

Poster Setup: Saturday starting at 8:00 a.m. Posters should be removed by 6:30 p.m.
Oral Presentations: attend your session 20 min in advance to load your file onto the computer

Schedule at a Glance

Friday, December 10	
7-10 p.m.	Mixer and Registration (Atrium, Biosciences Complex)
Saturday, December 11	
8:30-9:30 a.m.	Registration, coffee (Atrium)
9:15-9:30 a.m.	Opening Remarks (Rm 1103)
9:30-10:15 a.m.	Plenary Talk: Maria J. Harrison, Boyce Thompson Institute, Cornell University, "The arbuscular mycorrhizal symbiosis: an underground association" (Rm 1103)
10:15-10:30 a.m.	<i>Coffee and refreshments</i> (Atrium)
10:30-11:15 a.m.	Plenary Talk: Normand Brisson, University of Montreal "The Whirly Transcription Factors: Defense Gene Regulation and Beyond" (Rm 1103)
11:30-1:00 p.m.	<i>Lunch and posters</i> (Atrium)
1:00-2:30 p.m.	Oral presentations (concurrent sessions; Rms 1102, 1103)
2:30-2:45 p.m.	<i>Coffee and refreshments</i> (Atrium)
2:45-4:00 p.m.	Oral presentations (concurrent sessions; Rms 1102, 1103)
4:00-6:00 p.m.	Posters, awards, and light reception (Atrium)
6:00 pm -?	Unofficial pub crawl

Organizing Committee: W.A. Snedden (Queen's), Lynn O'Malley (Queen's)

The CSPP would like to thank the generous support of our sponsors, Performance Plants (www.performanceplants.com) and VWR.



ORAL PRESENTATIONS

Sessions 1A (Rm 1102) and 1B (Rm 1103) are concurrent from 1:00-2:30pm

Sessions 2A (Rm 1102) and 2B (Rm 1103) are concurrent from 2:45-3:45pm

Session 1A: 1:00-1:15pm

Involvement of DIR1, a putative lipid transfer protein, in long distance signaling during Systemic Acquired Resistance

Robin K. Cameron, Melody Neumann, Zhiying Zhao, Asif Mohammed, Karen Haines

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Systemic acquired resistance (SAR) is elicited in response to local necrotizing infections which induce the production of a long distance signaling molecule that is perceived in distant tissues resulting in resistance to normally virulent infections in distant parts of the plant. Our previous studies using *dir1-1* (defective in induced resistance), a SAR-defective mutant, indicate that *dir1-1* can perceive the SAR signal present in petiole exudates (enriched for phloem sap) from wild type SAR-induced plants, but *dir1-1* exudates do not contain this signal (Maldonado et al, Nature 419: 399-403, 2002). Protein Gel Blot analysis demonstrated that DIR1 protein is present in petiole exudates of SAR-induced wild type, but not *dir1-1* plants. These results suggest that DIR1, which encodes a putative lipid transfer protein (LTPs contain a lipid binding pocket), may be involved in the production of the SAR mobile signal or in transporting a lipid signal to distant tissues to establish SAR. DIR1 can be detected in intercellular washing fluids of wild type, but not *dir1-1* plants indicating that the predicted cell wall signal sequence does localize DIR1 to the cell wall. We hope to address how DIR1, a cell wall-associated protein translocates, such that it is found in petiole exudates after SAR induction and suggesting that DIR1 may be transporting a lipid signal via the phloem to distant tissues during SAR. A number of approaches are being taken, including characterization of transgenic *dir1-1* plants containing a signal sequence-less DIR1 gene fused to the GUS reporter gene, under the control of the native DIR1 promoter and native DIR1 promoter-GUS lines to determine the cellular location of DIR1 before and after SAR induction. The results of preliminary experiments with these transgenics will be presented and should provide more information about DIR1 and its role in long distance signaling during SAR.

Session 1B: 1:00-1:15pm

Pectins determine the physical properties of the pollen tube cell wall in the apical growth zone

Elodie Parre^{1,2}, Jean-François Bolduc^{1,3}, Anja Geitmann¹

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The cell wall is one of the structural key players regulating pollen tube growth, since plant cell expansion depends on an interplay between intracellular driving forces and the controlled yielding of the cell wall. Pectin is the main cell wall component at the growing pollen tube apex. We therefore assessed its role for pollen tube growth and cytomechanics using the enzymes pectinase and pectin methyl esterase (PME). Pectinase activity was able to stimulate pollen germination and tube growth at moderate concentrations whereas higher concentrations caused apical swelling or bursting in *Solanum chacoense* pollen tubes. This is consistent with a modification of the physical properties of the cell wall affecting its extensibility and thus the growth rate as well as its capacity to withstand turgor. To prove that the enzyme induced effects were due to the altered cell wall mechanics, we subjected pollen tubes to micro-indentation experiments. We observed that cellular stiffness was reduced and visco-elasticity increased in the presence of pectinase. These are the first mechanical data that confirm the influence of the amount of pectins in the pollen tube cell wall on the physical parameters characterizing overall cellular architecture. Cytomechanical data were also obtained to analyze the role of the degree of pectin methyl-esterification, which is known to exhibit a gradient along the pollen tube axis. This feature has frequently been suggested to result in a gradient of the physical properties characterizing the cell wall and our data provide for the first time mechanical support for this concept. The gradient in cell wall composition from apical esterified to distal de-esterified pectins seems to be correlated with an increase in the degree of cell wall rigidity and a decrease of visco-elasticity. Our mechanical approach that includes modeling of the cell using finite element analysis provides new insights concerning the mechanics of pollen tube growth and the architecture of living plant cells.

Session 1A: 1:15-1:30pm

Protein Phosphorylation in Amyloplasts Regulates the Formation of Protein Complex Assemblies Involving Key Starch Biosynthetic Enzymes

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Protein phosphorylation is a widespread regulatory mechanism controlling many cellular processes in living organisms. The focus of our current research is to understand the role of protein phosphorylation in the regulation of starch metabolism in plants; in particular, the process of storage starch biosynthesis which occurs in amyloplasts. On-going research involves identifying ^{32}P -ATP-labelled phosphoproteins using quadrupole-orthogonal acceleration time of flight mass spectrometry (Q-TOF-MS), and understanding the effects of phosphorylation/dephosphorylation on enzymes in the pathway. A number of key enzymes in the biosynthetic pathway are readily phosphorylated when intact amyloplasts from developing wheat endosperm are incubated with ^{32}P -ATP, including starch branching enzymes (SBEs), starch synthases (SSs), and a plastidial form of starch phosphorylase (SP). Results with isolated amyloplasts indicate that phosphorylation state influences the formation of a number of protein complexes in these organelles. Amyloplast protein complexes were isolated using co-immunoprecipitation with peptide-specific antibodies, and components of the complex identified using Q-TOF-MS. One of the complexes identified contains SBEI, SBEIIb, and SP. The formation and integrity of this amyloplast protein complex is phosphorylation-dependent. The functional significance of multi-enzyme complexes in the pathways of starch metabolism in plants will be discussed.

Session 1B: 1:15-1:30pm

Plant Nucleoside Diphosphate Kinase: a housekeeping and regulatory protein?

