Montreal (http://www.tourisme-montreal.org/)

9th Canadian Plant Biotechnology Conference

May 12th-15th, 2014

McGill UNIVERSITY

Montreal, Quebec, CANADA

Conference website: http://www.mcgill.ca/iapbcanada2014

Conference contact: iapbcanada2014@mail.mcgill.ca

Organized by
International Association for Plant Biotechnology
Canadian Section

INTERNATIONAL ASSOCIATION FOR PLANT BIOTECHNOLOGY
I A P B

The 9th Canadian

Plant Biotechnology Conference

May 12 – 15, 2014

La Plaza - Holiday Inn Montreal Midtown, Montreal, Quebec

Organizing Committee

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Krystyna Klimaszewska (Canadian Forest Service)
Surinder Singh (McGill University)

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Exhibitors:

Génome Québec

Norgen Biotek Corp

Qubit Systems Inc.

Conference Venue

Holiday Inn Centre-Ville La Plaza
420 Rue Sherbrooke Ouest
Montreal, QC, Canada H3A 1B4
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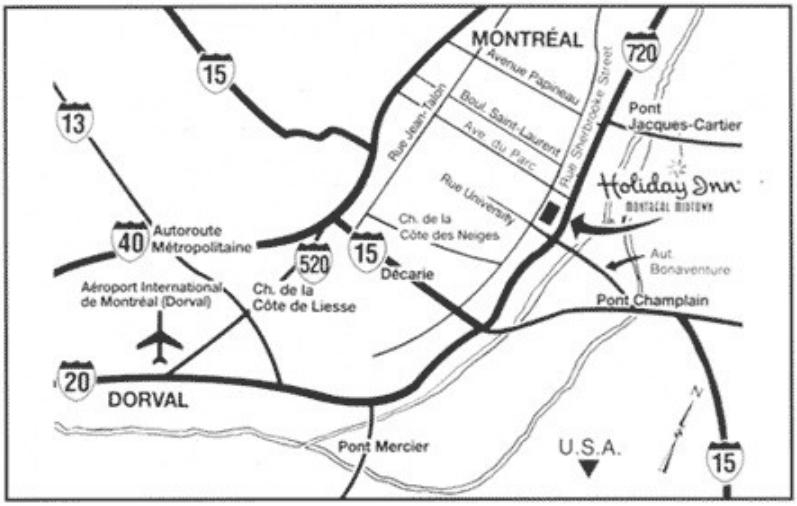

9TH CANADIAN PLANT BIOTECHNOLOGY CONFERENCE
MONTREAL, MAY 12-15, 2014

MONDAY, MAY 12

6:00 - 8:00 pm Registration

TUESDAY, MAY 13

8:00 - 11:00 am & 1:00 - 3:00 pm: Registration Desk Open

8:00 am - Continental Breakfast

8:45 am - Welcome Address
Dr Jaswinder Singh, Co-Chair 9th CPBC, Department of Plant Science, McGill University
Dr Ian Strachan, Associate Dean (Graduate Education), McGill Faculty of Agriculture and Environmental Sciences

9:00 am - 12:00 pm - SESSION I:
PLANT REGENERATION AND GENETIC ENGINEERING
Session Chairs: Dr Dan Brown (Canadian Centre for Agri-food Research in Health and Medicine), Dr Danielle Donnelly (McGill University)

9:00 - 9:45 am - Keynote Speaker:
Dr Peggy G. Lemaux, Department of Plant & Microbial Biology, University of California, Berkeley
"Alteration of thioredoxin expression in cereals leads to unexpected improvements in grain properties - but where are they?"

9:45 - 10:00 am - Christie Lovat, Department of Plant Science, McGill University
"Somatic embryogenesis as a breeding tool in American chestnut (Castanea dentata (Marsh.) Borkh.)"

10:00 - 10:15 am - Marie-Josée Cardinal, Department of Plant Science, McGill University
"Towards Introducing Ac/Ds Transposons into Hordeum spontaneum to Capture Genetic Diversity"

10:15 - 10:30 am - Christina Larder, Department of Plant Science, McGill University
"Screening Quebec-bred potato genotypes for functional food properties"

10:30 -11:00 am - Coffee & Networking Break

11:00 - 11:30 am - Wenbin Li, Key Laboratory of Soybean Biology in Chinese Education Ministry, Northeast Agricultural University, China
"Transgenic soybean confers drought tolerance"

11:30 - 11:45 am - Priti Maheshwari, Agriculture and Agri-Food Canada, Lethbridge, Alberta
"Zinc-finger nuclease Technology for novel trait development in Triticale cv. Ultima"

11:45 am - 12:00 pm - Wenzislava Ckurshumova, Department of Cell and Systems Biology, University of Toronto
"Auxin response factor functions in plant regeneration - molecular control of de novo shoot formation"

12:00 - 12:15 pm - Stephen Hunt, Qubit Systems Inc., Kingston, Ontario
"Application of high throughput phenotyping to study early phase drought stress"

12:15 - 12:30 pm - Vinay Panwar, Agriculture and Agri-Food Canada, Morden, Manitoba
"Host-induced gene silencing of Puccinia triticina genes shows enhanced resistance to leaf rust disease in transgenic wheat plants"

12:30 - 1:30 pm – Lunch Buffet

1:30 - 2:30 pm – Poster Session I – Even Numbered Posters Presented

2:30 - 5:30 pm - SESSION II:
BIOMATERIALS AND MOLECULAR FARMING

Session Chairs: Dr Rima Menassa (Agriculture and Agri-Foods Canada-London), Dr Abdelali Hannoufa (Agriculture and Agri-Foods Canada-London)

2:30 - 3:15 pm – Keynote Speaker:
Dr Marc-Andre D'Aoust, Medicago Inc, Quebec City, Quebec
"Medicago: plant-made influenza vaccines and beyond"

3:15 pm - 3:45 pm - Rima Menassa, Agriculture and Agri-Foods Canada, London, Ontario
"Transplastomic plants for high level production of pharmaceuticals and industrial enzymes"

3:45 - 4:15 pm - Coffee & Networking Break

4:15 - 4:30 pm - Kishor Duwadi, Department of Biology, University of Western Ontario
"Silencing of selected cysteine protease genes improves recombinant protein accumulation in transgenic tobacco plants"

4:30 - 4:45 pm - Melissa Bredow, Department of Biology, Queen's University
"Secretion of antifreeze protein isoforms from the freeze-tolerant perennial grass, Lolium perenne and their ability to confer freezing tolerance"

4:45 - 5:00 pm - Sean Miletic, Department of Biology, University of Western Ontario / Agriculture and Agri-Foods Canada, London, Ontario
"A plant-made vaccine to protect cattle against Shiga toxin-producing Escherichia coli"

5:00 - 5:15 pm - Reza Saberianfar, Department of Biology, University of Western Ontario / Agriculture and Agri-Foods Canada, London, Ontario
"Role of elastin-like polypeptide and hydrophobin for protein body formation in Nicotiana benthamiana leaves"

5:15 - 5:30 pm - Won-Sik Kim, Norgen Biotek Corp., Thorold, Ontario
"The importance of sample preparation for plant miRNA purification"

5:30 - 5:45 pm - Dominique Michaud, Département de phytologie, Université Laval
"Tomato cystatin SlCYS8–A versatile expression partner for recombinant proteins in plant biofactories"

5:45 pm – Free Evening to Enjoy Downtown Montreal

WEDNESDAY MAY 14

8:00 - 11:00 am & 1:00 - 3:00 pm: Registration Desk Open

8:00 am - Continental Breakfast

9:00 am - 12:00 pm - SESSION III: MOLECULAR BREEDING AND OMICS

Session Chairs: Dr Jean-Benoit Charron (McGill University), Mr Surinder Singh (McGill University)

9:00 - 9:45 am - Keynote Speaker:
Dr. Peter Pauls, Department of Plant Agriculture, University of Guelph
"Shared Synteny between Bean and Soybean Leads to Understanding of Gene Function and Molecular Selection Tools in Both Crops"

9:45 - 10:05 am - Banyar Aung, Agriculture and Agri-Food Canada, London, Ontario
"The miR156-SPL gene network regulates flowering and shoot branching in alfalfa"

10:05 - 10:25 am - Surinder Singh, Department of Plant Science, McGill University
"Functional characterization of barley malting quality QTL"

10:30 - 11:00 am – Coffee & Networking Break

11:00 - 11:30 am - P. Srinivasa Rao, International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India
"Molecular breeding approaches to enhance sweet sorghum (Sorghum bicolour L. Moench) productivity"

11:30 - 11:50 am - Xuan Yang, Department of Biology, University of Western Ontario / Agriculture and Agri-Foods Canada, London, Ontario
"Optimizing Gluconacetobacter diazotrophicus Inoculation Methods and Assessing the Involvement of Candidate Plant Genes in Colonization of G. diazotrophicus in Brachypodium distachyon"

11:50 am - 12:10 pm - Liyao Ji – Department of Plant Science, McGill University
"Functional food value of cooked Columbian potato (Solanum tuberosum Group phureja)"

12:10 - 12:30 pm - Parham Haddadi, INRA -Institut Jean-Pierre Bourgin, Versailles, France
"Genome-wide analysis of natural quantitative variation in response to water-deficit stress in Arabidopsis thaliana"

12:30 - 1:30pm – Lunch Buffet

1:30 - 2:30 pm – Poster Session II – Odd Numbered Posters Presented

2:30 - 5:30 pm - SESSION IV:
METABOLISM AND METABOLIC ENGINEERING

Session Chairs: Dr Jean Rivoal (IRBV, Universite de Montreal), Dr Tamara Western (McGill University)

2:30 - 3:15 pm - Keynote Speaker:
Dr. Vincenzo De Luca, Department of Biological Sciences, Brock University
"Discovery and metabolic engineering of iridoid/secoiridoid and monoterpenoid indole alkaloid biosynthesis"

3:15 - 3:30 pm - Konstantinos Aliferis, Department of Plant Science, McGill University
"Advanced High-throughput Metabolomics in Biomarker-Assisted Plant Selection"

3:30 - 3:45 pm - Pramod Kumar Rathor, Department of Environmental Sciences, Dalhousie University
"Arabidopsis thaliana Glycine Rich-Ribosome Binding Protein 3 (AtGR-RBP3) is a negative regulator of salinity stress"

3:45 - 4:15 pm - Coffee & Networking Break

4:15 - 4:35 pm - Jean Rivoal, Institut de Recherche en Biologie Végétale, Université de Montréal
"Manipulation of cytosolic nucleoside diphosphate kinase affects carbon metabolism and growth in transgenic Solanum tuberosum roots"

4:35 - 4:55 pm - Owen Rowland, Department of Biology, Carleton University
"Production of Commercially Relevant Fatty Alcohols and Wax Esters in Engineered Oilseed Crops and Microorganisms"

4:55 - 5:15 pm - Dan Riggs, Department of Biological Sciences, University of Toronto
"KISS ME DEADLY proteins modulate phenylalanine ammonia lyases to alter partitioning of secondary metabolites"

6:00 - 7:00 pm – Reception

7:00 - 9:00 pm –Banquet & Awards for Distinguished Plant Biotechnologist & Student Presentations
(Band performing: Ste-Anne-en-Gigue)

THURSDAY MAY 15

8:00 - 11:00 am Registration Desk Open

8:00 am - Continental Breakfast

9:00 am - 12:00 pm - SESSION V:
COMMERCIALIZATION OF PLANT BIOTECHNOLOGY
Session Chair: Dr Yafan Huang (Performance Plants Inc, Kingston)

9:00 - 9:45 am - Keynote Speaker:
Mr. Dave Smardon, President & CEO, Bioenterprise
"Agriculture is the new wave"

9:45 - 10:10 am - Dr. Shujun Yang, Sr. Research Scientist, Performance Plants Inc.
"A novel receptor-like protein kinase involved in regulation of water use efficiency in Arabidopsis"

10:10 - 10:35 am - Dr. Anker P. Sørensen, Vice President, New Business, Keygene Inc.
"Crop Improvement with KeyGene"

10:45-11:15 am - Coffee & Networking Break

11:15 - 11:40 am - Dr. Kevin Gellatly, Regulatory Affairs Manager, Monsanto Canada Inc.
"Facilitating a Path to Market for Biotech Crops"

11:40 am - 12:05 pm - Mr. Jeffrey Seitz, Vice President, D-Mark Biosciences
"Expediting commercialization: acceleration and innovation of sample preparation methods for generating high quality data"

12:15 - 12:30 pm - Closing Address

Dr Lining Tian, Co-Chair 9th CPBC, Agriculture & Agri-Food Canada, London, Ontario

12:30 pm Conference Closed – Enjoy Montreal!

