Western Regional Meeting

February 18-19, 2020

Coast Canmore Hotel

Canmore, Alberta

Program

Tuesday				
Time	**Room**			
9:00	Crocus	**Plenary**		**Plenary: AL Samuels**
10:00-10:30	Arnica	**COFFEE**		
10:30	Crocus	Kwon	Moonhyuk	Biochemical elucidation of psychoactive natural products, salvinorin and salvidivin, in *Salvia divinorum*
10:50		Morris	Jeremy	A single residue determines substrate preference in benzylisoquinoline alkaloid N-methyltransferases
11:10		Salama	Eman	Development of hairy root system in lettuce (Lactuca sativa) to assess CRISPR/CAS9 genome-editing.
11:30		Tasnim	Sifat	Functional Characterization of genes with potential roles in the biosynthesis of heartwood terpenoids in Western redcedar
11:50		Utomo	Joseph	Developing a microbial platform for investigating Taxol biosynthesis pathway
12:15	Arnica	**LUNCH**		
1:00	Crocus	Fortier	Colleen	Mountain pine beetle associated fungal pathogen-induced changes in phenolic biosynthesis of pine and the effects of water limitation
1:20		Alberts	Mitchell	Measuring the uptake and partitioning of isotopically-labelled naphthenic acids in willow root and shoot tissue
1:40		Hodgins	Connor	Biosynthesis of off-flavour metabolites in *Pisum sativum* (pea)
2:00		Cooke	Janice	Identifying the molecular basis of resistance to *Cronartium harknessii* , the causal agent of western gall rust, in lodgepole and jack pine
2:20- 2:35	Arnica	**COFFEE**		
2:40	Crocus	Amini	Safoora	The characterisation of transcriptome from different developmental stages of *Rafflesia cantleyi* floral buds
3:00		Fox	Adam	Development of a designer RNA-binding protein to target the metabolism of endogenous mRNAs in plant cells.
3:20		Toth	Ryan	Rhizobiales-like Protein Phosphatase 2 and Type One Protein Phosphatases interaction with the D Group Mitogen Activated Protein Kinases in *Arabidopsis thaliana*
3:40		Ro	Dae-Kyun	Developing multiplex genome-editing platforms in yeast (*Saccharomyces cerevisiae*) using a single gRNA-mediated CRISPR-Cas9
4:00		Atugala	Dilini	Establishing an in vivo approach to identify interactions between plant RNA-binding proteins and their mRNA targets
4:30-6:30	Arnica	**Poster session**		
Wednesday				
9:00	Crocus	**Keynote**	Yeaman	The genomic basis of adaptive and plastic responses to climate in conifers
10:00		Scandola	Sabine	Directing Development of Next-Generation Spectrally-Controlled Plant Growth
10:20		Harris	Neil	Can differences in metal specificity of durum wheat P_{1B}-ATPase 3 (TdHMA3) transporters explain the cadmium-specific phenotype of QTL *Cdu-B1*?
10:40		Belmonte	Mark	RNA interference protects Canadian crops against fungal attack
11:00	Arnica	**Coffee & Award Presentations**		
11:30	**Brown Bag and Field Trip**			

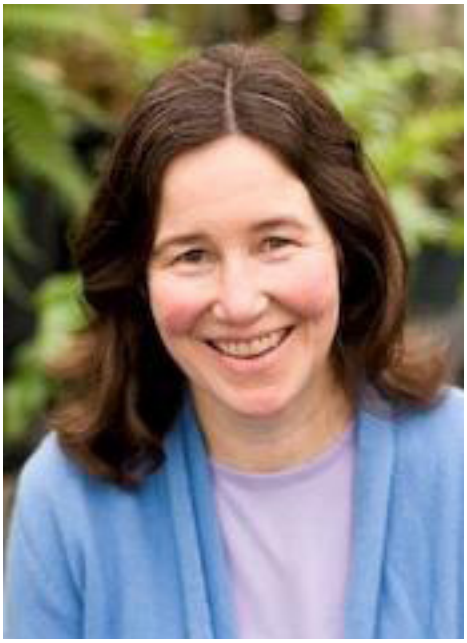
Posters
Abstracts starting on page 15

Poster	First Name	Last name	Title
1	Kristian	Caldo	Probing the biochemistry of benzylisoquinoline alkaloid biosynthesis for synthetic biology applications
2	Colleen	Fortier	Defining the window of phenological opportunity for spruce budworm herbivory of white spruce: determining whether lignin and epicuticular waxes are the major components contributing to needle toughness during spring bud burst
3	Dennis	Campbell	Using immunofluorescence to investigate possible changes in pectin structure in leaves of the *P. sativum* Argenteum mutant.
4	Guanqun (Gavin)	Chen	Fusion of Arabidopsis acyl-CoA binding protein 6 and an algal diacylglycerol acyltransferase 1 (DGAT1) enhances the performance of DGAT1
5	Jayde	Johnson	Regulatory mechanisms for an ancient chloroplast protein phosphatase discovered in the model plant *Arabidopsis thaliana*
6	Chris	Joshna	Capturing and characterizing the RNA binding protein repertoire of the *Brassica napus* root mRNA interactome
7	Aleksei	Sorokin	*Agrobacterium tumefaciens* - mediated Transformation of Cannabis (*Cannabis sativa*) seedlings
8	Ankita	Thapar	Investigating the effects of plant growth regulators on pod height in soybean
9	Glen	Uhrig	Sequential Multi-PTM quantitative proteomic analysis of osmotic and salinity stress in *Arabidopsis thaliana* roots
10	Chris	White-Gloria	Shewanella-like phosphatase 1 as the antagonist to the major chloroplastic protein kinase Casein Kinase 2

PLENARY TALK
TUESDAY FEBRUARY 18, 9:00AM

LACEY A SAMUELS
UNIVERSITY OF BRITISH COLUMBIA

The Unique Cell Biology of Cannabis Glandular Trichomes



Dr. Lacey Samuels completed her PhD at the University of British Columbia. After a post-Doctoral fellowship at the University of Colorado, Dr. Samuels accepted a position in the Department of Botany, University of British Columbia. The Samuels lab approach is to integrate cell biology with molecular biology and biochemistry. The flowers of *Cannabis sativa* L. (cannabis) are used medicinally and recreationally by humans because they contain specialized cannabinoid metabolites. The metabolites are produced in hairs (glandular trichomes) on the flower. Using techniques in advanced microscopy and molecular biology, the Samuels lab is discovering the unique properties of cannabis trichomes, which is critical information for molecular breeding, targeted engineering, atnd optimized harvest and processing of this important plant.

