently in plants. How plants do so is unclear, but the current hypothesis proposes that *GNOM*-dependent auxin transport and signaling control vein patterning. However, unlike complete loss of *GNOM* (*GN*) function, inhibition of both auxin transport and signaling fails to prevent vein patterning, suggesting the existence of at least one more, *GN*-dependent vein-patterning pathway. Here, we combined cellular imaging, chemical induction and inhibition, and molecular genetic analysis to show that the residual, *GN*-dependent vein-patterning pathway depends on the movement of an auxin signal through plasmodesmata (PD). PD permeability is regulated during leaf development: permeability is initially high throughout the leaf, then lowers between vein and non-vein cells, but remains high between vein cells. Unregulated PD aperture leads to defects in auxin transport and signaling; in turn, inhibition of auxin transport or signaling enhances vein patterning defects caused by unregulated PD aperture. *GN* controls regulated PD aperture, and simultaneous interference with auxin transport, auxin signaling, and regulated PD aperture phenocopies complete loss of *GN* function. Therefore, we have identified all the main vein-patterning pathways in plants as well as an unprecedented mechanism of vascular network formation in multicellular organisms

P15. A proteome-level investigation into Plasmodiophora brassicae resistance in Brassica napus canola

Dinesh Adhikary[1], Devang Mehta[1], R. Glen Uhrig[1], Habibur Rahman[1], Nat N. V. Kav[1]

[1]University of Alberta

Clubroot disease of canola is caused by *Plasmodiophora brassicae* Woronin, a soil borne, obligate, biotrophic protist. This disease causes significant loss to canola yield and the continuous evolution of new pathotypes necessitates the development of new resistant canola cultivars. The identification of differentially abundant proteins (DAP) is an attractive approach to gain insights into the plant-pathogen interactions as well as in the future development of gene specific markers for use with developing clubroot resistant cultivars. In this study, *P. brassicae* pathotype 3 was used to challenge clubroot susceptible (CS) and clubroot resistant (CR) canolaseedlings. Root samples were collected at 7-, 14-, and 21-days post inoculation (DPI), protein samples were extracted, digested with trypsin and subjected to LC-MS/MS analysis. A total of 937 DAP in the CR line and 784 DAP in the CS line ($q < 0.05$) were detected upon challenge with the pathogen, across all time points investigated. DAPs identified revealed potential roles for calcium dependent signaling pathways in pathogen perception and the activation of defense related responses. In addition, proteins related to dehydrins, reactive oxygen species (ROS) biochemistry, thaumatin, and phytohormones were also identified among the DAPs. Interestingly, among the DAPs 74 putative proteins orthologues of CR genes and QTLs associated with 9 CR loci in four chromosomes including chromosome 3 and 8 were detected in our samples. In conclusion, our results have provided additional insights into the molecular mechanisms that may be involved in mediating resistance to *P. brassicae* in canola.

P16. Plant regeneration and cytological study of leaf calluses of pearl millet cultivars Vg272, IP8182 and ICP501.

Venkata Lakshmi Tetali[1], Krishna Rao Mantha[2]

[1]Science teacher, [2]Professor

The study was carried with the intention to discern the cytology of leaf callus and standardize the differentiation of calli of 3 cultivars of pearl millet (Pennisetum glaucum (L)R.Br.). Leaf explants of cultivars Vg 272, IP8182 and ICP501

were cultured in MS basal medium supplemented with 1-5 mg/l of 2,4-D and/ 5mg/l NAA. Among the 3 cultivars, 21-23% of leaf explants callused in all the media in 3-4 weeks. The initial callus was of two types (i) glistening white with mucilage and/ (ii) white friable callus. The friable callus initiated in MS+2.5 and 5 mg/l 2,4-D metamorphosed into compact nodulated callus in 12 weeks in the cvs Vg 272 and IP8182; in two weeks 10% of the Vg 272 compact calli from 2.5 mg/l differentiated into plantlets in MS basal medium. Vg 272 callus in NAA supplemented medium (5mg/l) was rhizogenic. The one month old calli raised in 5 mg/l 2,4-D revealed similar mitotic index of diploid and tetraploid cells (3.93% and 3.84%) in IP 8182 and (0.70 and 0.79) in Vg272 of ~ 1119 cells counted. The differentiated plantlets were diploid (2n=14) and tetraploid plantlets were not obtained. The study indicates that although diploid and tetraploid cells could be induced in 2.5mg/l 2,4-D media, the culture conditions for further differentiation of diploid and tetraploid plantlets appeared to be different in these two genotypes Vg 272 and IP8182 of pearl millet.