KEYNOTE & ORAL ABSTRACTS

Session I: Plant Regeneration and Genetic Engineering

K1: Alteration of Thioredoxin Expression in Cereals Leads to Unexpected Improvements in Grain Properties - But Where Are They?

Peggy G. Lemaux
Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720

Work primarily with cereals (barley, wheat, sorghum) has established thioredoxin *h* (Trx *h*) as a central regulatory protein in seeds. Trx *h* acts by reducing disulfide (S-S) groups of diverse, yet specific, seed proteins (storage proteins, enzymes and enzyme inhibitors). Early *in vitro* protein studies, demonstrating effects of Trx on enzymatic activities associated with seeds, were complemented with studies in barley grain in which Trx *h5* was overexpressed in the endosperm. This grain showed accelerated germination and early or enhanced expression of starch-degrading enzymes, traits important to the malting and brewing industry. More recent studies have used transgenic approaches in wheat to alter Trx *h* expression. In one study, Trx *h5* overexpression, driven by a hordein promoter and its protein body targeting sequence, led to changes commensurate with earlier *in vitro* work, *i.e.*, increased solubility of disulfide proteins and decreased allergenicity. In a second study with Chinese collaborators in wheat, an antisense Trx *h9* construct, driven by an α-gliadin promoter, resulted in underexpression of that gene in the cytosol. The engineered grain showed effects opposite those in barley grain overexpressing Trx *h5*. Germination was retarded and spikes from field-grown material showed a dramatic delay in preharvest sprouting, had increased grain yield irrespective of humidity conditions, and baking quality was identical to nonengineered grain. Lastly, overexpression of Trx *h5* in sorghum grain increases protein digestibility, likely due effects on the sulfur-rich seed storage proteins. In total, these results provide evidence that Trx *h* levels in cereal endosperm are linked to seed properties and to potential applications. But, none of these applications are in the marketplace for several reasons, one of which relates to reluctance of the market to embrace genetically engineered foods. Scientists have a responsibility to know the facts about the technologies that are being used and to engage the public in dialogue.

O1: Somatic embryogenesis as a breeding tool in American chestnut (Castanea dentata (Marsh.) Borkh.)

Christie Lovat, Danielle J. Donnelly
McGill University, 21 111 Lakeshore Road Ste-Anne-de-Bellevue, QC, H9X 3V9

Somatic embryogenesis is a technique which generates entire plants from small tissue explants. An indirect effect of this technique is the production of somaclonal variants; somatic plantlets which have a different phenotype than their source plant (Scowcroft 1985). Little work has been done investigating methods to regulate the generation of somaclonal variants (Scowcroft 1985). This has prevented somatic embryogenesis from becoming a breeders' tool. American chestnut (Castanea dentata (Marsh.) Borkh.) is a tree native to eastern North America (Burnham 1988). In the 1900s, the introduced fungal species Cryphonectria parasitica (Murrill) Barr. devastated C. dentata, and it has yet to recover (Burnham 1988). It is the goal of this study to investigate techniques to both minimize and maximize the production of somaclonal variants of C. dentata. A range of explant types will be investigated for their effect on the production of somatic variants. As well, the somatic embryogenesis of C. dentata will be refined to improve embryo production and plantlet survival. Regenerated plants will be screened for the three most problematic fungi affecting C. dentata. This study will demonstrate that somatic embryogenesis can be an invaluable breeders' tool, and may result in somatic variants of C. dentata with improved disease tolerance.

O2: Towards Introducing *Ac/Ds* Transposons into *Hordeum spontaneum* to Capture Genetic Diversity

Marie-Josée Cardinal and Jaswinder Singh
Department of Plant Science, McGill University, Sainte Anne de Bellevue, QC H9X 3V9

Domestication of cultivated plants has triggered erosion of genetic diversity of important stress related alleles. Researchers highlight the potential of using wild accessions as a gene source for improvement of cereals such as barley, which has major economic and social importance worldwide. Based on the introduction of the maize *Ac/Ds* transposon system for gene identification in cultivated barley, the objective of this research is to investigate the response of *Hordeum spontaneum* accessions in tissue culture to standardize parameters for introduction of *Ac/Ds* transposons into the wild barley genome through genetic transformation. We investigated the response of 10 wild barley genotypes for callus induction, regenerative green callus induction and regeneration of fertile plants. The activity of exogenous *Ac/Ds* elements was observed through a transient assay on immature wild barley embryos/callus. Transformed calli were identified using hygromycin as a selectable agent in the media and by the expression of GFP. Forty six bombardment experiments were performed on 3952 pieces of callus (3-5 mm each) in 10 genotypes of wild barley. The transformation frequency of putative transgenic lines based on the GFP reporter system ranged from 0-23.19% in wild barley genotypes. Molecular screening for *Ac/Ds* transgenic plants are being performed for further confirmation.

O3: Screening Quebec-bred Potato Genotypes for Functional Food Properties

Christina Larder[1, 2], Atef Nassar[1,2] Stan Kubow[2] and Danielle Donnelly[1]
[1]Plant Science Department McGill University, 21,111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, H9X 3V9, Canada; [2]School of Dietetics and Human Nutrition, McGill University, 21,111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, H9X 3V9, Canada

Potato (Solanum tubersum L.) is a staple crop that plays an important role in human nutrition. Potato starch contains rapid digestible starch (RDS) that rapidly increases blood glucose, slowly digestible starch (SDS) that causes a slow blood glucose increase and resistant starch (RS) that does not yield absorbable glucose. The RDS, SDS and RS content of foods partly determine the glycemic load (GL), which is related to improved blood glucose control and chronic disease prevention. An increase in the ratio of amylose:amylopectin, degree of phosphorylation, concentration of total soluble protein and polyphenols in potatoes could result in decreased RDS and increased SDS and RS, which lead to lower GL. Potato starch when cooked and cooled causes a rearrangement of amylose and amylopectin called retrogradation resulting in increased RS. In vitro hydrolysis of food starch is an important tool to predict the in vivo glycemic impact of foods. The in vitro enzymatic digestion methodology described herein can be used to screen advanced potato selections for genotypes with more SDS and RS (lower predicted GL). These techniques enabled identification of superior genotypes in two Quebec breeding programs. The outcome will be Quebec potato cultivars that can be promoted for their reduced GL.

O4: Transgenic soybean confers drought tolerance

Lei Bo[1], Dinghui Wang[1], Zhen Li[1], Youzhi Ma[2],Hongxia Zhang[3] and Wenbin Li[1]
[1]Key Laboratory of Soybean Biology in Chinese Education Ministry, Northeast Agricultural University, Harbin, P. R. China 150030; [2] Chinese Agricultural Academy; [3]Chinese Science Academy

Drought is the serious threat to the sustainability of soybean production. Breeding drought-tolerant soybean prove to be a faster track toward preventing soybean yield loss. The drought-resistant genes GmNFYB1, TaDREB3a, ThIPK2 were transferred into cultivated soybean. GmNFYB1 was selected from GmNFYB family which was up-regulated by drought and ABA. Transgenic soybeans showed higher ratio of root/shoot and well developed root system. Water and proline content of leaf increased under drought condition. Transgenic soybean produced more effective pods and seeds than non-transgenic controls. TaDREB3a was isolated from wheat, encoded a member of the ERF/AP2 transcription factor family protein. Transgenic soybeans with TaDREB3a gene confer a strong tolerance to drought condition, showing a significant yield advantage in two arid regions with different drought scales. The physiological index results indicated that proline content, SOD activity and CAT activity were obviously higher than controls. ThIPK2 was an inositol polyphosphate kinase gene homolog isolated from Thellungiella halophila. Transgenic soybean with ThIPK2 gene displayed water deficit- and oxidative-tolerance compared to non-transformed controls. The impacts of GmNFYB1, aDREB3a, ThIPK2 transgenic soybeans on environment, biodiversity and animals were also analyzed, which proved the security of these genes applied to produce transgenic soybean.

O5: Zinc-finger nuclease Technology for novel trait development in *Triticale* cv. Ultima

Priti Maheshwari and Francois Eudes
Agriculture and Agri-Food Canada,5403 - 1 Avenue South / 5403 1e avenue sud Lethbridge, Alberta, Canada T1J 4B1

Zinc-finger nucleases (ZFNs) are powerful tools for site-specific genome editing enabling broad range of genetic modifications by inducing DNA double-strand breaks that stimulate error-prone non-homologous end joining or homology-directed repair at specific genomic locations. In the present investigation Nanocarrier mediated co-delivery of DNA encoding site specific Zinc Finger Nucleases, a single strand donor DNA and an Homologous Recombination related RecA protein was attempted in microspores derived from a double haploid line of triticale cv. Ultima to facilitate analysis of non-GMO OMT gene knockouts in the progeny for a novel trait development. Three candidate ZFN sequences were designed for the exon 1 and 2 of OMT gene of Ultima. The Donor DNA which is a mutated version of the wild OMT gene will contribute towards the site targeted mutation of Ultima OMT first exon resulting in loss of gene function. The resulting Triticale OMT gene knockouts exhibiting low lignin straw would enhance the competitiveness of triticale feedstock for the energy, biochemical and biomaterial value propositions.

O6: Auxin response factor functions in plant regeneration - molecular control of de novo shoot formation

Wenzislava Ckurshumova, Naden T. Krogan, Tatiana Smirnova and Thomas Berleth
Department of Cell and Systems Biology, University of Toronto

In vitro regeneration of complete organisms from diverse cell types is a spectacular property of plant cells. Hormone-exposure protocols that trigger the de novo formation of either roots or shoots from callus tissue demonstrate the importance of auxin and cytokinin signaling pathways, and genetic differences in these pathways may contribute to the highly divergent responsiveness of plant species to regeneration protocols. We show that signaling through MONOPTEROS (MP)/AUXIN RESPONSE FACTOR 5 is necessary for the formation of shoots from Arabidopsis calli. Most strinkingly, variants of MP, but not of other ARFs, turned out to be sufficient for promoting de novo shoot formation through pathways involving the homeobox transcription factor SHOOT MERISTEMLESS (STM) and AP2 domain transcription factor CYTOKININ RESPONSE FACTOR2 (CRF2). Our findings provide an entry point to better address the molecular genetics and transcriptomics underlying divergent regeneration properties in various tissues and species. We also demonstrate how transcription factor design can selectively improve regeneration properties without interfering with other growth properties of the plant.