TUESDAY 10:30-10:45

Biochemical elucidation of psychoactive natural products, salvinorin and salvidivin, in *Salvia divinorum*

MOONHYUK MK KWON; NGO, I; PELOT, KA; CHIORENE, S; PARK, K; PAN, CH; VEDERAS, JC; ZERBE, P AND RO, DK

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The clerodane diterpenoids are the large subclass of C20 diterpenoid natural products found most often in the mint family (Lamiaceae). Among many clerodane diterpenoids, salvinorin A and its structural analogs (e.g., salvinicin and salvidivin) from Salvia divinorum are of particular interest due to their psychotropic activities. Salvinorin A is the first non-nitrogenous opioid that selectively acts as a kappa-opioid receptor agonist, thus making it an important lead-structure to develop mood-controlling drugs. Despite its importance, the biosynthesis of salvinorin A and its analogs remain unknown. To elucidate the salvinorin A biosynthesis, 57 million transcript reads were generated by Illumina sequencing from S. divinorum trichome. From the assembled transcriptomic data, the type II diterpene synthase catalyzing the biosynthesis of a unique kolavenyl diphosphate (clerodane skeleton) from geranyl geranyl diphosphate (GGPP) was functionally identified. However, the type I diterpene synthase that further catalyzes the formation of kolavenol, a direct precursor of salvinorin A, could not be identified, although three strong candidate cDNAs were examined. Taking advantages of the fact that yeast endogenous phosphatase can still produce kolavenol from kolavenyl diphosphate, we further investigated oxidative modifications of kolavenol by expressing several cytochrome P450 (CYP) cDNAs in the yeast engineered to produce kolavenol. From these screenings, two additional CYP genes responsible for the oxidative modifications were identified, and chemical structures of their enzymatic products were determined by NMR. Surprisingly, one of the CYPs catalyzes the synthesis of salvidivin backbone directly from kolavenyl diphosphate, bypassing the type I diterpene synthase. Collectively, these results advance our knowledge of salvinorin A biosynthesis and provide a new catalytic insight in the clerodanoid biosynthesis

TUESDAY 10:50-11:05

A single residue determines substrate preference in benzylisoquinoline alkaloid N-methyltransferases

JEREMY S MORRIS; YU, L AND FACCHINI, PJ

Department of Biological Sciences, University of Calgary, Calgary, AB

N-methylation is a common feature in the biosynthesis of many plant specialized metabolites. Early in the biosynthesis of benzylisoquinoline alkaloids (BIAs), coclaurine *N*-methyltransferase (CNMT) catalyzes a reaction which converts the secondary amine (*S*)-coclaurine into the tertiary amine (*S*)-*N*-methylcoclaurine. Subsequent enzymatic steps yield the core intermediate (*S*)-reticuline, from which branch pathways for the biosynthesis of morphine, noscapine, sanguinarine and other end-product BIAs diverge. In some branch pathways, such as that leading to the taxonomically widespread and ecologically significant alkaloid magnoflorine, an additional *N*-methylation yielding quaternary BIAs is catalyzed by reticuline *N*-methyltransferase (RNMT). Despite the clear functional contrast, analysis of primary sequence information has been unable to accurately distinguish CNMT-like and RNMT-like enzymes from each other, necessitating screening via costly *in vitro* enzyme assays for each non-model plant examined. Furthermore, even with the recent emphasis on structural characterization of BIA NMTs, the features and mechanisms underlying the CNMT-RNMT functional dichotomy were unknown. We report the identification of structural variants tightly correlated with CNMT-like or RNMT-like activities and show through reciprocal mutagenesis that a single residue can act as a switch between functions. Yeast-based in vivo screening of a putative BIA NMT library confirms that this discovery allows for accurate prediction of activity strictly from primary sequence information. Our results highlight the unusually short mutational distance separating ancestral CNMT-like enzymes from more evolutionarily advanced RNMT-like enzymes, and thus help explain the widespread yet sporadic occurrence of quaternary BIAs in plants. Comparison of this functional switch in BIA NMTs to analogous features in bacterial MT enzymes provides evidence of convergent evolution at the molecular level.

TUESDAY 11:10-11:25

Development of hairy root system in lettuce (*Lactuca sativa*) to evaluate CRISPR/CAS9 genome-editing.

EMAN SALAMA[1,2]; AHMED[1], A; HODGINS, C[1]; GHONEIMY,E[3]; ABDEL-WAHAB, A[4], ABDEL-RAHMAN,R[2]; SALEM,M[3] AND RO, DK[1]

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Hairy roots induced by *Agrobacterium rhizogenes* has been employed for a variety of purposes, such as metabolic engineering, recombinant protein production, and phytoremediation. Furthermore, hairy root system can be developed as a tool to rapidly evaluate the efficiency of CRISPR-Cas9 construct. In our experiments, we optimized hairy root production system in lettuce (*Lactuca sativa*) using five different *Agrobacterium rhizogenes* strains (K599, AR10, AR1193 R1000 and R1200). The levels of expression in hairy roots were examined by green fluorescent protein marker, and it was determined that *Agrobacterium rhizogenes* AR10 is the most suitable strain for the hairy root induction in lettuce. This strain was subsequently used to evaluate CRISPR-Cas9 in lettuce. We designed a CRISPR-Cas9 construct, comprised of the silence-resistant version of CaMV35S and multiple gRNA linked by tRNA sequences for self-cleavage, to knock out the gene encoding cis-prenyltransferase binding protein at 37 °C. Its genomic DNA sequences were determined, and three gRNA-recognition sites were selected, based on the in vitro Cas9 cleavage assays. Six individual hairy roots were induced after infecting lettuce with the CRISPR-Cas9 constructs with 3 gRNAs. Sequencing the PCR-amplicon of the locus showed that five hairy roots have mutations induced by CRISPR-Cas9. Cloning individual clones followed by Sanger sequencing further showed that all 3 gRNA sites possess insertion or deletion mutations. In summary, hairy root method in lettuce is a speedy approach to evaluate the efficiency of CRISPR-Cas9 constructs prior to time-consuming stable transformations through tissue cultures.