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Nucleoside diphosphate kinase (NDPK) is a ubiquitous enzyme catalyzing the interconversion of NDPs and NTPs. NDPK isoforms have been described in the cytosol, mitochondria and chloroplasts, but no consensus exists on the function(s) of this enzyme in plants. We isolated a cDNA encoding *Solanum chacoense* cytosolic NDPK (cNDPK), which was expressed as a 6xHis-tagged protein in *E. coli*. The recombinant protein was purified by affinity chromatography on a Ni²⁺-agarose column. Kinetic analysis of recombinant cNDPK suggested that the enzyme is strongly regulated by the NDP to NTP ratio. A polyclonal antibody generated against recombinant cNDPK was specific for the cytosolic isoform as shown from immunoprecipitation experiments and western blot analysis of chloroplasts and mitochondria preparations from *Solanum tuberosum*. DEAE fractogel analysis of NDPK activity in root tips, leaves, tubers and potato cell cultures suggests that cNDPK constitutes the bulk of extractable NDPK activity these tissues. Activity measurements, western blot analysis as well as immunolocalization experiments performed on potato root tips and apical buds demonstrated that cNDPK was predominantly localized in the meristematic zones, in young root endoderm, in provascular tissues, as well as in the epidermal layer of the apical region. These data suggest that NDPK plays a specific role in cell division and/or early growth in plant meristems. All NDPKs are known to autophosphorylate on a conserved His residue. Phosphoaminoacid analysis of recombinant cNDPK indicated that it was also autophosphorylated on a conserved Ser residue. In addition to this autophosphorylating activity, we also found that plant cNDPK can phosphorylate several other proteins in vitro, suggesting that cNDPK may be involved in their regulation. The identity of these targets is currently under investigation.

Session 1A: 1:30-1:45pm

High root biomass production in anchored *Arabidopsis* plants grown in axenic sucrose supplemented liquid culture

Marie-France Héту, Linda J. Tremblay and Daniel D. Lefebvre

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There are many benefits in growing *Arabidopsis* in solution base media, especially when large amounts of root tissue are required for molecular and biochemical studies. Roots grown in soil are brittle and tend to break easily when removed from their substrate. We have developed an axenic liquid culture system that simplifies growing large amounts of roots from intact plants, while avoiding contaminating organisms. This technique consists of germinating seeds on stainless steel screens placed on half strength semi-solid Murashige and Skoog (MS) medium containing 2% sucrose. The screens anchor and support the plantlets in an upright position while keeping the roots and shoots separate. The seedlings are then easily transferred with forceps to wide-mouth 125 mL Erlenmeyer flasks containing 10 mL of half strength MS liquid medium and 1% sucrose and onto a shaker under fluorescent lights. After three days the volume is increased to 15 mL and the sucrose to 3%. After which, the sucrose is removed for a minimum of 48 hours to permit further experimental manipulation. The medium is changed every 3-4 days to replenish nutrients. The presence of sucrose in the media dramatically increases the plants' biomass and large amounts of root tissue can easily be harvested.

Session 1B: 1:30-1:45pm

Functional characterization of selected members of the Arabidopsis plant U-box Arm proteins

Jennifer Salt, Yashwanti Mudgil, Donna Yee, Dr. D. Goring

University of Toronto, Department of Botany

In Brassica, the ARC1 (Arm Repeat Containing 1) protein has been shown to be a positive effector and a downstream component of the S-Receptor Kinase (SRK) in the self incompatibility response. Following the activation of ARC1 by SRK, it acts to promote the degradation of an unknown target via ubiquitination through its U-Box domain thus preventing self pollination. Arabidopsis contains 41 Plant U-Box proteins (AtPUBs) that based on sequence similarity are predicted to be related to ARC1, all of which contain both a U-Box domain as well as a set of Arm repeat motifs. These Arm repeats are involved in protein-protein interactions, and in Brassica are involved in the interaction of ARC1 with SRK. Our lab is interested in determining the function of selected members of this family in self compatible Arabidopsis. To this end, we have screened for T-DNA insertions in selected members, and have been characterizing those mutants that display a phenotype with a single insertion. This has included both overall phenotype analysis as well as germination assays under different conditions. To investigate the potential role that these proteins may have in selective protein degradation, ubiquitination assays have been completed for some members, indicating that they are indeed E3 ubiquitin ligases similar to ARC1. In addition, yeast two-hybrid assays have been done to investigate the potential interaction of AtPUBs with Arabidopsis S-Domain kinases, which share homology with Brassica SRK. Results have indicated that the AtPUBs may interact with selected S-Domain kinases and may be participating in signal transduction cascades that regulate various aspects of growth and development in Arabidopsis.

Session 1A: 1:45-2:00pm

Does the IMMUTANS gene product act as an alternative electron sink in *Arabidopsis thaliana*?

D. Rosso¹, A.G. Ivanov¹, A. Fu,² S.R. Roderme², D. Maxwell¹, & N.P.A. Huner¹

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Chlororespiration has been defined as a chloroplastic respiratory electron transport chain (ETC) that interacts with the photosynthetic ETC. A component of the chlororespiratory pathway is believed to be encoded by the IMMUTANS gene, the presumed plastid terminal oxidase (PTOX). We hypothesize that plants in which IMMUTANS has been over-expressed (OVEX) should exhibit increased capacity to act as a sink for photosynthetic ETC compared to WT and a plant which lacks the IMMUTANS gene (CS 3157). To assess potential functional differences in photosynthetic intersystem electron transport between WT, OVEX, and knockout mutant, we used the absorbance change at 820 nm (A820/A870; A820/A820) as a measure of the oxidation-reduction state of P700. We found that plants in which the IMMUTANS gene has been over-expressed (OVEX) had 50% greater oxidation-reduction of P700 compared to WT. Interestingly, the extent of photo-oxidation of P700 for the knockout mutant exhibited an even greater extent of photo-oxidation of P700. Overall, these data indicate that IMMUTANS does play a role as a competitive alternative electron sink to photosynthetic electron transport. We also investigated whether IMMUTANS would exert a photoprotective role by keeping the plastoquinone pool oxidized under photoinhibitory conditions such as high light and low temperature. We hypothesized that the over-expressers should keep the plastoquinone pool oxidized in the presence of high light and low temperature, in comparison to WT and knockout mutants. Our data indicate that the IMMUTANS gene product does not protect the photosynthetic ETC from photoinhibition, since all plants exhibited the same susceptibility to photoinhibition.