O7: Application of high throughput phenotyping to study early phase drought stress

Stephen Hunt[1], Klára Šimková[2], Sharmila Madhavan[2], Zuzana Benedikty[2] ,
Diana Santelia[2] and Martin Trtílek[2]
[1]*Qubit Systems Inc., 1573 John Counter Blvd., Kingston, Ontario, Canada K7M3L5*
[2]*Photon Systems Instruments, Drásov, Czech Republic.*

Recently we have used the PlantScreen Plant Phenotyping System developed by Qubit Systems Inc. to study the response of various plants to drought stress conditions, including Arabidopsis starch-related mutants. We have optimized the screening conditions and the image processing analyses to obtain quantitative assessment of complex traits such as growth, development and photosynthetic status. By using combinations of chlorophyll fluorescence imaging, thermal imaging and morphological image analysis we have recognized a set of parameters that could serve as early stress markers for characterization of plant performance under adverse environmental conditions.

O8: Host-induced gene silencing of *Puccinia triticina* genes shows enhanced resistance to leaf rust disease in transgenic wheat plants

Vinay Panwar [1], Pierre Fobert[3], Mark Jordan[1], Brent McCallum[1], and Guus Bakkeren[2]
[1]*Morden Research Centre, Agriculture and Agri-Food Canada, Morden, MB, R6M 1Y5, Canada*
[1]*National Research Council Canada, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9, Canada*
[3]*Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, BC, V0H 1Z0, Canada,*

Rust fungi are among the most economically important plant pathogen. Their obligate biotophic nature obviates many established genetic methods for developing effective resistance resources. Rust fungal genomics is now entering an advanced phase leading to prediction of many genes important for infection and growth. Recently, in a proof-of-concept study, we demonstrated the use of host-induced gene silencing (HIGS) as a powerful reverse genetics tool to study the function of predicted pathogenicity genes of rust fungi using transient RNA-interference (RNAi) approaches. Here we show that we can achieve HIGS in transgenic wheat plants by knocking down the expression of two selected leaf rust fungus, Puccinia triticina (Pt) genes, namely MAP-kinase (PtMAPK1) and cyclophilin (PtCYC), resulting in effective disease suppression. Transgenic wheat plants expressing RNAi constructs for the target Pt genes showed accumulation of corresponding small interfering RNA (siRNA) molecules. Disease suppression suggests successful translocation of silencing molecules targeting Pt genes from transgenic wheat plants into interacting fungi. In some generated wheat lines, fungal biomass was reduced to 20% of control plant infections. The results indicate that engineering and expression of essential rust fungal genes in host plants can be used as an effective strategy for developing rust disease resistance in wheat.

SESSION II: BIOMATERIALS AND MOLECULAR FARMING

K2: Medicago: plant-made influenza vaccines and beyond

Marc-André D'Aoust
Medicago inc. 1020 Route de l'Église, Bureau 600, Québec (Qc), Canada, G1V 3V9

Medicago is pioneering the development of a new generation of vaccines based on virus-like particles (VLPs) using a plant-based manufacturing technology. The company's main pipeline products include pandemic and seasonal influenza vaccines. Our influenza vaccine substance consists of influenza VLPs which are particles of approximately 130 nm in size constituted of virus hemagglutinin anchored in a phospholipid bilayer (envelope). These VLPs present a major advantage over whole inactivated or split viruses as they mimic the cognate virus antigenic surface, thereby triggering a strong and long-lasting immune response, while being not infectious. The pandemic vaccine development program, based on H5 VLPs, is currently in clinical development with a phase II trial in human. Current clinical results indicate that the pandemic vaccine is safe and induces protective levels of antibodies.
The presentation will focus on how the transient expression technology has been brought to commercial scale, on the clinical development of the influenza vaccine and on the high-throughput screening platform developed to accelerate the discovery of new vaccine candidates.

O9: Transplastomic plants for high level production of pharmaceuticals and industrial enzymes

Rima Menassa, Igor Kolotilin, Angelo Kaldis and Hong Zhu
Agriculture and Agri-Food Canada, 1391 Sandford St., London, ON, N5V 4T3.

To achieve economical production of recombinant proteins, a production system must be scalable, produce high levels, be safe, and be able to carry out post-translational modifications. In this presentation I will make the case for transplastomic tobacco as a production platform for recombinant proteins. We built a suite of chloroplast expression vectors for optimizing the production of a bacterial and two fungal xylanases. We found that different configurations of the cis control elements had significant effects on gene expression and protein accumulation. This comparative analysis allowed us to identify the best vector for production and purification of a subunit vaccine for enterotoxigenic E. coli (ETEC), the causal agent of post-weaning diarrhea of piglets. F4 fimbriae of ETEC are highly stable proteinaceous polymers, mainly composed of the major subunit FaeG, a candidate subunit vaccine. We used a variant of the FaeG protein, FaeGntd/dsc, engineered for expression as a stable monomer by N-terminal deletion and donor strand-complementation (ntd/dsc). Transplastomic plants produced FaeGntd/dsc very efficiently at 2g/kg fresh leaf weight. Purified rFaeGntd/dsc was able to bind to porcine epithelial F4 receptors and competed with F4+ ETEC for receptor binding. We purified 25 g of rFaeGntd/dsc , and are currently working on formulation for oral immunization of piglets this fall.

O10: Silencing of selected cysteine protease genes improves recombinant protein accumulation in transgenic tobacco plants

Kishor Duwadi[1], Ling Chen[2], Rima Menassa[1, 2], Sangeeta Dhaubhadel[1, 2]
[1]*Department of Biology, University of Western Ontario, London, ON, Canada*
[2]*Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON, Canada*

Plants are an attractive host system for pharmaceutical protein production. Many therapeutic proteins have been produced and scaled up in plants at a low cost compared to the conventional production systems. The main technical challenge during this process is to produce sufficient level of protein in plants. Low yield is generally caused by proteolytic degradation during expression and downstream processing of recombinant proteins. One approach to overcome proteolytic degradation involves creation of stable transgenic lines with reduced proteolytic activity. Recently, it has been found that cysteine protease (CysP) inhibitors show protective effect on human immune-regulatory interleukin-10 (IL-10) produced in transgenic tobacco plants. To identify CysP gene(s) involved in IL-10 accumulation, the DFCI Nicotiana tabacum gene index was searched which revealed a total of 55 putative CysPs. Based on their expression in leaf tissue, 10 candidate CysPs were selected for further characterization. Overexpression and silencing constructs were made for all10 candidate CysPs in order to study the effect of the selected CysP genes in tobacco lines that overexpresses IL-10 protein (WT-IL-10). Agrobacteria-mediated plant transformation technology was utilized to generate transgenic lines with reduced CysP and increased IL-10 accumulation. Using Enzyme linked immunosorbent assay it was found that recombinant protein yield could be increased up to 1.6 fold in T0 CysP silenced plants in comparison to the level present in WT-IL-10 tobacco plants.

O11: Secretion of antifreeze protein isoforms from the freeze-tolerant perennial grass, *Lolium perenne* and their ability to confer freezing tolerance

Melissa Bredow, Barbara Vanderbeld, Lena Dolman, Kyle Lauersen, Sharon Regan and Virginia K. Walker
Department of Biology, Queen's University, Kingston, ON

Temperate perennials must prevent freezing-induced damage. As a consequence, some plants express antifreeze proteins (AFPs), which constrain the growth of apoplastic ice-crystals through a process called ice-recrystallization inhibition (IRI). These proteins are also capable of modestly lowering the freezing point of intercellular fluids. Lolium perenne (Lp) is a freeze-tolerant grass that expresses four AFP isoforms. The fully processed LpIRI3 protein, LpAFP, has been extensively characterized. Following cold acclimation, AFP activity has been identified in the apoplast. This localization is presumably led by N-terminal signal sequences. One isoform, LpIRI2, appears to have undergone almost a complete deletion of the N-terminal domain, including any recognizable signal sequence. Rather than acting as a pseudogene or non-functional allele, LpIRI2 may be secreted via a non-classical pathway or function intracellularly. Fluorescently-tagged LpIRI2 and LpIRI3 transgenic lines have been developed in both Arabidopsis thaliana and the freeze tolerant rye grass, Brachypodium distachyon, to visually localize AFP expression. Additionally, LpAFP, LpIRI3, and LpIRI2 expression lines have been generated in A. thaliana and assessed for freezing tolerance using electrolyte leakage and survival assays. Although other IRI isoforms are likely classically secreted, we believe that LpIRI2 is secreted in the absence of a recognizable signal sequence, as seen in some defense-related proteins.

O12: A plant-made vaccine to protect cattle against Shiga toxin-producing *Escherichia coli*

Sean Miletic[1,2] Angelo Kaldis[2], Jacqueline MacDonald[2], Antoine Leuthreau[2] and Rima Menassa[2]
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The overuse of antibiotics in livestock rearing has set off international alarms as antibiotic-resistant bacteria become more prominent, posing a major threat to public health. This highlights the need for alternatives to control and limit the spread of pathogenic bacteria. Shiga toxin-producing Escherichia coli (STEC) are a common pathogen infecting thousands of humans and animals every year. Immunizing cattle herds against STEC can reduce contamination in the food supply and reduce human infection. Despite this, current vaccines only offer serotype-specific protection. The goal of this project is to produce a plant-made vaccine to provide multiple-serotype protection against STEC in ruminants. Immunogenic antigens from different serotypes were produced transiently in the leaves of Nicotiana benthamiana. Proteins were targeted to different intracellular compartments and were fused to hydrophobin or elastin-like polypeptide (ELP) tags to promote protein accumulation. These STEC genes were also bombarded into the plastid genome of Nicotiana tabacum for stable, high-yield protein accumulation. Accumulating antigens will then be tested for their immunogenicity and efficacy in challenge experiments in a goat ruminant model.

O13: Role of elastin-like polypeptide and hydrophobin for protein body formation in *Nicotiana benthamiana* leaves.

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Elastin-like polypeptide (ELP) and hydrophobin (HFBI) are two types of fusion tags shown to increase the accumulation levels of recombinant proteins and induce the formation of protein bodies (PBs) when transiently expressed in Nicotiana benthamiana leaves. PBs are endoplasmic reticulum (ER) derived organelles originally found in seeds and required for accumulation of large amounts of proteins. The mechanism by which the ELP and HFBI fusion tags induce the formation of PBs is not well understood.
We have studied the effects of several elements involved in the process of PB formation. Our results show that: 1. ER retrieval of proteins is required for PB formation 2. PBs remain part of the ER endomembrane system after maturation and actively exchange their content through ER 3. HFBI induces PBs smaller in size compared to ELP 4. PB size and protein accumulation levels are co-dependent factors involved in the formation of PBs 5. PB formation is a passive mechanism and proteins targeted to the secretory pathway can get sequestered into the PBs. We have utilized this property of PBs as a tool in order to increase the accumulation levels of erythropoietin (a low accumulating protein) by co-expression with PB-inducing proteins.

O14: The importance of sample preparation for plant miRNA purification

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MicroRNAs are endogenous 20 to 24 nucleotide noncoding RNAs that play crucial posttranscriptional regulatory roles in plant and animals. Tremendous efforts are currently being undertaken to understand the profile of the entire miRNA population of a biological sample, which will provide useful information on miRNA activity. Many miRNA discovery tools, including micro arrays and Next-gen-based sequencing, have made it possible to comprehensively and accurately assess the entire miRNA repertoire.This presentation deals with the importance of sample preparation on downstream applications. A prerequisite for obtaining successful results from these approaches is an efficient method for total RNA purification without bias. The choice of the method of RNA purification is critical to the outcome of downstream analysis. This is made more significant in variations of the plant specimens and the high phenolics, starch and other inhibitors co-isolating with the RNA. The three most popular RNA purification methods (spin columns using Silicon Carbide, spin columns employing silica membrane and phenol/chloroform extraction) are compared in this talk in terms of quality, quantity and small RNA recovery from difficult and moderately challenging plant samples. Examples of microRNA study cases will be also discussed to highlight the importance of the RNA purification method used for different plant species.