P17. Screening and identification of microorganisms to suppress clubroot disease symptoms in canola

Ananya Sarkar[1], Anna Kisiala[2], R. J. Neil Emery[2], Habibur Rahman[1], Nat N. V. Kav[1]

[1]University of Alberta, [2]Trent University

Clubroot disease caused by the soil-borne protist, *Plasmodiophora brassicae*, is a significant threat to canola production in Canada, resulting in 10-15% yield losses. Controlling this disease remains a challenge and we have explored the possibility of using microorganisms belonging to *Bacillus*, *Pseudomonas* and *Trichoderma* genera to suppress clubroot disease symptoms in canola. We screened 33 microorganisms belonging to these genera against four *P. brassicae* pathotypes for their disease suppressive potential under greenhouse conditions. Among these, 10 strains exhibited disease suppression efficacy and/or plant-growth promoting properties. Cellular (pellet) and cell-free (supernatant) fractions from these 10 strains were further evaluated for beneficial effects and, four strains were effective against three pathotypes, while six were effective against all four pathotypes ($p<0.05$). To determine whether secreted phytohormones, including cytokinins (CK) were responsible for the observed effects, we characterized the phytohormone profiles in the pellet and supernatant fractions of these strains using LC-HRMS/MS. Total CK levels (pmol/g) were higher in the supernatants than the pellets ($p<0.001$), with *cis*-zeatin (cZ) and isopentenyladenine (iP) being the most dominant. Among the strains evaluated, *Trichoderma virens* accumulated the highest levels of total CKs in the supernatant, and contained significantly higher levels of cZ in both fractions. This might give *Trichoderma* an advantage over all other beneficial strains, since cZ is a stable nucleobase and plays important roles in plant stress responses. Clubroot disease progression has been known to alter CK levels in the host, and microbes with signature CK profiles might be useful tools to combat this disease.

P18. Implementing a sustainable tree and plant production system: Floating field

Rodney Sidloski[1], Ali Baghdadi[2]

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Demand for tree, horticulture, and grain production worldwide resulted in hydroponic greenhouses and vertical gardens in recent decades but their relatively high cost has not been competitive with conventional plant production systems.

The invention of the floating tree and plant production and live storage system has facilitated a cost-effective alternative to a conventional greenhouse and outdoor nursery systems. The technology, apparatus, and process have been patented for all vascular and non-vascular plants for both the production, the multi-year live storage of tree seedlings and other perennial plants, and the extreme water savings that this system provides.

This system which in its simplest form utilizes outdoor natural water bodies to produce several million field ready tree or vegetable field ready seedlings per acre of water surface, eliminates costly greenhouse infrastructure, irrigation systems, heating and ventilation systems, nutrient feeding, ph balancing apparatus, and fungus control, all integral with greenhouse plant production.

The extreme water-saving potential for plant and tree production in arid and semi-arid regions facing water scarcity is noted. The low-cost live storage of trees and perennial plants in frozen water surfaces provides solutions for countries with sub-zero winter temperatures.

The floating field container root production system requires aggressive root development to ensure a cohesively

formed plug of root and soil for successful field transplanting throughout the growing season. The use of plant growth regulators can play an important role in promoting seedling root development in many hardwood trees which contain less natural rooting hormones.

P19. Down-regulation of MsTAC1 in alfalfa confers morphological alterations and improved tolerance to drought

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Drought poses a severe threat to alfalfa (*Medicago sativa* L.), which is a popular forage legume with relatively high yields and superior nutritional quality. As such, there is a critical need to develop alfalfa germplasm with improved tolerance to water deficit. *TELOMERASE ACTIVATOR 1* (*TAC1*), encoding a zinc finger transcription factor, was found previously to negatively regulate responses to various types of abiotic stress in Arabidopsis. In this study, a homolog of *AtTAC1* was identified in alfalfa, and RNAi genotypes were generated to examine its role in drought resilience. Under well-watered conditions, TAC1-RNAi genotypes with confirmed down-regulation of the target gene were found to exhibit higher fresh shoot weight, dry shoot weight, and root volume, as well as delayed flowering, compared to empty vector controls (EVs). TAC1-RNAi alfalfa genotypes also exhibited increased tolerance to drought, as evidenced by a significant reduction in the soil moisture content at which the plants wilted, as well as higher survivability and improved recovery after severe drought, compared to EVs. Moreover, TAC1-RNAi genotypes displayed a significant decrease in detached leaf water-loss compared to EVs, which corresponded with a significant reduction in stomatal density. Taken together, our results suggest that *MsTAC1* will be a useful target gene for inactivation using CRISPR/Cas as a means of producing drought tolerant, transgene-free alfalfa germplasm downstream.