TUESDAY 11:30-11:45

Functional Characterization of genes and enzymes in the biosynthesis of heartwood secondary metabolites in Western redcedar

SIFAT TASNIM AND MATTSSON, J

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Western redcedar (*Thuja plicata*; WRC), is an economically important conifer species in the province of British Columbia, Canada. WRC heartwood is valuable for its natural rot resistance. Paradoxically, logged trees are frequently culled because of extensive heartwood fungal rot. One of the defense mechanisms of WRC against pathogens includes the production of secondary metabolites (e.g. terpenoids) in the form of oleoresin. There is evidence that high heartwood levels of terpenoid-derived tropolones, in particular β-thujaplicin, correlate with rot resistance. However, it takes more than 15 years of growth before this specialized terpenoid can be quantified. There is therefore a need for early genetic prediction of heartwood rot resistance in growing trees. Currently, the genetic basis of β-thujaplicin biosynthesis pathway is unknown in any plant species. We have used an RNA-seq approach to identify genes expressed at the site of heartwood formation. Among them, we identified six putative terpene synthases. We generated recombinant protein and tested their enzymatic activities. We found that these enzymes produce two monoterpenes e.g. terpinolene and 3-carene, both of which may be precursors to tropolones. To our surprise, we found that one enzyme produced the sesquiterpene alcohol elemol, hitherto linked to termite resistance in another tree species, as well as three monofunctional diterpene synthases that produce sandaracopimaradiene/syn-stemod-13(17)-ene, levopimaradiene and copalyl diphosphate which is a key intermediate for diterpene biosynthesis. These genes and enzymatic activities have not been previously reported in WRC and provide an opportunity to assess their potential roles in heartwood rot resistance in this species.

TUESDAY 11:50-12:05

Developing a microbial platform for investigating Taxol biosynthesis pathway

JOSEPH UTOMO; CHAVES, F; BAEK, S; KWON, M; MARTIN, VJJ; RO, DK

Department of Biological Sciences, University of Calgary, Calgary, AB

An important anticancer drug, Taxol, belongs to the diterpenoids, a 20-carbon-backbone subclass of terpenoid superfamily and is derived from a common precursor, geranylgeranyl diphosphate (GGPP). Despite that most enzymes in Taxol biosynthesis pathway have been discovered, there are some missing links in the pathway. Unfortunately, the chemical synthesis of its precursor, taxa-4,11-diene (taxadiene), is difficult to achieve, hindering the investigation of Taxol biosynthesis pathway. Additionally, most of the subsequent steps in Taxol biosynthetic pathway are catalyzed by cytochrome P450s in endoplasmic reticulum and would be difficult to be expressed in prokaryotes. To tackle these problems, we used yeast (S. cerevisiae) as a microbial platform to produce taxadiene. Expression of taxadiene synthase (TmTDS) from Taxus media in yeast showed a low titer of taxadiene since yeast only use GGPP for protein prenylation. Three main strategies were employed to increase taxadiene titer. First, we built and expressed a plasmid to increase flux towards terpenoid pathway. Second, we expressed and identified the most suitable GGPP synthases for diterpene production. Third, we used codon optimized TmTDS and tagged TmTDS with maltose binding protein to improve the expression and solubility of TmTDS. Combining these strategies resulted in more than 1,800-fold increase of taxadiene compared to wild-type yeast expressing TmTDS alone. Integration of IPP plasmid genes into yeast genome by CRISPR/CAS9 gave a similar level of taxadiene production with plasmid-based overexpression. This integration relieved the availability of plasmid selection marker, allowing us to further investigate other modifying enzymes such as cytochrome P450s, in the future.

TUESDAY 1:00-1:15

Mountain pine beetle associated fungal pathogen-induced changes in phenolic biosynthesis of pine and the effects of water limitation

COLLEEN E. FORTIER[1], ADRIANA ARANGO-VELEZ[1,2], MIRANDA J. MEENTS[1,3], AND JANICE E. K. COOKE[1]

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The current outbreak of mountain pine beetle (MPB; *Dendroctonus ponderosae*) has advanced beyond its native host, lodgepole pine (*Pinus contorta* var. *latifolia*), to a new host, jack pine (*Pinus banksiana*). Evidence suggests that jack pine has not experienced MPB outbreaks previously. Lack of a historic relationship might indicate that jack pine has fewer specialized defense mechanisms against MPB and their most pathogenic fungal associate, *Grosmannia clavigera*. Environmental changes associated with climate change likely have played a role in facilitating MPB range expansion, and water limitation could compromise plant defenses during droughts. We are examining differences in defense responses between lodgepole and jack pine, and how water limitation impacts these responses, focusing on phenolic defenses which accumulate in xylem lesions formed following MPB attack or *G. clavigera* inoculation. Putative pine phenolic biosynthesis genes were used to mine transcriptomic datasets generated from xylem and phloem tissue following inoculation. A subset of genes was selected for qRT-PCR analysis. Transcript profiles revealed that some branches of phenolic biosynthesis, like stilbene and flavonoid biosynthesis, showed strong gene regulation in response to inoculation, while water deficit often reduced transcript abundance. Additionally, lodgepole and jack pine showed subtle differences, primarily in the timing of their responses, which may be more apparent in metabolite profiling currently underway using HPLC. Early results show dramatic changes in phenolic profiles following fungal inoculation. We hypothesize that these observed differences in composition and quantity of phenolic defenses between species and under water deficit contribute to differential susceptibility to MPB and fungal associates.

TUESDAY 1:20-1:35

Measuring the uptake and partitioning of isotopically-labelled naphthenic acids in willow root and shoot tissue

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Extraction of bitumen from surface mined oil sands in northern Alberta produces large volumes of toxic oil sands process-affected water (OSPW) stored in tailings ponds. OSPW contains a group of organic compounds referred to as naphthenic acids (NAs) that are primary contributors to OSPW toxicity. While research into OSPW remediation has received much attention, the current knowledge of biological remediation techniques is limited. Phytoremediation is an approach that utilizes the ability of plants to remove and degrade contaminants from soil and ground water. The removal of NAs from OSPW by plants has been indirectly measured through NA concentrations in solution. However little work has been done to directly measure NA uptake into plant tissue. Using isotopically labelled NAs, we have shown that structurally diverse NAs are taken up by a native willow species, sandbar willow (*Salix interior*), using autoradiography and high-resolution mass spectrometry through direct measurement of NAs in plant tissue. NAs were taken up at different rates with structurally simple NAs being taken up more readily than structurally complex NAs. Additionally, differences in uptake were observed between plants grown in hydroponic media compared to soil contaminated with NAs. The results presented suggest that willow can remove NAs from liquid media and soil, implicating that this plant species is a candidate for OSPW phytoremediation. Future research probing the efficacy of NA uptake by other plant species and further metabolomics studies tracking the conversion of NA-derived carbon into plant metabolites will bring clarity to plant NA uptake and degradation mechanisms.