O15: Tomato cystatin SlCYS8–A versatile coexpression partner for recombinant proteins in plant protein biofactories

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Tomato cystatin SlCYS8 is a useful protease inhibitor model in the study of plant–insect interactions, and a good candidate for the development of pest-resistant transgenic crops. Here I discuss the potential of this protein as a versatile coexpression partner for the stabilization, high-yield expression and post-harvest purification of clinically useful recombinant proteins produced in plants. The strong inhibitory effects of SlCYS8 against endogenous proteases in host plant tissues make this protein an attractive coexpression partner for the *in situ* stabilization of protease-susceptible proteins, especially during their migration along the cell secretory pathway. The very high production and translation rates of slcys8 transcripts in plant cells also make this protein useful as an N-terminal translational fusion partner to boost the production of recombinant proteins expressed otherwise at lower levels. The intrinsic robustness and well defined structure of this protein make it useful, finally, for the design of fusion variants and affinity chromatography procedures that allow for an efficient, single-step purification of the expressed proteins following extraction. These multiple uses of SlCYS8 in plant systems are illustrated here with mammalian and human proteins transiently expressed in agro infiltrated leaves of the well characterized expression host *Nicotiana benthamiana*.

SESSION III: MOLECULAR BREEDING AND OMICS

K3: Shared Synteny between Bean and Soybean Builds Understanding of Gene Function in Both Crops

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The availability of whole genome sequences for common bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L. Merr) has enabled detailed comparisons to be made of their genomes. These studies confirm that for most genes in common bean, two homologous genes occur in soybean. The DNA sequence correspondence between common bean and soybean agrees to a large extent with previously reported patterns of shared synteny between these two species and supports conclusions that the soybean genome was duplicated after the establishment of Phaseolus and Glycine from a common progenitor.
We used *in silico* analyses to identify the genomic locations of over 100 phenylpropanoid pathway genes in common bean and soybean and examined putative relationships between these genes and genes for seed coat colour and flower colour. A similar approach was used to characterize genes for enzymes in the folate synthesis pathway as well as a gene potentially related to yield in both crops. The high degree of shared synteny between the two species facilitated the identification of gene function and allowed information from a highly characterized crop like soybean to be associated with specific loci and genes in bean.

O16: The miR156-SPL gene network regulates flowering and shoot branching in alfalfa

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MicroRNA156 (miR156) regulates various aspects of plant development, including juvenile to adult transition, shoot branching, and flowering time by post-transcriptional silencing of members of the SQUAMOUS PROMOTER-LIKE (SPL) gene family. Here, we investigated miR156 function in Lotus japonicus (Lotus) and Medicago sativa (alfalfa). In Lotus, miR156 cleaves transcripts of two SPL genes, Lj-SPL1 and Lj-SPL2. Lotus plants overexpressing miR156 showed enhanced branching, delayed flowering time, and reduced nodulation. The latter was caused by repression of a number of nodulation-related genes. We also cloned and over-expressed an alfalfa homologue of a miR156 precursor and identified its target genes, comprising three SPL genes (SPL6, SPL12 and SPL13) and two members of the WD40 family (WD40-1 and WD40-5). Transgenic alfalfa plants overexpressing miR156 showed up to a 2.5-fold increase in vegetative biomass, enhanced shoot branching and shoot length, reduced internode length resulting in more nodes, increased secondary branches and leaves per stem, higher leaf trichome density, and a moderate delay in flowering time. The multitude of traits affected by miR156 are likely due to the large network of genes regulated by miR156 and its target genes. Thus, these genes can be exploited to improve yield and quality of alfalfa biomass.

O17: Functional characterization of barley malting quality QTL.

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Among various functional genomics tools to characterize genes in plants, transposon-based approach offers great potential, especially in barley and wheat, which possess large genomes and genetic transformation is not a routine. Barley is a key ingredient in malting and brewing industry; therefore, gene discovery in relation to malting quality has an industrial perspective. Malting quality is a complex and quantitatively inherited trait. QTL2, one of the important QTL (quantitative trait loci) affecting malting quality traits has been located on chromosome 4H. We employed transposon- and synteny- based approaches to dissect QTL2 region of barley. A *Ds* transposon insertion line (TNP), TNP- 29 was previously mapped in the vicinity of QTL2. Reactivations of *Ds* transposon from this TNP line lead to identification of genes harboring in this QTL2 region. Alternatively, barley-rice synteny approach detected 24 candidate genes; one of them shows differential gene expression among malting and non-malting barley varieties. Biochemical analysis further confirms these findings. Integrated effort of saturation mutagenesis with *Ds* transposons and synteny- based approach will lead to a better understanding of malting quality traits and candidate genes that display quantitative variation.

O18: Molecular breeding approaches to enhance sweet sorghum (*Sorghum bicolour* L. Moench) productivity

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The rapidly growing population worldwide and the emerging economies in Asia, South America and Africa, coupled with urbanization and declining fossil fuel resources has led to increased attention on alternative sources of energy, economically efficient and ecologically sustainable. Sweet sorghum is the same species as grain sorghum (Sorghum bicolour L. Moench), except for the sweet juice in the stalk tissues. It has been traditionally used as livestock fodder due to its ability to produce excellent silage and, as grain sorghum, it grows well under warm, dry conditions. This feedstock has greater role to augment renewable energy through conversion of sweet juice or biomass/bagasse to biofuels. Both staygreen and brown midrib (bmr) traits are important for sweet sorghum popularisation as the former enhances terminal drought tolerance and the later enhances stover quality. Stay green (Stg) trait in sorghum is characterized by the plant's ability to tolerate post flowering drought stress. In sorghum, stay green QTLs and linked markers were identified which facilitates their introgression into elite cultivars. In sorghum, stay green QTLs 1, 2, 3, 4 and QTLs A, B, C were identified. These QTLs affect several water related traits under post flowering drought stress, although, it depends on the genetic background. Stg 1 and Stg 3 were found to be having role in water extraction, whereas Stg B was found to be responsible for high transpiration efficiency. This will further help estimate genetic variation in sweet sorghum germplasm and identify new/better alleles for target traits. ICRISAT has successfully transferred Stg3 and Stg B QTLs in to promising sweet sorghum cultivars like ICSV 93046, SPV 1411 and ICSV 25280 through backcross method of breeding (BC2F1s). Apart from biofuel production sweet sorghum is widely used as fodder. Hence, introgression of low lignin conferring bmr alleles offers ample opportunities for enhancing its utilization. Three mutants bmr 6, 12 and 18 are popular. The bmr 6 plants were shown to have limited cinnamyl alcohol dehydrogenase (CAD) activity, the enzyme that catalyzes the conversion of hydroxycinnamoyl aldehydes (monolignals) to monolignols; while bmr 12 plants have reduced activity of caffeic acid O-methyl transferase (COMT) that catalyzes the addition of a methyl group to 5-OH-conferyl alcohol in monolignol biosynthetic pathway. The bmr 6 and 12 alleles are being transferred to elite sweet sorghum cultivars viz. ICSV 93046, ICSV 25275, ICSV 25280, ICSV 25315 and introgression is at advanced stage (BC2F1). Low lignin sweet sorghum cultivars are expected to play greater role both in dairy and biofuel industries.

O19: Optimizing *Gluconacetobacter diazotrophicus* Inoculation Methods and Assessing the Involvement of Candidate Plant Genes in Colonization of *G. diazotrophicus* in *Brachypodium distachyon*

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To study the nitrogen fixation of Gluconacetobacter diazotrophicus in different monocot crops, research was conducted to establish and optimize methods for introducing G. diazotrophicus into Brachypodium distachyon, a model monocot for molecular biology and genomics research. The colonization of G. diazotrophicus in B. distachyon was established through different inoculation methods with or without N supply. While Colonization was successful in all the conditions, the efficiency was high under hydroponic condition without N supply. The results indicate G. dizatrophicus can be introduced into B. distachyon, but the colonization efficiency can be influenced by nitrogen level in the growth media and the medium texture (4.3%-81.9%). To investigate the involvement of plant gene regulation in G. diazotrophicus colonization, global gene expression was conducted using next generation sequencing to generate the differential gene expression profile. The preliminary RNA sequencing result found more than 100 genes are differently expressed upon G. diazotrophicus colonization. The gene ontology and pathway analysis of these differentially expressed genes are still ongoing to further narrow down the candidate plant genes that are important for successful G. diazotrophicus colonization.

O20: Functional food value of cooked Columbian potato (*Solanum tuberosum* Group *phureja)*

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Potato, due to its high consumption worldwide, can be exploited for the delivery of functional foods, especially for the poor who do not take any supplements. In this project, the functional food-related metabolites in cooked tubers of eight diploid potato genotypes from Columbia were explored. The plants were grown under field conditions in a randomized block design with eight genotypes and three replications. Tubers were harvested, lyophilized and stored at -80°C. Metabolites were extracted from flesh samples using 60% aqueous methanol and analyzed using an LC-HRMS system. The data files were processed using MZmine-2 software. The peaks were putatively identified based on accurate mass error (AME) < 5ppm and fragmentation patterns. A total of 302 metabolites were putatively identified and these metabolites belonged to different chemical groups such as phenylproponoids, flavonoids, alkaloids, fatty acids, and terpenoids. The functional food properties of these identified metabolites were searched in databases and literature. A total of 98 metabolites were associated with health-benefiting roles for humans, such as anticancer, anti-inflammatory, antioxidant, antitumor, antimicrobial, and antiviral.

O21: Genome-wide analysis of natural quantitative variation in response to water-deficit stress in *Arabidopsis thaliana*

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The genetic basis of most traits is complex, involving many genes that interact with each other and the environment. To date as an alternative to generating laboratory-induced mutants, it is relatively popular to use naturally-occurring variation among genetically distant Arabidopsis thaliana accessions as the source of quantitative genomics approaches, designed to map quantitative trait loci (QTLs) and try and resolve them at the gene level. The objective of our work is to apply genome-wide quantitative molecular genetics to both, a very integrative and classical quantitative trait (shoot growth) and a molecular trait a priori more directly linked to the source of variation (gene expression under cis-regulation), in both cases studied in interaction with the water-deficit stress. We are using a combination of our unique high-troughput phenotyping robot (the Phenoscope), fine-mapping, and complementation approaches to pinpoint a significant number of QTLs and eQTLs to the gene level and identify causative polymorphisms and the molecular variation controlling natural diversity.

SESSION IV: METABOLISM AND METABOLIC ENGINEERING

K4: Discovery and metabolic engineering of iridoid/secoiridoid and monoterpenoid indole alkaloid biosynthesis.

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The Madagascar Periwinkle (*Catharanthus roseus*) has been developed into an increasingly widely used model system to study the cell and molecular biology of monoterpenoid indole alkaloid biosynthesis MIA (1) Recent successes in the molecular and biochemical characterization of the iridoid/secoiridoid [2-4] and monoterpenoid indole alkaloid pathways and their transport [5] have been achieved through rapid comparative bioinformatics guided identification of candidate genes combined with *in planta* virus induced gene silencing approaches and functional characterization of selected genes in the *Catharanthus roseus* model system. These discoveries have led to the complete elucidation of the pathways for strictosidine biosynthesis, the precursor of thousands of biologically active iridoids and MIAs.These studies have broad implications for advancing investigations about the evolutionary and ecological roles played by these metabolites in Nature as well as for their use in biotechnological production of useful iridoids/MIAs.