Session 1B: 1:45-2:00pm

VAN3, an Arabidopsis novel type ArfGAP protein, functions in a vesicle transport governing vascular continuity

Koji Koizumi 1,4, Satoshi Naramoto 1, Shinichiro Sawa 1, Natsuko Yahara 2, Takashi Ueda 1,2 Akihiko Nakano 1,2, Munetaka Sugiyama 3, and Hiroo Fukuda 1

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Vein pattern varies among plant species, but a common basic mechanism is believed to underlie this spatial arrangement. To identify machineries responsible for spatial regulator of vein formation, we have isolated and analyzed van mutants of Arabidopsis that are characterized by fragmented minor veins. Here we report the molecular identification of the VAN3 gene and characterization of the VAN3 protein. First, we identified VAN3 by a map-based cloning. VAN3 encoded a novel type Arf GTPase-activating protein with multi-domains. To characterize the molecular nature of VAN3 protein, we examined the function of three domains: BAR, PH, and ArfGAP domains. We determined ArfGAP activity of VAN3 by an in vitro GTP/GDP exchange assay. VAN3 protein stimulated the hydrolysis of GTP to GDP binding to yeast Arf1p, indicating that VAN3 can function as an Arf GTPase activating protein regulating the Arf cycle between a GTP bound active form and a GDP bound inactive form in vesicle transport. PH domain is thought to determine the subcellular localization of a protein by mediating protein/lipid interactions. Fat western blotting analysis indicated that VAN3 bound PI-4-P with higher affinity than PI and PI4,5-P2. Yeast two hybrid analysis showed that VAN3 could self-associate through the BAR domain. These results suggest that both BAR domain and PH domain may play a cooperative role of determining the subcellular localization of VAN3 through dimerization of VAN3 and binding of PI4,5-P2 in the membrane. Double labeling experiments of VAN3-YFP with GFP subcellular markers revealed that most of the VAN3-YFP staining dots were colocalized to trans-Golgi network (TGN) marker, indicating that VAN3 locate to TGN. Interestingly, all of the TGN were not marked by VAN3-YFP. This suggests that VAN3 localized to distinct TGN specifically functioning in the transport of cargo that is essential for the accomplishing the continuous nature of vein network.

Session 1A: 2:00-2:15pm

Plastoquinol Terminal Oxidase (PTOX) Phylogenetic Distribution and Gene Expression Analyses in *Anabaena variabilis* PCC 7120 Lead to PTOX Function Hypotheses.

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The *immutans* locus discovered in *Arabidopsis thaliana* in 1999 is now known to encode a plastoquinol terminal oxidase (PTOX) localized to the thylakoid membranes of higher plant chloroplasts. PTOX displays some sequence similarity to the alternative oxidases of higher plant mitochondria. A search of sequence databases revealed PTOX orthologs in many eukaryotes including higher plants, green algae, red algae, and diatoms. PTOX was also identified recently for the first time in prokaryotes and is present in several species of cyanobacteria for which complete genomes are available, as well as cyanobacterial scaffolds present in the recently released metagenome of the Sargasso Sea environmental samples. We have confirmed the presence and expression of PTOX in *Anabaena variabilis* PCC 7120 (*Plant Mol. Biol.* 53: 865-876, 2003). *A. variabilis* is believed to contain two quinol oxidases; PTOX and cytochrome *bd* oxidase (*cyd bd*). The role of these oxidases in photosynthetic and respiratory metabolism was examined by observing temporal changes in gene expression in response to shifts in light intensity, glucose or fructose feeding, and the addition of the electron transport inhibitors KCN and paraquat. The transcripts of photosynthetic genes encoding PSII D1 protein (*psbA2*), PSI core protein (*psaA*), and the large subunit of Rubisco (*rbcL*) were utilized as controls. Based on the phylogenetic survey for PTOX and our experimental results we present some theories as to the possible function of the PTOX protein.

Session 1B: 2:00-2:15pm

Expression analyses of Arabidopsis Proline-rich Extensin-like Receptor Kinase (PERK) family members

Sarah K. Keatley, May Grace Aldea, and Daphne R. Goring

Department of Botany, University of Toronto

Receptor kinases represent an extensive gene family in plants with functions ranging from growth and development to pathogen defense and wound response. The PERK family of receptor kinases is characterized by an extracellular domain rich in proline and sharing sequence similarity to extensin cell wall proteins, followed by a single transmembrane domain and cytosolic kinase domain. The original member, PERK1, was isolated from *Brassica napus*, and 15 PERK-related members were subsequently identified in *Arabidopsis* (AtPERKs). RNA blot analyses on the 15 AtPERK genes revealed that while some members were found to have organ-specific expression, others showed more ubiquitous expression patterns. In an effort to more precisely examine expression patterns and gain information about their biological roles, AtPERK1, 2 and 3 promoter::GUS transgenic plants were analyzed by histochemical staining, and specificity not apparent from RNA blot analyses was revealed. AtPERK1 exhibited widespread vascular expression while AtPERK3 was restricted to pollen grains, hypocotyls, and major veins of rosette leaves. No consistent staining was observed for AtPERK2 promoter::GUS plants. To more directly study the biological functions of individual AtPERK members, we are currently screening for T-DNA insertions in these genes. To date, 21 homozygous T-DNA insertion lines representing 14 AtPERK genes have been isolated. These plants have been observed under general growth conditions for any defects and to date no obvious phenotypic defects have been found. To test for possible functional redundancy between AtPERK members, crosses between individual AtPERK T-DNA insertion lines are in progress.

Session 1A: 2:15-2:30pm

***In Vivo* Regulatory Phosphorylation of Phosphoenolpyruvate Carboxylase in Developing Castor Oil Seeds.**

William L. Turner, Karina E. Tripodi and William C. Plaxton,

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This study provides the first definitive evidence that a developing seed PEP carboxylase (PEPC) is subject to regulatory seryl phosphorylation *in vivo*. Two novel isoforms (PEPC1 & PEPC2) were recently purified and characterized from developing castor oilseeds (COS) (Blonde & Plaxton 2003 J Biol Chem 278:11867). The association of a common 107 kDa catalytic subunit (p107) with an immunologically distinct, but PEPC-like 64 kDa polypeptide (p64) leads to marked physical and kinetic differences between the PEPC1 p107 homotetramer and PEPC2 p107/p64 heterooctamer. We hypothesized that PEPC1 (inhibited by malate, Asp & Glu) and PEPC2 (desensitized to effectors) respectively support storage protein versus fatty acid (storage lipid) synthesis. Here we describe the production of anti-phosphorylation-site specific IgG to the conserved N-terminal phosphorylation-site (Ser6) of COS p107. Immunoblotting established that p107 phosphorylation is: (1) maximal in full cotyledon (stage VII) developing COS, (2) absent in fully mature COS, (3) more pronounced in PEPC1 than in PEPC2, (4) reversed following incubation of clarified COS extracts or purified PEPC1 or PEPC2 with bovine heart PP2A, and (5) not involved in possible PEPC1 and PEPC2 interconversion. Enhanced p107 phosphorylation during COS development was correlated with a >5-fold increase in I50(malate) value of PEPC activity present in desalted COS extracts. The p107 of stage VII COS became fully dephosphorylated *in vivo* 48 h following excision of intact pods containing developing COS, or following 72 h of dark treatment of intact plants. Ca²⁺-independent p107 PEPC kinase activity did not parallel p107 phosphorylation changes since it remained relatively constant throughout COS development, or 48 h following excision of intact pods of developing COS. We conclude that the *in vivo* phosphorylation status of COS p107 is: (1) modulated by photosynthate recently translocated from source tissue into developing COS, and (2) controlled by factors other than the synthesis/degradation of COS PEPC (p107) kinase.