TUESDAY 1:40-1:55

Biosynthesis of off-flavour metabolites in *Pisum sativum* (pea)

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Canada is predicted to produce 4.7 million tonnes of peas (*Pisum sativum*) in 2020, more than any other country. Peas are an attractive crop because of the association their roots have with nitrogen fixing rhizobia. Flour made from pea seeds is increasingly being used in health food products because it is gluten-free, high in protein, and has a low-glycemic index. The widespread usage of pea flour is hindered because of bitter and metallic “off-flavours” caused by the compounds saponin B and DDMP saponin. To produce saponin-free pea cultivars, two saponin biosynthetic genes [β-amyrin synthase (*BAS*) and β-amyrin 22-hydroxylase (*B22H*)] were targeted for gene knock-out using CRISPR/Cas9. *BAS* and *B22H* cDNAs were cloned and functionally expressed in yeast, to confirm their catalytic activity. The exact sequence and intron position of *BAS* and *B22H* was determined from isolated genomic DNA fragments and used to computationally design three guide RNAs (gRNA). The double stranded cleavage ability of the gRNA candidates was evaluated using recombinant Cas9 complexed with *in vitro* transcribed gRNA candidates. A plant transformation vector was cloned using the CaMV35S promoter to express Cas9 and the three gRNAs. The gRNAs were expressed as a single transcript by linking each gRNA to an auto-cleavable tRNA sequence. A pea transformation protocol has been developed and we are currently working to induce mutations in *BAS* and *B22H* of transformed peas. The genotype and metabolite profile of the generated transgenic peas will be examined to validate the success of the genome editing.

TUESDAY 2:00-2:15

Identifying the molecular basis of resistance to *Cronartium harknessii*, the causal agent of western gall rust, in lodgepole and jack pine

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[4] Natural Resources Canada, Canadian Forest Service, Canadian Wood Fibre Centre, Quebec QC

Cronartium harknessii ([J. P. Moore] E. Meinecke) is the causal agent of western gall rust. Lodgepole pine and jack pine are both hosts for *C. harknessii*, and we have demonstrated that lodgepole pine is more susceptible to this biotrophic pathogen than jack pine. The goals of this project are to (1) use a comparative approach to identify molecular mechanisms that render jack pine more resistant to *C. harknessii* than lodgepole pine, and (2) use a genetic approach to discover genomic signatures that differentiate *C. harknessii*-resistant and -susceptible lodgepole pine. To address the first goal, we are carrying out RNA-Seq to compare lodgepole and jack pine transcriptomic responses to *C. harknessii* inoculation. To address the second goal, we conducted large-scale resistance screens with 76 families of *C. harknessii*-inoculated seedlings, and also collected samples from naturally *C. harknessii* infected mature lodgepole pine stands comprising about half of these families. Good correlations were found between seedling and mature tree gall incidence data. A total of 1920 individuals were genotyped using a pine Affymetrix Axiom 50K single nucleotide polymorphism (SNP) array. Following data quality assessment analyses, 1085 individuals from the seedling resistance screen and 667 individuals from the mature tree trials are being used for genomic selection. To complement this quantitative genomics approach, we plan to carry out RNA-Seq on resistant versus susceptible families of lodgepole pine to determine whether loci associated with *C. harknessii* resistance correspond to genes that show differential responses to *C. harknessii* inoculation in resistant versus susceptible lodgepole pine.

TUESDAY 2:40-2:55

The characterisation of transcriptome from different developmental stages of *Rafflesia cantleyi* floral buds

SAFOORA AMINI[1,2], KHADIJAH ROSLI[1,2], MOHD-FAIZAL ABU-BAKAR[3] , HALIMAH ALIAS[3] , MOHDNOOR MAT-ISA[3] , MOHD-AFIQ-AIZAT JUHARI[4] , JUMAAT HAJI-ADAM[4] , HOE-HAN GOH[5] , KIEWLIAN WANID[1,2]

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Rafflesia cantleyi is a specialised holoparasitic plant with dramatic morphological modifications. It has a reduced vegetative structure, which only appears as a flower and it possesses an unusual life cycle, where its floral bud development takes up to nine months. However, little information is available on the underlying mechanisms that govern flower development in this organism. To gain a global perspective on the flower development in *R. cantleyi*, transcriptome data were generated from three developmental stages of its floral buds, representing the early (FBS1), mid (FBS2) and advanced (FBS3) developmental stages that were used to profile the floral bud transcriptomes and identify differentially expressed transcripts (DEGs) between the three floral bud stages (FBS1 vs. FBS2, FBS2 vs. FBS3, and FBS1 vs. FBS3). A total of 89,690 transcripts were obtained by de novo assembly of 91.63 million clean paired-end reads through the Trinity analysis pipeline. A total of 2,312 and 3,961 DEGs were identified for FBS1-FBS2 and FBS2-FBS3 comparisons, respectively. A total of 972 and 1,769 transcripts were up-regulated; while 1,340 and 2,192 transcripts were down-regulated in FBS1-FBS2 and FBS2-FBS3, respectively. In the FBS1-FBS3 comparisons, 4,642 DEGs were identified with 1,999 up-regulated and 2,643 down-regulated. Using K-mean clustering, DEGs in 12 co-expression clusters with different patterns were identified. The centroid values of each cluster which show the expression patterns of each cluster were used to compare the relationships between clusters, and three major groups were identified. These three major group showed correlations to three developmental stages. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analyses have revealed that DEGs are involved in hormone signal transduction, light signalling, and the specification of floral organ identity. Among these DEGs are transcription factor families involved in flower development, such as ARF, MADS-box, bHLH, and MYB families. Furthermore, DEGs associated with hormone auxin (IAA), cytokinin (CK), gibberellic acid (GA), jasmonic acid (JA) and as well as flowering pathways such as photoperiod and autonomous pathways were identified. The phytohormone-related DEGs suggest similar roles of phytohormones during flowering process and highlight the possible role of GA and JA in *R. cantleyi* flowering. A total of 628 transcripts exhibiting sequence similarities to Arabidopsis flowering-related genes include SVP, LFY, and members of ABCE model genes were differentially expressed during flower development. In addition, floral organ-specific expression patterns of ABCE model genes showed the relationship between ABCE model genes expression and floral morphology in *R. cantleyi*. Based on this, one ABCE model was proposed.

TUESDAY 3:00-3:15

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

ADAM FOX, CHI ZHANG, MITCHELL ALBERTS AND DOUGLAS MUENCH

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PUF proteins are a conserved group of sequence specific RNA-binding proteins that bind to RNA in a modular fashion. The RNA-binding 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, organization and amino acid sequence identity. We reported the identification of a novel RNA consensus sequence for an Arabidopsis PUF protein that contains an atypical RNA-binding domain. The Arabidopsis PUM23 (APUM23) consensus sequence was ten nucleotides in length, contained a centrally located UUGA core element, and had a preference for 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 have engineered APUM23 to alter its RNA target sequence, and are using the advanced structural backbone of APUM23 to determine its RNA target specificity *in vivo*. We are also testing this designer PUF to alter the metabolism of target mRNAs by fusing it to effector domains, such as RNases and translational enhancers.