O22: Advanced High-throughput Metabolomics in Biomarker-Assisted Plant selection

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In addition to the classical protocols for plant selection, innovative, high-throughput and cost effective methods are required for precise plant breeding in order to successfully respond to the challenges of modern times. Being closer to the phenome compared to other "omics", metabolomics represents an excellent tool for the robust classification of plant cultivars and their selection based on desired traits. The use and integration of data from various analytical platforms combined with advanced bioinformatics software and species-specific metabolite libraries enable the recording, deconvolution, and monitoring of the analyzed metabolomes and their fluctuation in response to biotic and abiotic stimuli. The discovery of the corresponding metabolite-biomarkers of effect could be used for the development of validated protocols for the discrimination between cultivars of plants and their selection following a biomarker-assisted strategy. Here, as a proof of concept, advanced high-throughput metabolomics protocols will be presented for the biomarker-assisted selection of major crops such as, soybean (*Glycine max* L.) and potato (*Solanum tuberosum* L.) for resistance against the soil fungal pathogen *Rhizoctonia solani*, and willow cultivars (*Salix sp.*) on the basis of their phytoremediation capacity.

O23: *Arabidopsis thaliana* Glycine Rich-Ribosome Binding Protein 3 (AtGR-RBP3) is a negative regulator of salinity stress

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Salinity is one of the major abiotic stresses that affect crop production. Mechanism(s) of plant tolerance to salinity is complex and is mediated by multiple biochemical pathways. Our earlier studies have shown that Ascophyllum nodosum extracts (ANE) impart salinity tolerance in plants. To understand the molecular mechanism of salinity tolerance induced by ANE, the whole genome transcriptome of Arabidopsis thaliana was examined through microarray analysis. Analysis of ANE-induced transcriptome under salinity stress led to the identification of a number of positive and negative regulators of salinity tolerance in Arabidopsis. We systematically screened Arabidopsis single gene knockout mutants corresponding to genes that were significantly down regulated in ANE-mediated salinity tolerance. In this study we observed that plant carrying a mutation in Glycine Rich-Ribosome Binding Protein 3 (AtGR-RBP3) exhibited a marked salt tolerance compared to wild type Arabidopsis Col-0. Arabidopsis mutants rbp3-1 and rbp3-2 accumulated significantly less Na+ in the leaf tissue as compared to wild type plants when grown under high salt condition. Also, an increase in sodium: potassium (Na+/K+) ratio was observed in wild type as compared to rbp3-1 and rbp3-2. We served an increase in the expression of stress responsive genes; RD29A, RD22 and SOS1. Subcellular localization study revealed that AtGR-RBP3 was localized in the endoplasmic reticulum. Taken together, these results indicate that AtGR-RBP3 is a negative regulator of salinity stress in Arabidopsis.

O24: Manipulation of cytosolic nucleoside diphosphate kinase affects carbon metabolism and growth in transgenic *Solanum tuberosum* roots

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In plants, nucleoside diphosphate kinase (NDPK) isoforms have been described in the cytosol, in mitochondria and in plastids. A variety of functions have been assigned to plant NDPK isoforms, but no consensus exists about their importance in metabolism. In this study, we report the effects on carbon metabolism of the genetic manipulation of cytosolic NDPK in transgenic potato (Solanum tuberosum) roots. Sense and antisense NDPK constructs were introduced in potato using Agrobacterium rhizogenes to generate a population of root clones displaying a 40-fold variation in NDPK activity. We used a targeted metabolomic approach as well as measurement of various hysiological parameters to understand the impacts of this modification on metabolism. Root growth and respiration were positively correlated with NDPK expression levels. No significant variation was observed in the adenylates pools among transgenic roots. However, different NDPK expression levels led to shifts in the pools of important intermediates in primary carbon metabolism and in root starch content. Changing the level of NDPK expression also led to pleiotropic effects on the activity of other enzymes and positively affected glycolytic flux. The implication of cytosolic NDPK in the control of root carbon metabolism will be discussed in the light of these data.

O25: Production of Commercially Relevant Fatty Alcohols and Wax Esters in Engineered Oilseed Crops and Microorganisms

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Fatty alcohols and wax esters are used in a wide number of commercial applications. Primary fatty alcohols are found as ingredients in detergents, cosmetics, pharmaceutical and agrochemical formulations, as well as food products, typically acting as surfactants, thickening agents or emulsifiers. The excellent lubrication properties and stabilities of wax esters (fatty alcohols esterified with fatty acids) make them of especially high value, in particular as high-performance machinery lubricants and automobile transmission fluids. There is considerable interest in producing wax esters from genetically engineered organisms with qualities that are customized to fit specific industrial roles. One way to develop such designer wax esters would be to engineer alcohol-forming fatty acyl reductase (FAR) enzymes to specifically produce fatty alcohols of desired physical properties and which can then be incorporated into 'tailor-made' wax esters. FAR enzymes have distinct substrate specificities with regard to chain length and saturation. Engineering of FAR proteins would be aimed at both optimizing total fatty alcohol production and modifying substrate chain-length/saturation specificity. We will report on our recent advances in characterizing and engineering FAR enzymes as well as advancements in the high-level production of wax esters in oilseeds and microbes.

O26: KISS ME DEADLY proteins modulate phenylalanine ammonia lyases to alter partitioning of secondary metabolites

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Regulated proteolysis is an important regulatory mechanism known to modulate many aspects of plant metabolism and development. In an Arabidopsis microarray screen to identify targets of the master regulator BREVIPEDICELLUS, we discovered nine up-regulated genes implicated in protein degradation. Two of these encode very similar F-box proteins of the KISS ME DEADLY clade, known to play a role in negatively regulating cytokinin signaling. Yeast two hybrid analyses revealed that the KMD genes interact with phenylalanine ammonia lyase (PAL), the gateway enzyme of the phenylpropanoid pathway. The KMD proteins also interact with SKP proteins, allowing them to function in ubiquitin ligase complexes. We isolated single, double and triple kmd mutants and showed that these plants exhibit lower PAL activity and reddish stems and leaves, indicative of aberrant accumulation of anthocyanins. Cytological and biochemical assays revealed higher anthocyanin and lignin levels, and conversely, plants overexpressing KMD contain less of these phenylpropanoid products. Based on our data and microarray data mining, we propose that these F-box proteins play a role in sensing nutrient availability and exert their functions to coordinate the flow of primary metabolites into secondary metabolism. As such, the KMD proteins might be exploited to control pathway flux and provide a means to reduce lignin levels in biofuel feedstocks.

SESSION V: COMMERCIALIZATION OF PLANT BIOTECHNOLOGY

K5: Agriculture is the new wave

Mr. Dave Smardon
President and CEO, Bioenterprise

O27: A novel receptor-like protein kinase involved in regulation of water use efficiency in Arabidopsis

Dr. Shujun Yang
Sr. Research Scientist, Performance Plants Inc.

Water use efficiency (WUE) is an integrative plant performance trait closely associated with plant drought tolerance and productivity. A mutant, *hwe116,* is identified from an EMS-induced *Arabidopsis* mutant population showing significant higher WUE than the wild-type *Columbia-0*. Genome-wide mapping and map-based cloning led to the determination of a major QTL and the identification of a novel gene encoding a receptor-like protein kinase (RLK), AtPK220, within this QTL responsible for the higher WUE phenotype of *hwe116*. The point mutation in the catalytic domain of AtPK220 protein abolishes the protein kinase activity. AtPK220 has a short signal peptide and multiple transmembrane domains at its N-terminal, and is found to target primarily to plasma membrane of root cells. *AtPK220* is highly expressed in root pericycle and endodermis, and its expression in roots could quickly be repressed once plants were subject to osmotic stress. Consistently, down-regulation of *AtPK220* via RNAi in *Arabidopsis* enhanced WUE of the transgenic plants. Furthermore, transcriptome analysis with the *hwe116* mutant and RNAi-silenced plants suggests that *AtPK220* might function in sensing extracellular water potential and to relay the signal to downstream genes for regulation of water transport across different cell types.

O28: Crop Improvement with KeyGene

Anker P Sørensen
Vice President, New Business, Keygene Inc.

DNA technologies are developing with a dramatic acceleration; especially high-throughput sequencing technologies are revolutionizing the DNA research arena. We may ask ourselves what the implications are for molecular plant breeding in terms of genetic research as well as trait and variety improvement programs. The current challenge for plant geneticists clearly lies in the ability to integrate and aggregate the different and large data sources, in order to make firm and robust associations between the phenotypic variability and the genotypic variability, after which these can immediately be exploited by modern plant breeders. Novel technologies for generation of mutant alleles of interesting plant genes are in development and will increase the genetic variability of germplasm available for variety improvement programs. We will demonstrate some of the approaches that KeyGene has taken in order to assist in the crop improvement programs. KeyGene uses two platforms: KeySeeQ and <Crop>Pedia for discovery of genes related to trait expression. KeySeeQ is applied to discover trait influencing genes based on plant transcriptome analysis. <Crop>Pedia is a comprehensive knowledge platform for fast and effective marker development and gene research. It supports novice and experienced molecular biologists in generating knowledge from data. Novel variation in elite germplasm is generated using KeyPoint® Mutation Breeding. An array of new alleles in identified candidate genes are created in a high throughput way.

O29: Facilitating a Path to Market for Biotech Crops

Dr. Kevin Gellatly
Regulatory Affairs Manager, Monsanto Canada Inc.

Commercialization of biotech crops requires navigating the global regulatory environment. Gaining cultivation or import approvals requires providing country regulators with data packages tailored to an assortment of asynchronous laws, regulations and requirements. Tech developers must also abide by international guidelines for evaluating biotech product safety. Monsanto's technology development process is divided into four distinct phases: discovery, proof of concept, early development, advanced development and regulatory submissions. Extensive scientific evidence is compiled to prove food, feed and environmental safety of biotech products for global regulatory agencies. Gene/protein and crop safety assessments occur throughout the product development process. A commitment to stewardship requires the responsible management of a product throughout its life cycle from concept to discontinuation. Biotech crops continue to be proven safe and have the support of all major scientific organizations. To date there are hundreds of peer-reviewed publications on the characterization and safety of transgenic crops.

O30: Expediting commercialization: acceleration and innovation of sample preparation methods for generating high quality data

Mr. Jeffrey Seitz
Vice President, D-Mark Biosciences.

Plant Biotechnology is forever evolving and continues to innovate rapidly and significantly. This allows the researcher to achieve more consistent and reproducible data more quickly. With extensive experience working with Canada's Genomics research community, D-Mark Biosciences will reflect on a solution based approach to molecular and genomic plant science, identifying some of the most innovative, next generation molecular research tools that address challenges in pre-commercialization. Both technological advancements and better business practices will be discussed.

POSTER ABSTRACTS

P1: Plant-made vaccines as an alternative to antibiotic treatments: Transient expression in *Nicotiana benthamiana* and generation of stable transplastomic *Nicotiana tabacum*

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During the last decade, antibiotics resistance appeared in numerous pathogens. The use of antibiotics is not seen as a sustainable solution in the field of health but also in agriculture and agri-food industry. One of the most striking examples is the Escherichia coli O157:H7 case, an enterohemorrhagic strain which causes serious health issues. This strain is found in the digestive tract of cows which are healthy carriers, but E. coli O157:H7 is pathogenic for humans when found in wastewater or in meat. The use of vaccines, as a preventive treatment, can be an interesting alternative to antibiotics in the way that they stimulate the immune system of the individual to fight the infection. In addition to health problems, economic issues must be taken into account. Vaccines can be an alternative only if they are cheap enough to encourage farmers to use them. It is in this context that plant-made vaccines can make a contribution by reducing the cost of production. In this project, two strategies for the production of protein subunits derived from different serotypes of enterohemorrhagic E. coli are used. The first one is the use of agroinfiltration for transient expression in Nicotiana benthamiana leaves. The second strategy is the generation of stable transplastomic Nicotiana tabacum plants.