Session 1B: 2:15-2:30pm

Expressed Sequence Tags from the Yukon Ecotype of *Thellungiella salsuginea* Reveal Little Overlap in Gene Expression in Response to Cold, Drought and High Salinity

Annie C.E. Wong, Yong Li, Brett Whitty, Sajjad R. Akhter, C. Diaz-Camino, J. Brandle, G. Brian Golding, Elizabeth A. Weretilnyk, Barbara A. Moffatt, and Marilyn Griffith

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The Yukon ecotype of *Thellungiella salsuginea*, a close relative of *Arabidopsis*, tolerates soil salinity of 500 mM NaCl, freezing to -21°C, and droughts that reduce its relative water content to as low as 24%. In this study, we analyzed 6578 ESTs from cold-, drought-, and salt-stressed cDNA libraries of *Thellungiella*, which were annotated as 3508 unigenes, to identify the mechanism of abiotic stress tolerance. When the translation products of *Thellungiella* unigenes were compared with the *Arabidopsis* protein database, the median E value obtained in the cold cDNA library was 1.3E-77, while the drought and salinity cDNA libraries were 3.3E-64 and 2.7E-66, respectively, which indicates higher conservation of cDNAs associated with cold stress between *Arabidopsis* and *Thellungiella* versus those recovered in the drought- and salt-cDNA libraries. All unigenes obtained from cold, drought and salinity libraries were classified according to the Gene Ontology Consortium; however, many of the *Arabidopsis* transcripts (30%) have not been yet assigned to a specific functional category. This limited our ability to assign a role to 55.2% of the *Thellungiella* sequences. It has been proposed that plants use common pathways to respond to different abiotic stresses. Among the *Thellungiella* unigenes, we identified 195 (5.4%) common transcripts between cold and drought libraries, 135 (3.7%) between salinity and cold, and 89 (2.4%) between drought and salinity. Surprisingly, only 140 unigenes (3.9%) were expressed in all three libraries and 70% are of unknown function. Only 8% of these genes have already been classified in *Arabidopsis* as responsive to either biotic or abiotic stimuli. Our data show that *Thellungiella*'s abiotic stress tolerance is a function of both novel genes and differences in gene expression compared with *Arabidopsis*, and that we currently have no information about the function of many of the genes that are involved.

Session 2A: 2:45-3:00pm

The ethylene receptors in Arabidopsis show non-redundant roles in differentially regulating seed dormancy

Yee D.¹, Chiwocha S.², Thurston G.¹, McPhee D.¹, Kozela C.¹, Beardmore T.³, Abrams S.⁴, and Regan S.¹.

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The involvement of the plant hormone ethylene in controlling seed dormancy in *Arabidopsis thaliana* has been analyzed as part of a larger objective to understand the functional significance of hormone cross-talk in plants. Until now, ethylene has been implicated in seed dormancy and germination without a more precise role to define its involvement. However, through our work, ethylene has been shown to have a definitive role in dormancy. Previous research has shown that the ethylene receptors in *Arabidopsis thaliana* act as negative regulators that may interact with other signalling pathways involved in the seed's development from dormancy through to germination. To understand their specific roles during seed development, the five ethylene receptors found in *A. thaliana* were studied using dominant gain-of-function mutants that confer ethylene insensitivity. Developing seeds from wild-type and mutant plants were isolated and tested for germination potential. Initial results from wild-type experiments identified four distinct seed developmental phases: A) pre-viable; B) precocious germination; C) dormancy; and D) germination-ready. Comparison of the germinability of seeds from the ethylene receptor mutants revealed, for the first time, distinctive non-redundant roles for two ethylene receptors during seed development. Specifically, two of the ethylene receptors were found to control the entrance into and exit out of seed dormancy.

Recent advances in small molecule profiling have allowed for the unprecedented ability to simultaneously monitor the levels of abscisic acids, auxins, cytokinins, gibberellins, and several metabolites of these hormones. Through collaborative work at NRC-PBI, the use of recently developed liquid chromatography-electrospray ionization tandem mass spectrometry method to simultaneously analyse various hormones and metabolites during seed development has revealed results substantiating the non-redundant characteristic of the two ethylene receptors involved in controlling dormancy. Furthermore, it has allowed for a potentially insightful visualisation of a more complex network of hormone cross-talk that likely occurs during dormancy.

Session 2B: 2:45-3:00pm

Characterization of a phosphate-starvation inducible vacuolar purple acid phosphatase from *Arabidopsis thaliana* suspension cell cultures

VELJANOVSKI, V. (1) and PLAXTON, W.C. (1,2)

(1) Departments of Biology and (2) Biochemistry, Queen's University, Kingston, ON, K7L 3N6.

Acid phosphatases (APs) function in the production and recycling of Pi, a crucial but limiting macronutrient for plant growth and metabolism. Quantification of immunoblots revealed that the ~4-fold increase in AP activity following 7-d of Pi-deprivation of *Arabidopsis* suspension cells was paralleled by a similar increase in the amount of an anti-(tomato purple AP (PAP))-IgG immunoreactive 54-kDa polypeptide. An AP from the Pi-starved (-Pi) cells was purified 957-fold to homogeneity and a final phosphoenolpyruvate-hydrolyzing specific activity of 421 units/mg. The final preparation was pink, indicating that it is a PAP. Blast analysis of its N-terminal sequence demonstrated that it is encoded by 1 of 29 putative *Arabidopsis* PAP genes (AtPAP26; Li et al. 2002 J Biol Chem), and that a 30 amino acid transit peptide is cleaved from the preprotein following its translocation into the vacuole. A proteomics study recently localized AtPAP26 to the cell vacuole of *Arabidopsis* suspension cells (Shimaoka et al. 2004 Plant Cell Physiol). It exists as a ~100 kDa homodimer of 54-kDa glycosylated subunits, and displayed a pH-activity optimum of 5.6, activation by Mg²⁺ and Ca²⁺, but potent inhibition by Zn²⁺, Fe²⁺, molybdate, and phosphate. Immunoblotting of roots and shoots from +Pi vs. -Pi *Arabidopsis* seedlings indicated that this PAP is specifically induced in the roots of the -Pi plants. This is the first bonafide Pi-starvation inducible intracellular PAP to be characterized from *Arabidopsis*. We hypothesize that it plays a pivotal role in Pi remobilization and scavenging from intracellular P-monoesters in -Pi *Arabidopsis* suspension cells and roots.

Session 2A: 3:00-3:15pm

A regulation of seed dormancy by ethylene receptors in Arabidopsis: A story in development, part II.

Thurston G.(1), Yee D.(1), Chiwocha S.(2), McPhee D.(1), Kozela C.(1), Beardmore T.(3), Abrams S.(4), and Regan S.(1)

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To gain insight into the role of ethylene in seed development, we compared the ability of a developing seed to germinate in wild type and ethylene receptor mutants from Arabidopsis. Germination profiles from developing gain-of-function receptor mutant seeds described a hypothetical model for the involvement of two receptors in the regulation of developmental seed dormancy. Preliminary germination profiles from developing loss-of-function receptor mutant seeds appear to support this model.

As most developmental processes are tightly orchestrated through hormonal co-ordination, we undertook a directed hormone profiling approach. This method investigated four of the five major classes of plant hormones and their metabolites including abscisic acids, auxins, gibberellic acids and cytokinins in developing wild type and gain-of-function receptor mutant seeds. Hormone profiles revealed marked differences throughout seed development and interestingly, offer a translation of our model to a hormonal level.