TUESDAY 3:20-3:35

Rhizobiales-like Protein Phosphatase 2 and Type One Protein Phosphatases interaction with the D Group Mitogen Activated Protein Kinases in *Arabidopsis thaliana*

RYAN TOTH; LABANDERA, A.M., UHRIG, R.G., MOORHEAD, G.B.

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Post Translational Modifications (PTM) are biological signaling mechanisms that govern the operations of life in many aspects, such as a proteins biochemical conformation, the expression of a gene, or an organism's response towards external stimulants. The most common PTM regulating 70% of *Homo sapiens* proteins is Reversible Protein Phosphorylation, a process in which a phosphoryl group is installed onto a protein resulting in numerous possible outcomes. Protein Kinases are the driving force behind protein phosphorylation, with Protein Phosphatases performing as the counterforce making Protein Phosphorylation reversible by removing a proteins phosphoryl group reverting the substrate to its original status. *Arabidopsis thaliana* uniquely only has one determined tyrosine phosphatase compared to the *Homo sapiens* genome containing 32. With that being considered, Rhizobiales-like Protein Phosphatase (RLPH), a unique bacterial-like phosphatase, which while bioinformatically characterized as a member of the PPP family, has been discovered to preferably dephosphorylate phosphotyrosine residues, specifically in the D Group Mitogen-Activated Protein Kinases (MPK) TxY motif. An engaging observation is that right next to the phosphotyrosine residue specifically dephosphorylated by RLPH2 is the Type One Protein Phosphatase (TOPP) regulatory binding RVxF motif, present in no other characterized MPK. In vitro binding assays have shown that TOPP does not interact with a dual phosphorylated MPK. From this, we hypothesize that RLPH2s dephosphorylation of the D Group MPKs TxY motifs phosphotyrosine residue allows for regulatory binding of the D Group MPKs to TOPP via the RVxF motif.

TUESDAY 3:40-3:55

Developing multiplex genome-editing platforms in yeast (*Saccharomyces cerevisiae*) using a single gRNA-mediated CRISPR-Cas9

DAE-KYUN RO, SIHYUN BAEK, KUNAL DALAL, JOSEPH UTOMO

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A budding yeast, *Saccharomyces cerevisiae,* is a versatile microbial platform to build synthetic metabolic pathways for production of diverse chemicals. To expedite the construction of complex metabolic pathways by multiplex CRISPR-Cas9 genome-edit, eight desirable intergenic loci, located adjacent to highly expressed genes selected from top 100 expressers, were identified and fully characterized for three criteria after integrating green fluorescent protein (GFP) gene - CRISPR-mediated GFP integration efficiency, expression competency assessed by levels of GFP fluorescence, and assessing growth rates of GFP integrated strains. Five best performing intergenic loci were selected to build the platform, and a synthetic 23-bp DNA comprised of 20-bp synthetic DNA with a protospacer adjacent motif (PAM) was seamlessly integrated into the five loci using CRISPR-Cas9 in a sequential manner. This process resulted in five different yeast strains harbouring either one, two, three, four, or five synthetic gRNA-binding sites in their genomes. On these pre-engineered yeast strains, simultaneous integrations of 2-, 3-, 4-, or 5-genes to the targeted loci were demonstrated with efficiencies from 84.8% to 98.0% using beet pigment betalain (3-gene pathway), kanamycin, and hygromycin resistance markers. Integrations of the multiple, foreign genes in the targeted loci with 100% precision were firmly validated by genotyping forty transformants. Our quintuple gene integration platform, referred to as SBY105, was used to generate a yeast strain synthesizing costunolide by a single transformation. This study demonstrates the effectiveness of single gRNA-mediated CRISPR platform to construct complex metabolic pathways in yeast.

TUESDAY 4:00-4:15

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

DILINI ATUGALA; JOSHNA, CR AND MUENCH DG

Department of Biological Sciences, University of Calgary, Calgary, AB

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. Here we discuss the purification of the plant RNA-binding interactome from Arabidopsis cell cultures, leaves and roots. Similar to other model organisms, the plant RNA interactome consists of a large proportion of proteins with non-classical and unknown RNA binding domains. We present validation data for these domain categorized RBPs (classical, non-classical and unknown). We have followed the UV crosslinking step by immunoprecipitation of RBPs of interest using specific antibodies and are in the process of identifying their target RNAs using deep sequencing of bound RNAs. Further, we are determining the functional role of these specific RBP-RNA interactions in plants that are exposed to environmental stresses, such as toxins, cold, heat shock and nutrient deficiency This research approach has provided us with a valuable tool to identify authentic RNA targets of interesting RBPs, and expands our understanding of the functional roles of RBPs in gene expression in plant cells.

KEYNOTE ADDRESS
WEDNESDAY FEBRUARY 19, 9:00AM

SAMUEL YEAMAN UNIVERSITY OF CALGARY

Sam Yeaman is an assistant professor at University of Calgary, where he holds an Alberta Innovates chair in Bioinformatics and computational biology. His research uses theoretical models and genomic data to understand the process of adaptation. In addition to research on theoretical models and statistical methods, his lab studies climate adaptation in conifers and sunflowers, antibiotic resistance in parasitic nematodes, freshwater adaptation in fish, and HIV adaptation to the human immune system. Dr. Yeaman completed a B.Sc. at Trent University and a Ph.D. at University of British Columbia under the supervision of Mike Whitlock, followed by postdoctoral research at University of Neuchatel and University of British Columbia. He is the recipient of an Early Career award from the Canadian Society for Ecology and Evolution and a Killam NSERC Emerging research leader award.

The genomic basis of adaptive and plastic responses to climate in conifers

Conifers display extensive evidence of local adaptation, with divergence among populations in a range of phenotypes, from budset to cold injury tolerance. They can also respond plastically to a wide range of climatic stresses, which can result in repatterning of gene expression. Here, we use comparative genomic approaches to study how both gene expression and adaptive differentiation respond to climate in lodgepole pine (*Pinus contorta*) and interior spruce (*Picea engelmanii, Picea glauca*, and their hybrids). We compare RNAseq expression profiles under 7 different environmental treatments and characterize genes that respond similarly vs. differently in their expression. We also explore the genetic basis of adaptive differences among populations using exome capture and phenotype- and environment-association analyses. We find 47 genes with strong signatures of convergent adaptation to climate in both species, despite 140 million years of independent evolution. Interestingly, the genes that are involved in convergent adaptation are also more likely to have conserved gene expression profiles. We are now expanding this study to explore climate adaptation in douglas-fir, western larch, and jack pine, as well as to study the basis of resistance to dothistroma in lodgepole pine, and Swiss needle cast in douglas-fir.