P20. Quantitative Proteome and PTMome Analysis of Arabidopsis thaliana Root Responses to Persistent Osmotic and Salinity Stress

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Abiotic stresses such as drought result in large annual economic losses around the world. As sessile organisms, plants cannot escape the environmental stresses they encounter but instead must adapt to survive. 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 to d) lacking. In addition to protein abundance and phosphorylation changes, evidence suggests reversible lysine acetylation may also be important for abiotic stress responses. Therefore, to characterize the 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 protein abundance, detecting significant changes in 245, 35 and 107 total proteins, respectively. Comparison with available transcriptome data indicates that transcriptome- and proteome-level changes occur in parallel, while post-translational modifications (PTMs) do not. Further, we find significant changes in PTMs, and protein abundance involve different proteins from the same networks, indicating a multifaceted regulatory approach to prolonged osmotic and salt stress. In particular, we find extensive protein-level changes involving sulfur metabolism under both osmotic and salt conditions as well as changes in protein kinases and transcription factors that may represent new targets for drought stress signaling. Collectively, we find that 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 proteome levels

P21. 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.

P22. Physiological, biochemical, and transcriptomic responses to salinity stress in Medicago sativa

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Alfalfa (*Medicago sativa* L.) is a widely cultivated perennial forage legume with great importance in the livestock industry due to its adaptability, high quality, high yield, and low fertilizer needs. Although alfalfa is moderately salt-tolerant, soil salinity is a key factor that limits its productivity. Salinization continues to affect a large percentage of irrigated crop land across the world. Thus, the development of saline-tolerant crop cultivars is vital for maintaining agricultural sustainability. The aim of this study is to elucidate the physiological, biochemical, and transcriptomic responses of *M. sativa* cultivar 'Beaver' to salinity stress in order to enable the downstream identification of potential targets for the genetic improvement of salt tolerance in alfalfa. Physiological and biochemical characteristics were assessed under salinity stress and control conditions, and RNA-Seq was performed in order to identify transcripts that were differentially regulated between treatments. Salt-treated plants showed significant changes in physiological and biochemical characteristics, including a reduction in plant height, shoot number, internode length, fresh and dry shoot weight, and relative water content, as well as increased proline and malondialdehyde content, compared to controls. Furthermore, RNA-Seq revealed a number of differentially regulated transcripts, including transcripts involved in redox, abiotic stress, and regulation of transcription. The findings of this study advance our understanding of the response of alfalfa plants to salinity stress, and provide insight that may drive future genetic improvement of salt-tolerance in alfalfa.

P23. In vitro evaluation of host defense peptides' antifungal and cytotoxic activities, and evidence of a new adjuvant peptide

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Host defense peptides (HDPs) can inhibit microorganism growth at micromolar concentrations and reduce disease susceptibility when expressed in plants. HDPs are small, cationic peptides with antimicrobial and/or immunomodulatory activity. This study aims to evaluate five HDPs of plant origin: BnPRP1, Ib-AMP 1Q, P4650, Shepherin 1, and Sm-985, *in vitro* for antimicrobial and cytotoxic activity to identify candidates for ectopic expression in plants. Antifungal activity was investigated by incubating conidia with various concentrations and combinations of peptides for 24 h. Spores were considered inhibited if the emerging mycelium was less than twice its length. To determine HDP cytotoxicity, mesophyll protoplasts were incubated with peptides for 24 h and subsequently stained to identify viable cells. In order of decreasing antifungal activity, the three most effective peptides were Sm-985, Ib-AMP 1Q, and Shepherin 1. Currently, the most effective peptide combinations were found to be Sm-985 and either Ib-AMP

1Q or P4650 (MIC 20 µM). Interaction ratios showed synergy between P4650, which alone had no antimicrobial activity, and Sm-985, Ib-AMP 1Q, and Shepherin 1, suggesting that P4650 had antimicrobial adjuvant activity. Among all HDPs tested, only P4650 was toxic to plant protoplasts, with ≥96% inhibition at 100 µM. Further assays will investigate activity of these peptides, alone or in combinations with each other, against additional pathogenic fungi and bacteria, as well as their cytotoxicity towards mammalian cells and plant protoplasts. The results from this study will identify novel HDPs for engineering disease resistance in crops and contribute to improving food security.