Session 2B: 3:00-3:15pm

Chromatin remodeling and the control of meristem growth in potato tubers emerging from dormancy.

R.D. Law and J.C. Suttle, USDA-ARS, Northern Crop Science Lab, Fargo ND (RDL, JCS) and Lakehead University, Department of Biology, Thunder Bay, ON (RDL; dlaw@lakeheadu.ca).

Variation in DNA architecture has emerged as the cardinal regulator of gene transcription. This dynamic process reversibly alters the three-dimensional environment of genes, allowing transcription as euchromatin and preventing it as heterochromatin. Shoot apical meristems undergo defined changes in gene expression during growth as photosynthetic tissues differentiate and mature. Potato tuber apical meristems (eyes) are a unique model system because they develop and arrest their growth at tuber maturity, only resuming growth after a period of dormancy. As the first step in elucidating biochemical mechanisms responsible for growth arrest and subsequent dormancy emergence, key indicators of eu- and hetero-chromatin were assayed in stored potato tubers. This revealed a series of molecular steps serving to activate gene expression and power new growth of meristems.

Session 2A: 3:15-3:30pm

Characterization of the *in vitro* proteolysis of phosphoenolpyruvate carboxylase from developing castor oil seeds by an endogenous cysteine endopeptidase

CROWLEY, V. (1), GENNIDAKIS, S. (1), and PLAXTON, W.C. (1,2)

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Two novel PEP carboxylase isoforms (PEPC1 & PEPC2) were recently discovered in developing castor oilseeds (COS) (Blonde & Plaxton 2003 JBC). The association of a common 107 kDa catalytic subunit (p107) with a distinct 64 kDa polypeptide (p64) leads to marked physical and kinetic differences between the PEPC1 p107 homotetramer and PEPC2 p107/p64 heterooctamer. Recent COS PEPC purifications unexpectedly resulted in the unwanted *in vitro* proteolysis of p107's N-terminus (during hydrophobic interaction chromatography), producing a truncated (98-kDa) polypeptide (p98). An endogenous asparaginyl thiol endopeptidase is the likely culprit causing partial p107 proteolysis. Immunoblotting was used to estimate proteolytic activity by the disappearance of p107 and concomitant appearance of immunoreactive p98 during incubations of clarified COS extracts or partially purified PEPC at 4°C. This proteolysis: (i) only occurred in the combined presence of a reduced thiol compound (i.e., 2 mM DTT) and high concentrations of sulphate ions (i.e. 20% ammonium sulphate), and (ii) was most pronounced in extracts from older stages of COS development. Dephosphorylated p107 appeared to be more susceptible to degradation than phosphorylated p107. The ability of various protease inhibitors (including Sigma's Plant Protease Inhibitor Cocktail) to prevent partial *in vitro* p107 proteolysis was assessed. All of the tested protease inhibitors were completely ineffective except for 2,2'-dipyridyl disulfide (DPDS), a relatively inexpensive and underutilized active site inhibitor of plant thiol proteases. The inclusion of 2 mM DPDS in buffers used during recent COS PEPC purifications has resulted in non-proteolyzed PEPC1 and PEPC2 preparations containing fully intact p107.

Session 2B: 3:15-3:30pm

Genetic Regulation of the Vascular Cambium in *Arabidopsis thaliana*

 ***Ertha Mullally and Sharon Regan***

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Wood is formed by the meristematic activity of the vascular cambium, a cylindrical meristem located between the xylem and phloem. Little is known about the regulation of the vascular cambium and there are no previous reports of mutants defective in its development. We have characterized a negative regulator of the vascular cambium, Clv 1, demonstrating that a *clv1* mutant of *Arabidopsis* produces significantly more secondary growth than wild-type. We have also identified an *Arabidopsis* mutant that, in contrast, lacks a cambium and appears to lack the ability to undergo secondary growth. This mutant is tentatively called cambiumless and it is postulated that the Cambiumless gene is a positive regulator of vascular cambium development. Using a map-based cloning approach we are in the process of identifying and characterizing this putative positive regulator of the vascular cambium.

Session 2A: 3:30-3:45pm

Does oxygen limit nitrogenase activity in soybean exposed to elevated CO₂?

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Department of Biology, Queen's University

Soybean (*Glycine max* L Merr) plants grown under control (360 mL/L) or elevated (800 μ L/L) CO₂ were assayed for various components of in vivo nitrogenase activity to test the hypothesis that increasing carbohydrate supply to nodules would increase the potential (i.e. O₂ saturated) nitrogenase activity and impose a more severe O₂ limitation on both nodule metabolism and total nitrogenase activity. Within 51 hours of elevated CO₂ treatment, significant increases relative to control plants were seen in total nitrogenase activity expressed per plant. After 6 days of elevated CO₂, the total nitrogenase activity per plant was 18% higher than that in plants in the control treatment. This was attributed to an initial increase in nodule size, and a subsequent increase in nodule number following plants exposed to elevated CO₂. However, after 9 d of elevated CO₂, the potential and total nitrogenase activities per g nodule dry weight were lower, not higher than corresponding values in plants in the control treatment. These results did not support the hypothesis. It was concluded that the metabolic capacity of the control nodules was not limited by carbohydrate supply, at least at the assay temperatures employed here.

Session 2B: 3:30-3:45pm

Differential expression of phosphate-starvation inducible intracellular and extracellular purple acid phosphatase isozymes from tomato

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Tomato purple acid phosphatases (PAPs) catalyze the hydrolysis of phosphate (Pi) from a wide range of P-monoesters under acidic pH. Recently, our group has described three novel PAPs: an intracellular heterodimeric and two secreted monomeric PAPs (IAP; SAP1 and SAP2, respectively) from Pi-starved (-Pi) tomato suspension cell culture. Enzyme activity assays and immunoblotting were employed to assess the influence of -Pi on the expression of IAP and both SAPs of tomato cell cultures and seedlings. The temporal PAP expression pattern was correlated with changes in media Pi, and intracellular free/esterified Pi concentrations. When tomato cells were transferred into -Pi media for 14-d, a 20-fold reduction in intracellular free Pi levels observed at d-6 was correlated with a 6- and 2.5-fold increase in SAP and IAP activity, respectively. Increased activity was correlated with *de novo* synthesis of PAP polypeptides. Immunoblots of cell culture filtrate (CCF) indicated that the 84 kDa SAP1 and 57 kDa SAP2 were initially detected 8-d following subculture into -Pi media. Quantification of immunoblots of clarified cell extracts indicated initial induction at d 6, and a 3-fold increase in the 63 kDa α - and 57 kDa β -subunits of the pre-existing heterodimeric IAP by d-11 in the -Pi cells. Treatment with 50 μ M jasmonic acid (JA) was correlated with a 2-fold induction in SAP1. The expression of the three PAP isozymes was observed in -Pi, but absent in +Pi tomato seedlings. The delayed onset of PAP activity and polypeptide induction in -Pi aerial tissues relative to roots was coincident with their respective dramatic reductions in intracellular Pi. Immunoblots of tomato seedling extracts probed with anti-(tomato SAP1) immune serum revealed the appearance of the antigenic 84 kDa SAP1 polypeptide in -Pi roots, but absent in stem and leaves. Following root-surface PAP activity staining, immunoblots revealed that SAP1 and SAP2 (or a related PAP) are secreted from -Pi and not +Pi roots. To our knowledge, this is the first report of differential expression of PAP isozymes in response to Pi-starvation, or JA treatment. This study also suggests that the Pi-starvation response of suspension cell cultures parallels that of whole plants.