P2: Use of somatic embryos of alfalfa for higher level of recombinant protein expression

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Plants have been explored as an inexpensive system for recombinant protein production. However, low protein yield in plant vegetative tissues has been an issue that limits the commercial utilization of plant expression systems. Somatic embryos resemble to zygotic embryos (seeds) in different aspects and high mass of somatic embryos can be induced from a small amount of plant tissues. Somatic embryos may also accumulate more proteins as in seeds. In this study, somatic embryo of alfalfa (Medicago sativa L.) was investigated for recombinant protein expression. Three heterologous genes: β-glucuronidase (GUS) which is a scientific reporter gene, and two molecular farming interesting genes, cholera toxin B subunit (CTB), and human interleukin 13 (hIL-13) were independently introduced into alfalfa via Agrobacterium-mediated transformation. Somatic embryos were subsequently induced from transgenic plants. Analyses showed that somatic embryos can store approximately two-fold more ectopically expressed proteins (as a percentage of total soluble protein) than vegetative organs such as roots, stems, and leaves. The foreign proteins CTB and hIL-13 could accumulate up to 0.15% and 0.18% of total soluble protein in alfalfa somatic embryos, respectively. These results indicate that somatic embryos can be a more efficient expression system to accumulate and produce heterologous proteins.

P3: Exploration of genetic diversity in wild and cultivated barley using gSSR and EST-SSR markers

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Barley (*Hordeum vulgare* subsp. *vulgare*) is an economically important cereal crop grown worldwide for feed and malt production, but its value as food is also increasing, due to its various health benefits. Wild barley (*Hordeum vulgare* subsp. *spontaneum*) is the progenitor of modern day barley cultivars, possessing a rich source of genetic variation for various biotic and abiotic stresses. Species specific molecular markers have great potential for efficient introgression of these important traits from wild to cultivated barley. In the present study, 140 microsatellite markers were screened to assess the genetic variation and species specific markers between wild and cultivated germplasm. From these 140, a polymorphic set of 48 genomic (gSSR) and 16 EST-SSRs amplified a total of 372 alleles in wild and 311 alleles in cultivated barley respectively. Cluster analysis discriminated all 47 accessions and classified wild and cultivated genotypes into two distinct groups, according to their geographic origin. Our analysis indicated that gSSRs were more informative than EST-based SSRs. Results from PCoA (Principle Coordinate analysis) analysis for species specific alleles clearly suggest that wild barley genotypes contain higher number of unique alleles. This study can be useful for MAS-based breeding programs for the improvement of cultivars.

P4: Precise transfer of useful variability from related non-progenitor wild species into wheat cultivars through induced homoeologous chromosome pairing

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A number of wheat–Aegilops substitution and addition lines for group 2 and 7chromosomes were used for precise transfer of chromosome fragments carrying genes for high iron and zinc uptake, transportation and sequestration of micronutrients in grains without linkage drag by induced homeologous pairing. Group 2 and 7 specific SSR marker analysis revealed introgression of alien chromosome. Wheat-Aegilops derivatives were crossed with homozygous ph1bph1b stock. Triticum aestivum cv. Pavon. In BC1 populations homozygous ph1bph1b plants were screened using ph1b specific psr2120 marker. Further plants with introgression were analyzed by AAS technique for micronutrient analysis. The plants with high micronutrient content with useful alien introgression will be backcrossed with elite wheat cultivars to produce BC2 population. Alternatively themonosomic 5B plants were crossed with non-progenitor Aegilops species and the F1 plants were screened for ±5B chromosome. Cytological analysis at meiotic stage showed reduced homoeologous pairing in +5B plants and high pairing in 5B deficient plants. This was further associated with morphological differentiation of the +5B and -5B plants. Finally, the plants with high micronutrientand homoeologous chromosomal pairing confirmed the effectiveness of both the above strategies to achieve the precise alien introgression which in the near future have the potential to be used as breeding material for generating biofortified high yielding wheat cultivars.

P5: Investigating hygroactuation using *Selaginella lepidophylla* as a model system

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Actuation is the process of generating controlled mechanical displacement in response to an external, non-mechanical stimulus. By studying biological actuators, it is possible to extract principles that can be used to create synthetic materials capable of reacting to stimuli in their external environment and adapt accordingly through a pre-determined mechanical response. Here, we propose to use Selaginella lepidophylla, a desiccation tolerant lycophyte, as a model for investigating hygroactuation. S. lepidophylla employs various physiological strategies in order to successfully survive water loss and prolonged periods of desiccation. These include extensive - and reversible- folding of its vegetative tissue at the macro-scale (branch curling), micro-scale (cytosolic vitrification and vacuolar filling), and nano-scale (cell wall folding). This investigation seeks to understand the mechanical properties that influence the hierarchical folding/unfolding of S. lepidophylla in response to changes in its relative water content. Living and dead tissue will be compared in order to differentiate between purely mechanical folding/unfolding mechanisms, as observed in dead tissue, and those that are coupled to biochemical processes, as commonly observed in living tissue. In addition, this investigation will seek to determine whether the mechanical folding/unfolding of S. lepidophylla vegetative tissue is reproducible over multiple cycles of wetting and drying.

P6: Seaweed extracts altered soybean gene expressions-transcriptome analysis

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Extracts of the brown seaweed Ascophyllum nodosum have been shown to increase root growth, stimulate shoot growth and branching, and enhance resistance to diseases. However, the molecular mechanisms of the improved plant growth remain largely unknown.
We investigated the effects of commercial A. nodosum extracts on growth and mRNA level in the transcriptome of Glycine max. The treated soybean seedlings exhibited significant increase in the length of primary root and number of lateral roots compared with controls. Transcriptome analysis showed up-regulated gene expression in photosynthesis, cutin biosynthesis, plant hormone signal transductions, and plant-pathogen interaction pathways after A. nodosum extracts treatment. This study suggests that plant transcriptome analysis is a valuable and effective approach to reveal the mechanism of plants growth promotion effects of seaweed extracts.

P7: Analysis of mucilage regulation and seed coat development in *Linum usitatissimum* (flax)

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Linseed flax (Linum usitatissimum L.) has long been cultivated to provide stem fibres for textiles and oil from the embryo. Further, the seed coat of flax produces a thick, pectinaceous mucilage that is used commercially as soluble fibre, emulsifiers, and a substitute for animal products in food. Flax mucilage is primarily composed of the pectin rhamnogalacturonan I (25%) and the hemicellulose arabinoxylan (75%). In order to understand the regulation of mucilage production in flax, we are correlating the expression of genes involved in polysaccharide biosynthesis with cytological changes of the flax seed coat mucilage secretory cells. Our results have both identified key time points in flax mucilage production and biosynthetic genes that are specifically upregulated during mucilage production and may serve as control points for regulating mucilage synthesis, as also seen in the well-studied model Arabidopsis thaliana. These results could serve as a starting point for tailoring flax mucilage quantity and/or general composition for specific purposes.

P8: Role of RNA-directed DNA methylation on the regulation of grain genes in barley

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Barley is an important cereal. It is utilized in animal feed and beer production. Owing to its importance, barley community has generated numerous genomic resources including large set of ESTs, TILLIING population, high density linkage and physical maps, and whole genome sequence. However, the regulatory mechanisms during barley grain development are still unknown.
Small RNAs control gene expression at transcriptional or post-transcriptional levels. In the process of small RNA pathways, the small RNAs bind to target mRNAs to inhibit protein synthesis. Furthermore, the gene expression is also regulated by RNA-directed DNA methylation (RdDM) triggered by small RNAs. Recently, key gene of RdDM has been shown to be involved in pre-harvest sprouting and dormancy in small grain cereals. The purpose of our current research is to investigate the role of RNA-directed DNA methylation in barley through transposon mutagenesis. From the repository of Ds transposon mutants, hvdcl3 mutants have been identified which can highlight the role of RdDM in the regulation of grain genes. Several homologous DCL3 genes have been found in barley. We are analyzing their functions in epigenetic regulation during barley development, especially reproductive phase. The results will improve the knowledge of epigenetic regulation in barley grain development.

P9: Exploration of Cellulose Synthase (CESA) genes in wheat for bio-energy traits

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Cellulose is the most abundant biopolymer on earth and therefore considered as renewable carbon source for bio-fuels and other bio-products. Cellulose in the primary and secondary cell walls of plants is synthesized by a multi-gene family called cellulose synthase (CESA). A thorough understanding of cellulose synthesis is crucial to design the cellulosic biomass for bio-energy purposes. Most of the research about the structure and function of these genes has been performed in model species such as Arabidopsis, rice and barley. However, until now, no comprehensive study has been reported about the gene structure, chromosomal location, phylogeny and evolution of this gene family in wheat. Twenty four full length genes homologous to eight HvCESA genes from barley were identified in wheat. Chromosomal location indicated the presence three homeologous copies of each gene encoded by the three genomes, A, B, and D of hexaploid wheat. Eight CESA genes were also identified from each of the diploid progenitors, Triticum urartu (AA) and Aegilops tauschii (DD). Phylogenetic and motif analysis showed the functional conservation of CESA gene family. Analysis of divergence revealed that the genes were evolved under purifying selection.

P10: Cytological stability and Molecular Variation in *Picea mariana* × *P. rubens* Hybrid Populations

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Interspecific hybridization can result in significant shifts in allele frequencies. The objective of the present study was to assess the level of molecular variation and cytological stability in populations of P. mariana × P. rubens hybrids derived from artificial crosses. Progenies from backcross populations created through a series of controlled pollinations among P. mariana and P. rubens trees across the hybridization index were analyzed. Several Inter Simple Sequence Repeat (ISSR) and Random Amplified Polymorphic DNA (RAPD) primers were used to amplify genomic DNA samples from each population. ISSR primers produced from 30% to 52% polymorphic loci. The level of polymorphism was higher with RAPD markers, ranging from 57% to 76%. Overall, the two marker systems generated similar levels of polymorphic loci for P. mariana and P. rubens populations. No significant differences were found among the P. mariana × P. rubens populations analyzed and between the hybrids and the parental populations regardless of the molecular marker used. Cytological analysis of P. mariana x P. rubens hybrids showed normal mitotic behavior at prophase, metaphase, anaphase and telophase. All the hybrids analyzed from different cross combinations were euploids. These findings confirm the genetic closeness of P. mariana and P. rubens species.

P11: Profiling the protective surface lipids of the oilseed crop *Camelina sativa*

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Camelina sativa is an emerging crop with seed oil composition suitable for biofuel production. Camelina is relatively drought-tolerant and requires less fertilizer than other oilseed crops. Various lipid- extracellular barriers of plants help to protect them against biotic and abiotic stresses. These barriers, which consist of solvent-insoluble polymeric frameworks and solvent-extractable waxes, include the cuticle of aerial plant surfaces and suberized cell walls found in periderms and seed coat. The chemical compositions and ultrastructural features of extracellular lipids extracted from aerial and subterranean tissues of Camelina were analyzed using gas chromatography and microscopy. This study provides qualitative and quantitative information on the waxes and lipid polyesters extracted from Camelina boundary tissues, allowing the relative activity of biosynthetic pathways in such tissues as well as the substrate specificities of key enzymes involved in the synthesis of various components to be assessed. Furthermore, this detailed description of the protective surface lipids of Camelina may provide insights into its drought-tolerant and pathogen-resistant properties, and also provide an additional source of high-value lipid components that can be extracted from the plant.