P24. Nitrate-regulated glutaredoxins act as negative regulators of lateral root growth

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Nitrogen is an essential nutrient for plants and acts as a chemical signal causing major transcriptional and developmental changes. Recent evidence suggests that glutaredoxins (GRXs) may be involved in plant nitrogen signalling. GRXs are small redox enzymes found in all eukaryotes and are generally involved in disulfide bridge reduction. Class III GRXs are unique to plants, but most remain functionally uncharacterized. This study focuses on two class III GRX genes, *AtGRXS6* and *AtGRXC11*, that are strongly transcriptionally upregulated by nitrate in the soil. To study these GRXs, we generated knockout lines for *AtGRXS6* and *AtGRXC11* individually, as well as a double *AtGRXS6*/*AtGRXC11* knockout mutant, using CRISPR Cas9-mediated mutagenesis. We have identified six independent *AtGRXS6*, *AtGRXC11*, and *AtGRXS6/AtGRXC11* mutants with defined insertion and/or deletion mutation sites by DNA isolation, PCR amplification of the target gene, and sequencing. Additionally, we created *AtGRXS6* overexpression lines, and demonstrated that these transgenic lines showed ~10-80-fold increases in *AtGRXS6* transcript abundance by using real time RT-PCR. We are now determining the phenotypic effects of the knockout mutations or overexpression lines, with a specific emphasis on root system architecture and typical developmental responses to nitrogen in the soil. Early results suggest that overexpression of these two glutaredoxins results in a marked decrease in lateral root growth. The long-term goal of this project is to better understand plant nitrogen use efficiency so that synthetic nitrate fertilizers can be applied more effectively in agriculture. This will minimize over-application of nitrogen fertilizers, which has significant detrimental effects on the environment.

P25. Roles of Class III Glutaredoxins in Plant Nitrogen Use Efficiency

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Glutaredoxins (GRXs) are small oxidoreductase enzymes that reduce disulfide bonds in target proteins. Class III GRXs are exclusively found in land plants, but most remain functionally uncharacterized. We previously found a cluster of class III GRX genes (*AtGRXS3/4/5/7/8*) that are transcriptionally activated by nitrate. This study focuses on determining how the *AtGRXS3/4/5/7/8* gene cluster influences patterns of gene expression and overall plant nitrogen use efficiency (NUE). Two *AtGRXS3/4/5/7/8* knockout lines, one *AtGRXS8* overexpression line, and wild-type plants were grown hydroponically under different nitrate conditions. To identify changes in gene expression, real-time PCR was performed on plants that were nitrogen-starved for 26 hours and then supplied with 5 mM KNO_3 for either 4 or 24 hours. To determine NUE, plants were grown under low nitrate (0.4 mM KNO_3) or high nitrate (9 mM KNO_3) conditions and biomass, protein, and nitrate content were quantified. We found no consistent alterations in patterns of gene expression of the target genes in relation to nitrate. However, our knockout plants showed increased shoot:root ratio when grown under high nitrate, but no change in nitrate or protein content. Our *AtGRXS8* overexpression plants showed reduced shoot:root ratio when grown under both nitrate conditions and reduced shoot nitrate content, but no change in total protein. These results suggest that *AtGRXS3/4/5/7/8* may play a role in determining how plants allocate energy between their root and shoot systems in nitrogen-rich soils. Future applications of these findings could have agricultural impacts to reduce fertilizer runoff and eutrophication by increasing NUE in crops.

P26. Transcriptome responses to reduced water availability in rice

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Drought stress is one of the main constraints to global food production. Understanding molecular mechanisms underpinning tolerance to drought is a major goal of plant science research. However, studying drought in controlled conditions requires the establishment of standardized, and physiologically relevant drought conditions. Here we aim (1) to establish standardized drought stress protocols for transcriptome studies in rice, (2) to determine how plants respond to narrow changes in soil water potential at physiological and transcriptome levels, and (3) to determine the transcriptional interactions coordinating the response to nearly stable water stress. We grew rice plants in three soil water conditions representing well-watered, moderate drought, and severe drought conditions for 10 days. Our results show that the mild and severe drought treatments elicited distinct global transcriptomic changes. We show that there is a quantitative shift in the magnitude of the differentially expressed genes identified and that different functional classes were invoked in response to the two drought stress treatments. We also identified transcription factor networks underpinning the observed changes in gene expression. Our method offers a roadmap to understanding the dynamic adaptive changes underlying the response to prolonged drought stress. This study provides valuable insight into potential transcriptional programmes, and regulatory networks coordinating the response of rice plants to nearly stable drought stress conditions.