POSTER PRESENTATIONS

Poster 1

Isolation and Characterization of PATCHY, a Gene Involved in Mucilage Release in the Arabidopsis Seed Coat

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In Arabidopsis, the epidermal cells of the outer ovule integument differentiate through a complex process into specialized cells that produce mucilage between the primary cell wall and plasma membrane. Upon imbibition the mucilage expands rapidly, breaking through the primary cell wall and enveloping the seed. A mutation in PATCHY (PTY) causes a peculiar phenotype where mutants have inconsistent mucilage release compared with wild type seeds. Cloning of PTY by plasmid rescue revealed a T-DNA insertion in a gene encoding a cell wall modification enzyme. Two independent Salk T-DNA knockout lines in the same locus produce a similar 'patchy' release phenotype, confirming the locus is involved in mucilage release. Preliminary expression analysis with qualitative RT-PCR shows expression of PTY in all tissues tested, including 4, 7, and 10 day old siliques, consistent with its proposed role in mucilage release and cell wall modification. A preliminary framework for the regulation of mucilage secretory cell differentiation has been proposed. The place of PTY within this framework is being investigated with double mutants and the results will be presented.

Poster 2

The Role of Alternative Oxidase in Modulating Carbon Use Efficiency and Growth, Preventing Oxidative Stress, and Maintaining Tissue Nutrient Status and Carbon Metabolism During Macronutrient Stress in Tobacco Cells

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When wild-type (wt) tobacco (*Nicotiana tabacum* cv. Petit Havana SR1) cells are grown under macronutrient (P or N) limitation, they induce large amounts of alternative oxidase (AOX), an inner mitochondrial membrane protein that constitutes a non-energy-conserving branch of the respiratory electron transport chain. To investigate the significance of AOX induction, wt cells were compared with transgenic (AS8) cells lacking AOX. Under nutrient limitation, the growth of wt cell cultures was dramatically reduced and carbon use efficiency (g cell dry weight gain g⁻¹ sugar consumed) decreased by 42 to 63%. On the other hand, the growth of AS8 cells was only moderately reduced by the nutrient deficiencies and carbon use efficiency values remained the same as under nutrient-sufficient conditions. A reduction in growth appeared to be an important mechanism to maintain nutrient status since the nutrient limitations more severely compromised the tissue nutrient status (P or N) of AS8 than wt cells. Northern analyses and a comparison of the mitochondrial protein profiles of wt and AS8 cells indicated that the lack of AOX in AS8 under P limitation was associated with increased levels of proteins commonly associated with oxidative stress and/or stress injury. Accompanying this, the level of electron transport chain components was consistently reduced in AS8 while tricarboxylic acid cycle enzymes did not show a universal trend in abundance in comparison to the wt. On the other hand, metabolite and enzyme analyses indicated that the absence of AOX in AS8 cells under N limitation was associated with pronounced shifts in carbon metabolism. We conclude that AOX respiration provides an important general mechanism by which plant cells can modulate their growth in response to nutrient availability and that AOX also has nutrient-specific roles in maintaining cellular redox and carbon balance.

Poster 3

Cytokinin oxidase activity throughout the development of R50, a pea mutant accumulating cytokinins

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Cytokinins (CK) are an adenine-based family of plant hormones, which regulates a number of pathways regulating growth and development. The levels of endogenous CK are regulated by both biosynthesis and catabolism and recent studies have shown that manipulation of either pathway significantly alters endogenous CK content. Isoprenoid and zeatin-type cytokinins are degraded by the enzymatic activity of cytokinin oxidase (CKX). The enzyme specifically cleaves the isoprenoid side chain, leaving adenine and the corresponding aldehyde. The pea nodulation mutant R50 (sym-16) displays a number of phenotypic traits that are indicative of increased CK content and this lead to the hypothesis that CKs may accumulate in this mutant. This was recently confirmed by an LC-MS-MS analysis of total CK content in the roots and shoots of R50 and in the wild-type (WT) line. It was further hypothesised that this accumulation of CKs in R50 may be a result of reduced biochemical activity of CKX. To investigate this topic further, the enzyme kinetics of CKX were measured throughout development in R50 and compared to those of the WT. Activity was detected by a colorimetric assay, following the protocol of Liberos-Minotta and Tipton (1995) as modified by Frébort et al. (2002). A significant decrease in CKX activity was noted in the roots and shoots of R50 seedlings and mature plants (9 and 17 days after planting, respectively). Interestingly, CKX activity in mature, dry and imbibed seeds of this mutant was comparable to that of the WT. Furthermore, the CKX activity profile was mirrored by the CK profile in both R50 and the WT. These results support the hypothesis that reduced activity of CKX is a major causative agent in the creation of the R50 phenotype.

Poster 4

***In vitro* manipulation of cereal genomes for increasing the frequency of recombinations in interspecific hybrids**

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Interspecific crosses are at present the main possibility for producing new germplasm for cereal breeding, but the efficiency of these methods is limited by a low recombination frequency. Somaclonal variability could be used for increasing the frequency of recombination in interspecific/ intergeneric hybrids of cereals, facilitating genomic rearrangements as a result of the 'in vitro' cell development (Phillips et al 1994).

In order to apply this method for producing durum wheat lines with improved traits (low to tolerance) we have used scutellar callus cultures of F1 embryos derived from a cross between several *Triticum aestivum* (genome ABD) lines and a *T. turgidum* var durum (genome AB) variety. The maternal parent (*T. aestivum*) was the donor for cold tolerance. Over 200 plants were regenerated using a medium-term (4-5 months) regeneration protocol (Cialacu, 1998). After 2 backcrosses with the recurrent parent (*T. durum*), over 60 recombinant lines were produced, characterized cytologically and biochemically and field-tested for improved resistance.

The percentage of recombinant lines showing an improved level of cold tolerance was higher for the genotypes derived from regenerated plants (24 %) than for the lines produced by conventional methods (11 %).