P12: Gene ontology analysis of *Plasmodiophora brassicae* pathogenesis in *Brassica napus*

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Damage to worldwide Brassica crops, as a result of infection by the protist Plasmodiophora brassicae Woronin (clubroot) accounts for approximately 10-15 % of total Brassica production. With very few ESTs of P. brassicae currently available in Genbank, a molecular insight into the pathogenesis of P. brassicae is a work in progress. We have generated a cDNA library from Brassica napus (canola: Westar) roots infected by P. brassicae pathotype 3 (Dr. G. Peng: AAFC, Saskatoon). Galls from heavily infected plants were collected 35 days post inoculation and a cDNA library was generated from total mRNA. Trimming of an original 10482 clones resulted in 4292 unique contigs, that were subsequently annotated and used to find all associated gene ontology (GO) terms using Blast2Go. Approximately 40 % of the annotated contigs are putative P. brassicae clones with the vast majority of these being previously undocumented. Approximately 6 % are candidate secretory proteins (SignalP 4.1), that may provide targets for blocking P. brassicae intracellular biotrophic parasitism. GO results for biological process, cellular component and molecular function for both host and pathogen will be presented along with preliminary putative secretory protein expression levels.

P13: Identification and characterization of population-diagnostic molecular markers in fragmented populations of *Betula papyrifera* (Marsh) from Northern Ontario

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White birch (Betula papylifera) is an open pollinate species that is dominant in the mining region of the Greater Sudbury after land reclamation. In fact, this species represents 65% of all trees in the Region. Because of the long-distance pollen dispersal, gene flow among the fragmented populations is expected to be high. We hypothesized that the exchange of genetic information between fragmented populations by range-wide paternal introgression is possible in wind-pollinated species such as B. papyrifera. On the other hand, the effects of heavy metal contamination from the mining activities on plant growth and population dynamics are well documented. In the present study, white birch (B. papyrifera Marsh.) populations from Northern Ontario (with emphasis on the Greater Sudbury Region) was assessed using Inter-Simple Sequence Repeat (ISSR). The level of polymorphic loci, Shannon index, Nei's genetic diversity, observed number of alleles, and gene flow were determined. The genetic variation of white birch from the Greater Sudbury Region was found to be low to moderate with the percent of polymorphic loci ranging from 24 % to 56 %. The gene flow was high as expected. Two population-diagnostic ISSR markers were identified. They were cloned, sequenced and converted to SCAR markers. The molecular data will be discussed within the context of heavy metal accumulations in soil and plants.

P14: Colonization of nitrogen fixing bacterium *Gluconacetobacter diazotrophicus* in corn plants grown in field-mimic environments

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Nitrogen is essential for all forms of life. Modern agriculture practices heavily rely on industrial fertilizers, especially nitrogen fertilizers. However, this approach is not cost effective and also results in significant environmental pollution problems. G. diazotrophicus is an endophytic non-nodule forming nitrogen-fixing bacterium and it can supply its natural host sugarcane with significantly amount of nitrogen. This bacterium may be used to achieve biological fixation in other monocot crops. In previous research, we showed G. diazotrophicus can be introduced into different corn plants grown in laboratory condition. In this study, we further investigated the establishment of symbiosis of G. diazotrophicus and corn in field-mimic condition. Plants were grown in soils collected from different agriculture fields and nitrogen species in soils were analyzed. Using PCR and reporter gene expression methods, we showed that G. diazotrophicus can be introduced into corn plants grown in different agriculture soils. In some colonized plants, G. diazotrophicus can be detected 100 days post inoculation, indicating that the G. diazotrophicus can positively adapt to corn in field mimic environments. Analyses showed that soils in different places contained different amounts of nitrogen. Bacterium colonization efficiency with respect to nitrogen level in environment is being evaluated.

P15: A nuclear-expressed and secreted recombinant plant antifreeze protein from *Chlamydomonas* cultures

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Transgenic algae offer the promise of relatively inexpensive production of recombinant proteins. The recent development of robust heterologous nuclear gene expression and a capability of secreting post-translationally modified recombinant proteins, with the minimal input of only sufficient light for photosynthesis is an exciting prospect. We have applied this strategy to the production of an antifreeze protein (AFP) from Lolium perenne (Lp), the perennial rye grass. After 'codon optimizing' the LpAFP sequence for Chlamydomonas reinhardtii, the protein-encoding region was ligated to an algal secretion signal sequence as well as a luciferase reporter. The resulting construct was transformed into a cell wall deficient strain. Algal transgenic lines showed variation in expression levels, likely due to the integration site. Recombinant protein with luciferase activity was recovered from culture medium of 'high producers' and shown to have AFP activity. LpAFP was successfully recovered from the medium by ultrafiltration and subsequently subjected to purification regimes. Unfortunately, production under purely photoautotrophic conditions was less reproducible. For practical applications, however, it is crucial to consider how to optimize the secretion of these recombinant proteins into water. Efforts to achieve this goal are currently ongoing.

P16: Expression of nitrous oxide reductase from *Pseudomonas stutzeri* in transgenic plants and activity thereof

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As the third most important greenhouse gas, nitrous oxide (N_2O) is a stable greenhouse gas and also plays a significant role in stratospheric ozone destruction. The primary anthropogenic source of N_2O stems from the use of nitrogen in agriculture. The bacterial enzyme nitrous oxide reductase (N_2OR) is the only known enzyme capable of catalyzing the conversion of N_2O to N_2. Therefore, to "scrub" or reduce N_2O emissions,
bacterial N_2OR was heterologously expressed inside the leaves and roots of transgenic plants. Previous studies shown the functional assembly of the catalytic centres of N_2OR is lacking when only nosZ is expressed in other bacterial hosts. More than 100 transgenic tobacco lines, expressing nosZ and nosFLZDY under the control of rolD promoter and d35S promoter, have been analyzed by PCR, RT-PCR and Western blot. The activity of N2OR expressed in transgenic plants, analyzed with the methyl viologen-linked enzyme assay, showed detectable N_2O reducing activity. The data indicated that expressing bacterial N_2OR heterologously in plants, without the expression of the accessory Nos proteins, could convert N_2O into inert N_2. This suggests that atmospheric phytoremediation of N_2O by plants harbouring N_2OR could be invaluable in efforts to reduce emissions from crop production fields.

P17: Optimizing *Gluconacetobacter diazotrophicus* Inoculation Methods and Assessing the Involvement of Candidate Plant Genes in Colonization of *G. diazotrophicus* in *Brachypodium distachyon*

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To study the nitrogen fixation of Gluconacetobacter diazotrophicus in different monocot crops, research was conducted to establish and optimize methods for introducing G. diazotrophicus into Brachypodium distachyon, a model monocot for molecular biology and genomics research. The colonization of G. diazotrophicus in B. distachyon was established through different inoculation methods with or without N supply. While Colonization was successful in all the conditions, the efficiency was high under hydroponic condition without N supply. The results indicate G. dizatrophicus can be introduced into B. distachyon, but the colonization efficiency can be influenced by nitrogen level in the growth media and the medium texture (4.3%-81.9%). To investigate the involvement of plant gene regulation in G. diazotrophicus colonization, global gene expression was conducted using next generation sequencing to generate the differential gene expression profile. The preliminary RNA sequencing result found more than 100 genes are differently expressed upon G. diazotrophicus colonization. The gene ontology and pathway analysis of these differentially expressed genes are still ongoing to further narrow down the candidate plant genes that are important for successful G. diazotrophicus colonization.

P18: Willows responses to arsenic contamination: physiological and molecular analysis

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The toxicity of As in plants is mainly mediated by the competition between arsenate (AsV) and phosphate in metabolic processes. In addition, As could disrupt enzymatic activities by the binding of arsenite (AsIII) to thiol groups present in proteins. Regardless of the negative effect of As in plants metabolism, several species have shown the capacity to survive or avoid the stress associated to As. The objective of this study is to show that some of them are even able to extract, degrade, or immobilize As contaminants. Results from a four weeks hydroponic study with Salix purpurea 'Fish Creek' saplings showed that these shrubs are able to support up to 5 ppm As without showing any significant symptoms while taking up and accumulating As in its tissues. Physiological measurements, including photosynthesis, transpiration, and biomass production, were measured in plants exposed to 0, 5, 30 and 100 ppm of As. Preliminary differential gene expression analyses (RNA-seq) led to the identification of 164 differentially expressed genes in stem, 864 in leaves and 1348 genes in roots. These results will help us to develop a better understanding of the mechanism implied in As metabolism in willows and in plants in general.

P19: The miR156-SPL gene regulatory network controls flowering and yield in alfalfa

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MicroRNAs (miRNAs) are a class of regulatory small RNAs that play roles in almost all biological and metabolic processes in plants. In particular, miR156 is a novel regulator of flowering time, secondary metabolism and biomass production through regulation of members of Squamosa Promoter-Binding Protein-Like (SPL) genes. Advances in breeding programs aimed at improving such important agronomic trait as biomass yield would continue to secure alfalfa's position as the world's most valued livestock forage and low-input bioenergy crop. In this study, we investigated the function of miR156 in Medicago sativa (alfalfa). Of the five predicted target SPL genes encoded by the alfalfa genome, three (SPL6, SPL12 and SPL13) contain miR156 cleavage sites and their expression was downregulated in transgenic plants overexpressing miR156. These transgenic plants had reduced internode length, enhanced shoot branching, increased trichome density, a modest (3-5 days) delay in flowering, and elevated biomass production, though no significant effects of miR156 were observed on plant height, root length, or nodulation. The multitude of traits affected by miR156 may due to the network of genes regulated by the three target SPLs. Our observations imply that miR156 could be employed as a tool to improve quality and yield of alfalfa biomass.

P20: A rapid small-scale wheat microspore culture technique for screening multiple variables

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The value of doubled haploids in genetic analysis and modern plant breeding has been known for a long time. Goals of the WICT (Wheat Improvement through Cell Technologies) project of the Canadian Wheat Alliance include the development of an efficient isolated microspore culture system (IMC) for spring and winter wheat that is effective with a broad range of wheat cultivars, as well as the establishment of an effective and efficient microspore transfection protocol using nanocarriers. Both activities involve the testing of a wide range of variables, including different culture and media conditions, cultivars and nanocarrier:DNA complexes. Unfortunately, the regular large scale wheat microspore extraction protocol requires the use of numerous spikes, 10-12, in one extraction in order to process 3-4 treatments. Experiments were conducted to develop a small scale extraction protocol for wheat microspores which would allow numerous treatments to be initiated. With this method, up to 20 different extractions/treatments can be processed simultaneously using as few as 10 anthers per treatment. This has significantly reduced the time and labour required for testing multiple treatments in the production of doubled haploid plants.

P21: Involvement of histone deacetylase genes in Arabidopsis root transformation

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Bacterial virulence proteins as well as host proteins are involved in the delivery and integration of transgenes during Agrobacterium-mediated transformation. Histone deacetylation, mediated by histone deacetylases (HDACs), has been shown to be involved in the maintenance of heterochromatin in plants by histone modifications. Heterochromatic regions are characterized by reduced transcriptional activity due to the decreased accessibility by transcriptional machinery. Here, we hypothesize that HDACs may be involved in transgene integration by affecting the DNA accessibly. In order to study this, knockout and overexpression lines for the HDA6, HDA19, or HD2C HDAC genes will be inoculated with Agrobacteria. Transformation efficiency will be evaluated by screening root explants that have formed calli on a callus-inducing medium containing selection. GUS expression and PCR amplification will be used to confirm successful transformants. The transformation system and regeneration systems have been established, with 80% of WT calli staining positive for GUS. Mutant lines for HDA6, HDA19, and HD2C were ordered from ABRC have been propagated. Overexpression lines, driven by a 35s promoter, for HDA19 and HD2C have been obtained.