WEDNESDAY 10:00-10:15

Directing Development of Next-Generation Spectrally-Controlled Plant Growth

SABINE SCANDOLA[1], DEVANG MEHTA[1] AND RICHARD GLEN UHRIG[1]

[1]Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada
[2]G2V Optics Inc. Edmonton, Alberta, Canada

Plants highly depend on sunlight for their energy production, which takes place during photosynthesis. Additionally, they developed arrays of sensors that allow them to sense light variations such as dawn and dusk and anticipate changes in the duration of the days occurring through seasons so they can adapt their circadian rhythm and improve their survival. With classic approaches, it is difficult to study plant responses to different light stimuli as indoor lighting systems do not allow us to program light variations (spectra, photoperiod or intensity). Horticulture techniques employs a combination of high pressure sodium, metal halide or fluorescent lights that have fixed parameters. In recent years, light-emitting diodes (LEDs) have offered to indoor facility growers a competitive alternative: an energy efficient, stable and programmable lighting system. They are known for their reduced heating, long lifetime, stability and homogeneity. However, there is limited knowledge of how plants respond to growth under an LED-only light regime. In collaboration with G2V Optics LED technology, we were able to study plant morphology and gene expression in the plant model *Arabidopsis thaliana* and provide valuable information for the horticulture light market. We combined molecular biology, transcriptomics and computer vision (through PlantCV) to gain a high level of understanding based on how plants react to different type of light. Our findings demonstrates that transitional dawn and dusk, specific photoperiod and spectra are beneficial to plants as it improve particular phenotypic traits and trigger the expression of several genes involved in carbon fixation, water transport or cellulose biosynthesis and down-regulation of stress bio-markers.

WEDNESDAY 10:20-10:35

Can differences in metal specificity of durum wheat P_{1B}-ATPase 3 (TdHMA3) transporters explain the cadmium-specific phenotype of QTL *Cdu-B1*?

NEIL HARRIS

Department of Biological Sciences, University of Alberta, Edmonton, AB

The durum wheat (*Triticum turgidum* ssp. *durum*) P_{1B}-type Heavy-Metal ATPase 3 (*TdHMA3-B1*) is a tonoplast-localized cadmium (Cd) and zinc (Zn) transporter. We recently identified a disruptive duplication in the coding sequence of *TdHMA3-B1* (allele *B1b*) that abolished TdHMA3-B1 transport activity. The functional (*B1a*) and non-functional (*B1b*) alleles segregated perfectly with Cd accumulation in grain of a global collection of durum wheat germplasm, supporting the conclusion that functional differences in alleles *B1a*/*B1b* are responsible for the quantitative trait loci, *Cdu-B1*, that accounts for >80% of the phenotypic variation in Cd concentration in durum grain. A contradiction in these results is that TdHMA3-B1 shows Cd and Zn transport activity, whereas the *Cdu-B1* phenotype is Cd-specific. We hypothesize that *TdHMA3-B1* and its A-genome homoeolog, *TdHMA3-A1*, act redundantly to produce the Cd-specific *Cdu-B1* phenotype. In yeast complementation assays, *TdHMA3-A1* rescued Cd-sensitive phenotypes, but unlike *TdHMA3-B1*, *TdHMA3-A1* did not rescue Zn-sensitive phenotypes, which is consistent with the proposed hypothesis. The molecular basis for differences in metal transport specificity of TdHMA3-A1 and TdHMA3-B1 will be presented.

WEDNESDAY
10:40-10:55

RNA interference protects Canadian crops against fungal attack

MARK F BELMONTE, WYTINCK, N; WALKER, P, WHYARD, S.

Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba

Sclerotina sclerotiorum, the causal agent of white mold, infects over 450 species of plants worldwide. This fungal phytopathogen has become a major threat to crops including canola which contributes $26.7 billion to the Canadian economy. *Sclerotinia* is a persistent problem for canola growers that has traditionally been managed using broad-spectrum fungicides. However, current fungicide strategies have proven less effective and crop rotations fail due to the promiscuous host range of Sclerotinia and the formation of durable resting structures known as sclerotia. Thus, there is an immediate need to manage Sclerotinia using novel species-specific control methods. Our novel strategy exploits the inherent cellular defense process known as RNA interference (RNAi). Upon encountering a double stranded RNA (dsRNA) molecule, the cell processes the dsRNA specifically targeting transcripts with sequence homology. Using a re-designed bioinformatics approach, we identified *Sclerotinia*-specific target genes. RNAi knockdown was confirmed using quantitative real-time PCR on RNA isolated from fungal liquid cultures. dsRNA molecules were screened for growth inhibition on the plant using a system representative of field conditions that showed up to 85% reduction in lesion spread. We then generated transgenic plants over-expressing good quality dsRNA and showed a more profound and prolonged tolerance to the fungus. Finally, I will provide insight into the utility of next generation molecular fungicides and their applicability to control pathogens.

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POSTER ABSTRACTS

TUESDAY 4:30 PM - 6:30 PM ARNICA BALLROOM

POSTER 1 Probing the biochemistry of benzylisoquinoline alkaloid biosynthesis for synthetic biology applications

MOIZ KAPASI, IVETTE MENENDEZ, JACINTA WATKINS, NATALI OZBER, SIYU LIANG, KRISTIAN CALDO, JEREMY MORRIS, PETER FACCHINI

Department of Biological Sciences, University of Calgary, Calgary, AB

Opium poppy (Papaver somniferum) benzylisoquinoline alkaloids (BIAs) are among the most widely used plant secondary metabolites as pharmaceutical drugs. Opiates have various applications in medicine including in pain management (codeine, morphine), as vasodilators (papaverine), anticancer drugs (noscapine), and antimicrobial agents (sanguinarine). Due to the structural complexity of BIAs, their chemical synthesis remains to be a difficult endeavor and commercially impractical; hence, there has been great interest in producing these valuable compounds through synthetic biology. Our laboratory has been employing advanced tools in genomics, transcriptomics, and proteomics to understand how plants have evolved the ability to drive the accumulation of BIAs to very high levels. Using omics-based discoveries, we aim to generate alternative high-titer opiate production platform by reconstituting the BIA metabolic pathways in microbes.