Several studies have reported the production of superior lines by exploiting somaclonal variability in barley (Li et al, 2001) and wheat (Arun, 2003). Our results strongly suggest the utility of somaclonal variability for increasing the frequency of recombination in cereal crosses. Arun et al. 2003, *Euphytica* 132: 235-241, Cialacu, M., 1998, PhD Thesis, Li et al. 2001, *Euphytica* 121:349-356, Phillips, R.L. et al. 1994, *Proc. Nat. Acad. Sci. (USA)* 91:5222-5226

Poster 5

Alternative Oxidase Attenuates Plant Cell Death by Dampening the Mitochondrial Generation of Reactive Oxygen Species

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In animals, it is well established that the mitochondrion plays an active role in the signal integration and execution of programmed cell death (PCD). Recent research suggests that the mitochondrion may also play some role in plant PCD. However, plant mitochondria harbor additional electron transport chain (ETC) components that may alter the specific role of plant mitochondria in PCD. One such component is alternative oxidase (AOX), which catalyzes the oxidation of ubiquinol and reduction of oxygen to water. Previously, we established that antisense tobacco suspension cells lacking AOX (AS8) exhibit increased susceptibility to PCD induced by several different treatments (Plant Physiol. 129, 1908-1920, 2002). To further investigate this in planta, we used post-transcriptional gene silencing to generate tobacco plants lacking leaf AOX. While Complex III inhibitors (antimycin A or myxothiazol) had no obvious effect on wt tobacco leaves, transgenic (RI9) leaves exhibited extensive cell death in response to these treatments. Interestingly, co-treatment of these inhibitors with the antioxidant flavone dramatically reduced the extent of RI9 death, suggesting that the death is dependent upon elevated levels of reactive oxygen species (ROS). ROS are well established to play an important role(s) in mitochondria-dependent PCD in animals. The results further suggest that the protective effect afforded by AOX is not simply due to its ability to maintain energy metabolism during disruptions in mitochondrial electron transport at Complex III but rather that it protects against a ROS-induced cell death during such ETC disruptions. Other experiments with suspension cells extend these results and suggest that a chronic increased level of oxidative stress being experienced by cells lacking AOX contributes to their increased susceptibility to a wide range of death-inducing treatments. Overall, the results suggest that mitochondrial-generated ROS impact plant PCD pathways and that AOX can attenuate such death pathways by dampening ROS generation.

Poster 6

In the pollen grain aperture and the pollen tube cell wall callose has load-bearing function

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While callose is a well known permeability barrier and leak sealant in plant cells, it is largely unknown whether this cell wall polymer can also serve as a load-bearing structure. Since callose occurs in exceptionally large amounts in pollen, we assessed its role for resisting tension and compression stress in this cell. The effect of callose digestion in *Solanum chacoense* and *Lilium orientalis* pollen grains demonstrated that, depending on the species, this cell wall polymer represents a major stress-bearing structure at the aperture area of germinating grains. In the pollen tube it is involved in the cell wall resistance to circumferential tension stress, and despite its absence at the growing apex, callose is indirectly involved in the establishment of tension stress resistance in this area. To investigate whether or not callose is able to provide mechanical resistance against compression stress, we subjected pollen tubes to local deformation by micro-indentation. The data revealed that lowering the amount of callose resulted in reduced cellular stiffness and increased visco-elasticity thus indicating clearly that callose is able to resist compression stress. Whether this function is relevant for pollen tube mechanics, however, is unclear as stiffened growth medium caused a decrease in callose deposition. Together our data provide clear evidence for the capacity of cell wall callose to resist tension and compression stress in pollen thus demonstrating that this amorphous cell wall substance can have a mechanical role in growing plant cells.

Poster 7

Re-initiation of pollen tube tip growth: The sequence of cellular events leading to a new tubular outgrowth

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The anisotropic distribution of cell wall components is a characteristic feature of growing pollen tubes. While esterified pectins form the bulk of cell wall material at the apex, de-esterified pectins, cellulose and callose are present in the distal regions of the tube. We showed previously that this phenomenon correlates with a reduced cellular rigidity at the growing apex of the cell (Geitmann and Parre, Sexual Plant Reproduction 17:9-16). Addition of pectin methyl esterase causes the rigidification of the apex and the arrest of pollen tube growth thus indicating that a certain deformability of the apex is essential for the growth process (Parre and Geitmann, Planta, in press). It is unclear, however, whether this deformability is a required condition for tip growth or whether it is the result of the continuous secretion of esterified pectins. To examine this question we attempted to determine whether cellular rigidity at the site of pollen tube re-initiation from swellings decreases prior to formation of the outgrowth or only upon onset of tube formation. To do so we measured the time course of the local cellular stiffness at the site of re-initiation and compared it to other sites on the same swelling. Swelling and outgrowth of new tubular cells were achieved by application and subsequent removal of cytochalasin D, an agent that prevents actin polymerization and effectively arrests polar growth in pollen tubes. Therefore, the recovery of the actin cytoskeleton was monitored in parallel. Repeated micro-indentation measurements at different locations on the cellular swelling revealed that local cellular softening preceded the formation of a new tubular outgrowth and was a good indicator of the direction of the new growth axis. Implications of these findings are discussed in the context of the mechanism governing apical growth.

Poster 8

Evidence that the Plastid Translocon Tic40 Components Possess Modulating Capabilities

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The transport of proteins into the plastid is a process that faces changing cellular needs such as the situation found in different plant organs or developing tissues. The plastid translocon must therefore be responsive to the changing cell environment in order to deliver efficiently different arrays of structurally diverse proteins. Although the Tic40-related envelope proteins appear to be translocon components designed to address the varying needs of protein translocation, details of their involvement remain elusive. This study was thus designed to combine plant-based experiments and yeast mitochondrion-based approaches for unveiling clues related to how the Tic40 components may behave during the protein translocation process. The main findings related to how Tic40 proteins may work are: 1) Natural fluctuations are apparent in developing tissues, in different organs of the same plant, and in different species; 2) Transgenic Arabidopsis seedlings can tolerate functionally a wide range of variations in Tic40 levels, from partial suppression to excessive production; 3) The Tic40 proteins themselves exhibit configurational changes in their association with yeast mitochondria in response to different carbon sources; 4) The presence of Tic40 proteins in yeast mitochondria influences regulatory aspects of the mitochondrial translocon, and 5) The Tic40 proteins associate with mitochondrial translocon components involved in regulatory-like events. The combined data provide evidence that Tic40 proteins possess modulating capabilities.

 **ter 9**

A Plant-like Alternative Oxidase is Present in Several Animal Phyla and is Widespread Amongst Marine Eubacteria

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The mitochondrion of most eukaryotes has multiple electron transport components that increase the points of entry and/or exit of electrons, thus giving a branched nature to the respiratory chain. In plants and many other organisms, a prominent example is alternative oxidase (AOX), a non-energy conserving branch in the respiratory chain and an additional terminal oxidase for the exit of electrons. Besides its ubiquitous presence in the Plantae, AOX is also sporadically distributed in both the Fungi and the Protista. Recently, we have established the presence of AOX in two kingdoms for which it was previously not known to exist. Firstly, our genome database searches have found AOX genes in seven animal species from three different phyla (Mollusca, Nematoda and Chordata). The AOX genes are present in some pathogenic nematodes and several marine intertidal species. This is consistent with frequent reports of cyanide-resistant respiration in such animals. In *Ciona intestinalis* (sea squirt) and *Crassostrea gigas* (Pacific oyster), AOX is expressed in several different tissues. Phylogenetic analysis is consistent with the animal proteins having originated by vertical inheritance. We hypothesize that alternative oxidase is likely widespread in the Animalia and discuss some of the potential role(s) for such a branched respiratory chain. Significantly, we have also uncovered AOX genes in a wide range of Eubacteria (and possibly Archaea). In part, this work has made use of a large “environmental shotgun sequencing” dataset from the Sargasso Sea. We have also extended our AOX work to include a similar diiron carboxylate protein in the chloroplast called plastoquinol terminal oxidase (PTOX). In the Sargasso Sea, PTOX is widespread amongst strains of cyanobacteria closely related to the high-light adapted *Prochlorococcus marinus* MED4, as well as *Synechococcus*. Taken together, our results support the hypothesis that extant mitochondrial AOXs and chloroplast PTOXs arose from the endosymbiotic events that gave rise to mitochondria and chloroplasts, respectively.