P22: The application of cell-penetrating peptide transfection in soybean somatic embryos

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Cell-penetrating peptides (CPPs) are a class of small invasive peptide sequences. These peptides possess cellular entry and macromolecular transport capabilities. CPPs have recently been investigated as a novel approach for plant transfection and transformation. Soybean is an important crop to both Canadian and worldwide agriculture, and it is one of the more challenging plants to genetically alter. With the many difficulties encountered using conventional transformation methods in soybean the use of CPP-mediated transfection presents an alternative avenue that should be investigated. The aim of this research is to develop and optimize the application of this technology in soybean somatic embryos. The soybean somatic embryogenesis system has been established and a construct containing the GUS/YFP reporter genes driven by a CaMV 35S promoter has been developed. The effects of important factors such as various CPP-DNA concentrations, incubation times, and the use of a permeabilization treatment on the successful delivery of a plasmid cargo molecule are being evaluated. Preliminary experiments have shown positive transient GUS expression in soybean somatic embryos. This technology has the potential to be used for the modification of various soybean traits once developed.

P23: In vitro regeneration of lettuce (*Lactuca sativa* L.) from shoot buds depends on location, size and shape of explants

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The process leading to development of new varieties involves preservation of superior genotypes that perform well in the field. In vitro culture can regenerate important germplasm into disease-free plants. An experiment was conducted to determine the effect of size and shape of explants from apical and axillary buds on their capacity to develop into plantlets. A viable plantlet was defined as having at least one ≥1 cm root and leaf. Explants were extracted using four types of cuts: square, diametral, diamond and transverse. Buds were left with 3, 6 or 9 mm underlying pith tissue. Compared to axillary buds, apical buds produced 29% more plantlets with ≥1 cm leaves, 32% more plantlets with ≥1 cm roots, and 37% more viable plantlets ($P < 0.0001$) after 6 weeks on MS medium. After two weeks of acclimation, percentage of viable plantlets in relation to initial number of explants was higher (80%, $P < 0.0001$) from the 6-mm diamond-shaped than from the diametral-shaped (41-51%) and transverse-shaped (49%) explants, but not from the other diamond-shaped (71-76%) and the square-shaped (61-70%) explants. Diamond and square treatments were associated with highest root number to leaf number ratios ($P<0.05$) prior to acclimation, a critical stage when stomata become functional.

P24: Trade-offs between growth and defense in response to caterpillar herbivory

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In higher plants, gibberellins (GAs)/DELLA pathway controls plant growth and development while jasmonates (JAs) pathway plays a major role in plant defense against insect herbivores. To study the role of DELLA proteins in plant-insect interactions, we compared herbivory-induced defense responses between wild-type Arabidopsis and a quad-della mutant that has constitutively elevated GA responses. Previous studies in our lab has shown that the labial saliva (LS) of Spodoptera exigua caterpillars interferes with induced Arabidopsis defence responses. To determine the role of LS in JA-GA crosstalk, plants were subject to caterpillars with either intact or impaired LS secretions. Upon caterpillar herbivory, both wild-type and quad-della plants showed an early jasmonate burst and increased transcript levels of JA-dependent gene markers. However, we found that DELLA proteins are required for LS-mediated suppression of jasmonate levels. A JA marker gene AtPDF1.2 and a salicylic acid (SA) marker gene AtPR1 showed LS-specific expression pattern in the quad-della mutants but not in wide-type plants. Our results demonstrate that DELLA proteins are involved in plant responses to caterpillar LS, which suggests a link between GA/DELLA pathway and plant defense signalling.

P25:Expression Analysis of the Water Stress Inducible Promoter, Pwsi18, in the Model Monocot, *Brachypodium distachyon*

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The objective of this research is to identify regions within the promoter Wsi18 that contain cis-elements involved in drought induced expression. Wsi18 is induced by drought stress, salt stress and abscisic acid. It is active in the whole plant body during stress, and is also active in the whole grain during seed development, with a low level of activity otherwise. As of yet, it is unclear which sequences within the promoter are essential for drought induced expression from Wsi18. To identify these regions, promoter constructs in which fragments of Wsi18 have been removed were created and will be used to drive the expression of the reporter gene GUS. in silico analysis was used to identify consensus sequences of drought responsive cis-elements that have been identified in other promoters such as ABRE, DRE, and MYB cis-elements. Six constructs have been created in which the regions containing clusters of these cis-elements have been removed. A Brachypodium distachyon transformation system has been established, and constructs will be introduced into B. distachyon, where GUS expression will be assessed under drought conditions. Elucidating the cis-elements that are essential for drought inducibility will make it possible to modify and tune the expression driven by this promoter.

P26: Characterization of a cruciferin deficient mutant of Arabidopsis and its utility for overexpression of foreign proteins in plants

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Plant seeds have been exploited as bioreactors for the production to foreign materials, but stable, high level expression has been elusive, in part due to the intrinsic bias for producing the natural storage reserves in their typical proportions. To identify mutants governing seed filling, we screened a population of mutagenized Arabidopsis plants for a mutant that failed to fill its seeds. Here we report the identification of ssp1, a viable mutant that accumulates approximately 15% less protein than wildtype seeds. Molecular analyses revealed that ssp1 is due to the introduction of a premature stop codon in CRU3, one of the major cruciferin genes. Unlike many other reserve mutants or transgenic lines in which seed storage protein levels are reduced by antisense/RNAi technologies, ssp1 exhibits low level compensation by other reserves, and represents a mutant background that might prove useful for high level expression of foreign proteins. To test this hypothesis, we generated single insertion reporter (PHA) lines in ssp-1 and introgressed the reporter back into wildtype. The ssp1 lines consistently accumulated more PHA than the backcrossed counterparts, with increases ranging from 12% to 126%. This proof of principle study suggests that similar strategies in crop plants may improve the yield of foreign proteins of agronomic and economic interest.

P27:Understanding the role of the BdHD1 histone deacetylase in drought stress response in model monocot, *Brachypodium distachyon*

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Drought is one of the most devastating threats to plant productivity. In order to survive drought stress, plants respond with a series of physiological, cellular, and molecular processes. The reversible acetylation and deacetylation on histones, catalysed by histone acetyltransferases and histone deacetylases (HDACs), play an important role in regulation of gene expression. AtHDA19 found in Arabidopsis thaliana was reported to be involved in Abscisic acid (ABA) response and presumably drought stress. Brachypodium distachyon is being developed as a model monocot species for molecular biology and genomics research. We conducted genomic analysis of Brachypodium and identified BdHD1 which is 78.6% similarity with AtHDA19 in protein sequence. Further experiments showed that the transcription level of BdHD1 in wild type Brachypodium (Bd21) could be positively affected under drought stress. The homozygous plants of BdHD1 T-DNA insertion mutant line has been selected and used for gene knock-out study. Meanwhile, BdHD1 has been cloned from Brachypodium. Construct for overexpressing BdHD1 has been made and plants for overexpressing the gene are being developed to study the involvement of BdHD1 in drought tolerance. This study can help to understand drought tolerance mechanisms relating to BdHD1, which will provide knowledge towards the improvement of monocot crop drought tolerance.

P28: A chimaeric affinity tag for efficient expression and chromatographic purification of heterologous proteins from plants

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Plant biofactories represent a promising option for the heterologous expression of complex and challenging recombinant proteins. Dedicated tools are still lacking, however, to marry recently developed high-yielding expression vectors for transient expression with routine purification procedures for the resulting protein products. Here we present the'Cysta-tag', a novel affinity tag for immobilized metal affinity chromatography (IMAC) based on a plant cystatin known to stabilize heterologous proteins in the plant cell secretory pathway. As illustrated with the human serpin model alpha-1-antitrypsin transiently expressed in Nicotiana benthamiana leaves, the Cysta-tag may be used to purify heterologous proteins in native conditions from various subcellular compartments. Commonly used protease recognition sites for linking purification tags are differentially stable in this context, with those recognized by cysteine proteases being less stable than serine protease-cleavable linkers. A simple, one-step IMAC purification protocol results in recovery of up to 25% of the expressed protein at 90% purity. The Cysta-tag provides a means for simple, efficient and cost-effective recombinant protein purification from plant tissues, applicable in principle to a variety of commercially available IMAC-based purification tools.

P29: pGTQL: A Flexible Gateway-Compatible Vector System for Plant Functional Genomics

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Functional genomic research always needs to clone different DNA fragments into binary vectors, so as to express genes with different tags, from various promoters and with different levels. Gateway technology makes cloning, especially subcloning a gene in multiple expression vectors, much more efficient compare to traditional restriction enzyme based methods.We have developed a large collection of 27 Gateway-compatible binary T-DNA destination vectors called pGTQL vectors for a wide range of different applications in plant functional study including BiFC, subcellular localization, complementation, over-expression, promoter-reporter and purification. All pGTQL plasmids are binary vectors that suitable for Agrobacterium-mediated plant transformation. All these vectors are using the same cloning cassette, which eliminated the problem on frame shift caused by cassette change when using destination vectors from different systems.Some of these vectors have been tested in both dicot and monocot plants including Arabidopsis thaliana, Brachypodium distachyon and Nicotiana benthamiana. Our results show that such system is highly efficient and serves as a high-throughput platform for transient or stable transformation in plants. These vectors expand the current plant functional genomics research toolbox, streamline the analysis of gene function and bridge the gap between basic and applied research for the study of valuable agricultural traits.

P30: Natural variation for seed oil content and composition in *Camelina sativa* (L.) Crantz and *Camelina microcarpa* Andrz ex DC. (Brassicaceae)

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Camelina sativa is a promising oilseed crop in North America with potential value in food, livestock feed, biofuel, and bioproduct applications. The extent of natural variation of the seed oil content and fatty acid composition among accessions of C. sativa as well as other species in the Camelina genus have not been fully explored. We have investigated this natural variation among 69 C. sativa accessions and 26 accessions of Camelina microcarpa; a close relative of C. sativa with a naturalized population in Canada. The intra- and inter-specific variability of seed oil content within these species were different with average oil content values higher in C. sativa accessions (39.3 ± 3.7%) than in C. microcarpa accessions (33.5 ± 3.7%). We then categorized the species by their respective cytotype designations and found a positive relationship between oil content and degree of ploidy. We also screened the seed oil composition and found that C. microcarpa had higher proportions of α-linolenic acid (omega-3 fatty acid) than C. sativa. The variability observed within our collection of Camelina accessions offers incentive to investigate the effects of hybridization between these Camelina species on oil content and composition. Some accessions with key oil characteristics were also identified for further development in biofuel or food applications.

P31: Functional characterization of wheat NRT2 gene responsible for nitrogen uptake by virus induced gene silencing technique

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Wheat (*Triticum aestivum* L.) is a second major staple food crop of the world. It has a large genome (16000 Mb) with highly repetitive sequences and several DNA sequences in the genome of this crop are yet to be characterized. In the present study, virus induced gene silencing (VIGS) technique was used to determine the phenotype of wheat NRT2 gene which has been found to be responsible for nitrogen uptake, lateral root growth and increase in root length in the model plant *Arabidopsis thaliana*. Barley stripe mosaic virus (BSMV) based plasmids (pα, pβΔβ and pγ) were used for making the desired constructs. The wheat plants at two leaf stages were inoculated with the BSMV virus carrying the antisense construct of NRT2 gene. The phenotypes of plants were recorded 21 days after inoculation. The infected plants showed a decrease in root length and root mass as compared to control plants. The inoculated plants were also found to be less healthy as compared to the controls. Further studies using qRT-PCR will be done to confirm the suppression of NRT2 gene.

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