POSTER 2 Defining the window of phenological opportunity for spruce budworm herbivory of white spruce: determining whether lignin and epicuticular waxes are the major components contributing to needle toughness during spring bud burst

COLLEEN E. FORTIER[1], BIANCA M. SACCHI, EKATERINA STOLNIKOVA, MAIA DALL'ACQUA, JUAN A. ALDANA, AND JANICE E. K. COOKE[1]

White spruce (*Picea glauca*) is a keystone species of Canada's boreal forest, which has a wide distribution and is of great economic and ecological importance. Eastern spruce budworm (*Choristoneura fumiferana*, SBW) is considered one of the most important pests affecting spruce and balsam fir, causing defoliation, host growth loss, and loss of forest products. SBW show a preference for feeding on newly expanding foliage during bud burst, and phenological differences between some spruce and fir species have been shown to impact SBW feeding behaviour and larval fitness. Other studies have suggested that SBW feeding on older foliage may be limited by increases in mechanical toughness during needle development. In addition, deposition and compositional changes of epicuticular waxes surrounding the needles may contribute to defining a window of opportunity where SBW phenology overlaps with spruce bud burst phenology. To better define these phenological changes, I am examining the developmental profiles of foliar lignin and epicuticular waxes during the course of bud burst for mature trees and seedlings of white spruce. Our results indicate that lignin and epicuticular waxes increase substantially as bud scales are lost and needles begin a rapid expansion phase. These increases in lignin and epicuticular waxes correspond to increased mechanical toughness of the needles. Analyses of lignin and epicuticular waxes is currently underway using HPLC and GC-MS to determine if changes chemical composition are correlated with increases in foliar toughness, which may ultimately influence SBW feeding behaviour.

POSTER ABSTRACTS

TUESDAY 4:30 PM - 6:30 PM ARNICA BALLROOM

POSTER 3 Using immunofluorescence to investigate possible changes in pectin structure in leaves of the *P. sativum* Argenteum mutant.

DENNIS CAMPBELL; LA ROSA MONTES, D.; BIRD, DA

Department of Biology, Mount Royal University, Calgary, AB

The leaf epidermis of Argenteum (Arg) peas (*Pisum sativum*) is loosely adhered to the underlying mesophyll and can easily be peeled away. Cross sections of such leaves viewed under light microscopy reveals large air spaces between the two cell layers with only interspersed attachment points. This leads to the silver appearance of their leaves contrasted with the normal green of wildtype (WT) peas. However, the molecular mechanism responsible for this phenomenon has not yet been established. We hypothesize that variation in pectin composition or arrangement in the middle lamella, between the epidermis and mesophyll, is responsible for the loss of adhesion. The middle lamella is known to be responsible for the connection of adjacent cell layers and is rich in a variety of pectin polysaccharides, the most abundant of which being homogalacturonan, rhamnogalacturonan-1, and rhanmnogalacturonan-2. Using a bank of monoclonal antibodies raised against various pectic antigens, and immunofluorescence assays of leaf cross-sections, we seek to characterize and find any potential differences between the pectic content of both WT and Arg leaves.

POSTER 4 Fusion of Arabidopsis acyl-CoA binding protein 6 and an algal diacylglycerol acyltransferase 1 (DGAT1) enhances the performance of DGAT1

GUANQUN(GAVIN). CHEN[*1], Y XU[1], K.M.P. CALDO[1], L. FALARZ[1,2], K. JAYAWARDHANE[1]

[1]Dept. of Agricultural, Food and Nutritional Science, University of Alberta, Canada
[2]Dept. of Biological Sciences, University of Manitoba, Canada

Microalgal oils with triacylglycerols (TAGs) as the major storage lipids are broadly used as nutritional supplements and biofuels. Diacylglycerol acyltransferase (DGAT) catalyzes a final step in TAG biosynthesis and is considered a key target for improving oil content. Although a growing number of DGAT1s have been identified in some algal species, the improvement of DGAT1 performance via protein engineering remains largely untapped. Here we analyzed the structure-function features of the hydrophilic N-terminal domain of DGAT1 from the green microalga *Chromochloris zofingiensis* (CzDGAT1) and used protein-fusion to improve its performance. The N-terminal domain of CzDGAT1 was less disordered than those of the higher eukaryotic enzymes and its partial truncation or complete removal could substantially decrease the enzyme activity, suggesting its possible role in maintaining enzyme performance. The fusion of Arabidopsis acyl-CoA binding protein 6 (ACBP6) to the N-terminus of the full-length CzDGAT1 could enhance the enzyme affinity for acyl-CoAs and augment protein accumulation levels, which ultimately drove oil accumulation in yeast cells and tobacco leaves to higher levels than the native CzDGAT1. In summary, the results unravel the distinct features of the N-terminus of algal DGAT1 and provide a novel strategy to generate performance-enhanced DGAT1 via protein fusion, which may open a new vista in generating improved membrane-bound acyl-CoA-dependent enzymes and boosting oil biosynthesis in plants and oleaginous microorganisms.

POSTER ABSTRACTS

TUESDAY 4:30 PM - 6:30 PM ARNICA BALLROOM

POSTER 5 Regulatory mechanisms for an ancient chloroplast protein phosphatase discovered in the model plant *Arabidopsis thaliana*

JAYDE, J JOHNSON, AHMAD, V;UHRIG, RG; MOORHEAD, GB

Protein phosphatases are enzymes which remove phosphate groups from target proteins, directly opposing protein kinases in order to signal events in the cell. There are four families of these hydrolases and each is believed to have arisen from an independent evolutionary event. Recently, a new subclass of these enzymes was discovered by our lab. These novel enzymes are not well studied or understood but potentially play key roles in the cell biology of the model plant Arabidopsis thaliana. Specifically, Shewanella-like phosphatase 1 (SLP1) has been found to be localized to the chloroplast and many potential substrate proteins have been identified by a quantitative phosphoproteomic mass spectrometry study. However, the mechanism(s) for substrate recruitment and regulation are not known. I plan to use structural techniques, such as x-ray crystallography, to examine SLP1s structure for potential protein docking sites, regulatory sequences, or regulatory domains. I will also further validate substrates discovered in our recent phosphoproteomic mass spectrometry study, and identify any possible interactors using biochemical techniques. Specifically, Far Western blotting and immunoprecipitations will be used to identify possible binding partners. So far, I have developed a hypoactive mutant to use for substrate validation studies using immunoprecipitation techniques. I have preliminary crystal screen hits, and I have conducted tandem affinity purification experiments suggesting SLP1 can co-purify with other proteins, possibly regulators, in vitro.

POSTER 6 Capturing and characterizing the RNA binding protein repertoire of the Brassica napus root mRNA interactome

CHRIS CJ JOSHNA, ATUGALA, DILINI, DA, MUENCH, DOUGLAS, DM

RNA binding proteins regulate gene expression by determining the function and/or outcome of bound RNAs. The recent emergence of a novel proteome-wide approach known as" RNA interactome capture" has helped capture active protein-RNA interactions *in vivo*. This technique employs UV-induced crosslinking of proteins to RNA, oligo(dT) capture of protein-RNA complexes, and protein identification via mass spectrometry. The implementation of this technique in *Arabidopsis thaliana* has helped uncover a large and functionally diverse repertoire of RNA binding proteins, many lacking conventional RNA binding domains, and possessing no previously established relationship to RNA biology. This discovery of novel RNA binding proteins not only provides insight into how plant responses and cellular processes are regulated, but also suggests new modes of RNA interaction and new functionalities for RNA-protein interactions. The goal of this study is to expand the plant RNA binding protein repertoire, by identifying and characterizing RNA binding proteins in crop species like *Brassica napus* (Canola), an important Canadian oilseed crop that generates the highest farm revenue from crops across Canada. To date, our study has identified 464 RNA binding proteins constituting the Canola root mRNA interactome, of which many had not been previously linked to RNA biology. The identification and characterization of these RNA binding proteins can not only provide insight into plant development, but it can also enhance genetic engineering efforts that aim to improve stress tolerant traits in Canola, as a means to combat rising global temperatures and escalating occurrences of droughts and floods brought about by climate change.

POSTER ABSTRACTS

TUESDAY 4:30 PM - 6:30 PM ARNICA BALLROOM

POSTER 7 *Agrobacterium tumefaciens* - mediated Transformation of Cannabis (*Cannabis sativa*) seedlings

ALEKSEI SOROKIN, NARENDRA SINGH YADAV

University of Lethbridge

Transient expression plays a vital role to provide a fast method to study the gene of interest and subsequently leads the path to develop an improved plant variety with better agronomic traits. An efficient and reproducible method was established for transient expression in *Cannabis sativa* seedlings *using Agrobacterium tumefaciens*-mediated transformation. *Agrobacterium tumefaciens* strain EHA105 carrying the pCAMBIA construct is used to transform *Cannabis* seedlings and the GUS assay was used to detect the transgenics. In this protocol, we have used 1% hydrogen peroxide (H_2O_2) solution for seed sterilization, which allows to quickly obtain viable sterile seedlings hence significantly advantageous over mercuric chloride or bleach. We have optimized various steps of transformation to achieve high efficiency. The method established in this study has potential to be an important tool for gene-function studies and crop improvement in *Cannabis*.

POSTER 8 Investigating the effects of plant growth regulators on pod height in soybean

ANKITA THAPAR, PHAM ANH TUAN AND BELAY T. AYELE

Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada

Soybean production is affected by several biotic, abiotic and morphology related factors that result in economic losses. Low pod height is one of the major concerns that causes harvest losses in the production of some soybean cultivars, and such a loss accounts for an average yield reduction of about one to two bushels per acre. Some plant growth regulators are implicated in promoting internode elongation or the height of lowest pod bearing nodes by stimulating the enzymes responsible for cell elongation. This research investigates the effects of different plant growth regulators on internode elongation and the height of lowest pod bearing nodes in soybean. To this effect, the study involves the use of three cultivars of soybean with contrasting lowest pod height. Our results to date indicated that seed treatment with gibberellin containing solutions increased the heights of the cotyledonary and first nodes as compared to the untreated control samples in the cultivar with the lowest pod height. No significant effect of the treatment on total plant height and lengths of the subsequent internodes was observed. To understand the cause for the variations in height of lowest pods/nodes further, we performed expression analysis of the GA biosynthetic GA20ox and GA3ox genes in the internodes of the three cultivars. Our preliminary results indicated variation in the expression patterns of these genes across the three cultivars. Details of the results of the study will be discussed during the presentation.

POSTER ABSTRACTS

TUESDAY 4:30 PM - 6:30 PM ARNICA BALLROOM

POSTER 9 Sequential Multi-PTM quantitative proteomic analysis of osmotic and salinity stress in *Arabidopsis thaliana* roots

MEHTA, D., RODRIGUEZ M., TAN, M., AND ***UHRIG RG***

Department of Biological Sciences, University of Alberta, Alberta, Canada

Given our changing climate, understanding the molecular ramifications of drought-related stress is more relevant than ever. Osmotic and salinity stress are two major outcomes of drought that can have wide-ranging effects on agricultural productivity. Multiple studies across a wide-range of relevant plant models have examined the effects of osmotic and salinity stress at either the transcriptomic and proteomic level; however, few have examined these stresses concurrently and even fewer have explored the role of post-translational modifications (PTMs) in plant adaptation to these drought-stress conditions. Furthermore, the vast majority of studies have been interested in changes observed in the immediate term post-stress initiation, rather than longer-term responses. Here we present a concurrent, quantitative proteomic analysis of both osmotic and salinity induced changes in the acetylome, phosphoproteome and total proteome of *Arabidopsis thaliana* roots after 24 hours of stress. This has allowed us to draw direct comparisons to previously acquired transcriptomic data and correspondingly generate a more holistic foundation of how plant roots adapt to osmotic and salinity stresses as well as how roots may differentially respond to either stress at the proteome and PTM-level. Analysis of the acquired PTM and proteomic data resolved networks of dynamically changing PTMs and proteins involved in RNA processing, protein translation and primary metabolism localized to a diversity of subcellular compartments. Our findings indicate plant roots adjust core plant cell processes over the longer-term to adapt to osmotic and/or salinity stress, providing a series of new potential targets for further characterization.

POSTER 10 Shewanella-like phosphatase 1 as the antagonist to the major chloroplastic protein kinase Casein Kinase 2

CHRIS WHITE-GLORIA, AHMAD VAHAB, GLENN UHRIG, GREGORY MOORHEAD

In plants, like all other eukaryotes, reversible protein phosphorylation, controlled by protein kinases and phosphatases, is an important regulator of various aspects of cell biology. Fundamental to plant metabolism is the local chloroplast metabolism, a collection of processes tightly controlled by post translational modifications such as protein phosphorylation. Following our group's bioinformatic discovery of chloroplast-localized protein phosphatase SLP1, our phosphoproteomics study revealed that SLP1 regulates the phosphorylation of 126 proteins in the chloroplast. Upon alignment of phospho-sites, an acidic phosphorylation motif appears which is indicative of casein kinase (CK) phosphorylation. Interestingly, this implies that SLP1 is the protein phosphatase that opposes the action of major chloroplastic protein kinase CK2. This gives SLP1 a supposedly widespread role and places it at the center of chloroplast metabolism, making it an attractive phosphatase to inspect. My research combines a biochemical and molecular biology approach to investigate the role of SLP1 in the chloroplast.

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