Poster 10

Phytoremediation of a contaminated groundwater site.

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Phytoremediation is a new and emerging technology. To investigate the relationship between transpiration rates and the amount of contaminants released from trees, *Populus balsamifera*, *P. deltoides* x *nigra*, *P. nigra* (poplar) as well as *Salix nigra* and *S. interior* x *eriocephala* (willow) were planted in a contaminated groundwater plume. The groundwater was contaminated with many substances such as 1,4-dioxane, ethylene glycol, and chloroform. A tent-like structure was constructed and erected around each tree to trap substances released into the air by the leaves. The air was drawn out of the tent-like structure using an air pump connected to the charcoal trap over a period of 3-8 hours. CO₂ levels, temperature, and humidity were measured using Qubit System sensors (Qubit, Kingston, ON). Transpiration rates and water conductance were calculated. An unidentified compound was identified as being trapped in the charcoal tubes using gas chromatography flame-ionization device (GC-FID) detection. Also, it was determined that the plastic tent caused a decrease in transpiration and water conductance compared to reported values. Further investigations are being undertaken to determine the identity of the unknown compound (using mass spectrometry) and the amount being released by the trees in relation to whole-tree transpiration.

Poster 11

Recombinant Arabidopsis succinic semialdehyde reductase: expression, purification, substrate specificity and affinity

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Succinic semialdehyde (SSA), which is derived from the metabolism of gamma-aminobutyrate, can be converted to either succinate in a reaction catalyzed by SSA dehydrogenase or to gamma-hydroxybutyrate in a reaction catalyzed by SSA reductase. Previously, complementation of a SSA dehydrogenase-deficient yeast mutant with an Arabidopsis cDNA library allowed the identification of a putative SSA reductase cDNA. In the present work, this cDNA was highly expressed in *Escherichia coli*, and the recombinant enzyme was purified to homogeneity from the soluble bacterial fraction. The enzyme readily catalyzed the reduction of SSA, as well as the reduction of phenylglyoxal, 2-propylpentanoic acid, and 4-carboxybenzaldehyde in an NADPH-dependent reaction. However, the catalytic efficiency for SSA reduction was 13-59 times that for the other substrates tested, indicating that the enzyme is an NADPH-dependent SSA reductase. The enzyme displayed hyperbolic saturation kinetics for SSA, with a half-saturation constant of 1.5 mM, and sigmoidal saturation kinetics for NADPH, with a half-saturation constant of 25 μ M and a Hill coefficient of 2.2. Activity was decreased with increasing NADP levels. Enzymes of this type typically catalyze regulatory or rate limiting steps in metabolism. The physiological function of SSA reductase will be discussed.

Poster 12

Recombinant Arabidopsis GABA transaminase: expression, linked assay, substrate specificity and affinity

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Gamma-aminobutyrate (GABA) is a ubiquitous non-protein amino acid, which has been associated with stress response and signaling in plants. It is catabolized to succinic semialdehyde via GABA transaminase (GABA-T) activity, which can use either pyruvate or 2-oxoglutarate as an amino acceptor, producing alanine and glutamate, respectively. Recently, an Arabidopsis GABA-T cDNA, which possesses a mitochondrial signal peptide, was identified in our laboratory. In this study, expression of the full-length cDNA and a truncated cDNA lacking the signal peptide was compared using *Escherichia coli* BL21 (DE3) Rosetta pLysS (Novagen) cells, which co-expressed the GroEL/ES chaperone complex. Use of the truncated cDNA resulted in much higher expression of the recombinant protein and a 6-fold increase in activity. Linked assays, which used either purified recombinant succinic semialdehyde reductase or commercial lactate dehydrogenase, enabled determination of GABA-T activity in the forward and reverse directions via the turnover of NADPH and NADH, respectively. In the forward reaction with pyruvate as the amino acceptor, the enzyme was specific for GABA and did not utilize other amino donors such as glycine. The enzyme did not 2-oxoglutarate with any amino donors, supporting the previous suggestion that distinct proteins account for the pyruvate- and 2-oxoglutarate-dependent GABA-T activities reported in plants. The Michaelis-Menten constants for GABA, pyruvate, alanine and SSA were 1.5, 0.15, 4.1 and 0.019 mM, respectively. HPLC assays revealed that the enzyme utilizes GABA and alanine as amino donors when glyoxylate is the amino acceptor, thereby producing glycine. The physiological significance of these findings will be discussed.

Poster 13

Single gene products targeted to multiple subcellular locations



Erin Schoor and Barbara Moffet

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Methylation occurs in every compartment of plant cells, yet two key enzymes involved in this process, adenosine kinase (ADK) and S-adenosylhomocysteine hydrolase (SAHH), are thought to be only localized within the cytosol. Our research indicates that this is not the case. To investigate how the methyl cycle functions in compartments other than the cytosol we have examined the localization of the two isoforms of ADK and SAHH. Immunolocalization studies utilized 3-week-old *Arabidopsis thaliana* meristem and leaf sections of both wild-type Columbia and T-DNA tagged mutants deficient in either ADK1 or 2. Binding of antibodies specific for ADK and SAHH was then recognized by secondary antibodies conjugated to 7 or 15 nm gold beads, allowing the detection of their respective enzymes by TEM. Complementary studies are using the CaMV35S promoter to direct the expression of ADK and SAHH cDNAs fused to a GUS reporter gene, to track their movements throughout the cell. Both methods show that ADK and SAHH are present within the nucleus and chloroplast as well as the cytosol. The same cellular distribution was observed for both ADK isoforms, in leaf and meristem tissue. As well as increasing our understanding of how plant methylation reactions are maintained throughout cells, these results provide an interesting research system to study the localization of proteins containing no discernable targeting sequences.

Poster 14

Inhibition of microtubules with colchicine causes altered organelle positioning and reduced levels of nitrogen fixation within infected cells of *Glycine max* root nodules.

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A selective symbiotic relationship exists between soil bacteria and leguminous plants, in which soil bacteria induce the formation of nodules on leguminous roots. The bacteria are provided with carbon skeletons, and the leguminous host plant with a useable form of nitrogen. Atmospheric nitrogen is reduced to ammonia by the nitrogenase enzyme within the symbiosome for use by the plant. Within root nodules, extensive arrays of actin filaments and microtubules exist. In young and mature infected cells, these arrays may interact with symbiosomes and cellular organelles. The inhibition of microtubule polymerization by colchicine led to structural changes within developing *P. sativum* root nodule cells. Amyloplasts and mitochondria were normally located at the periphery of most infected cells. In colchicine treated cells, these organelles were found in clumps in the central cytoplasm of infected nodule cells. Vacuolar placement was also altered in comparison to control cells. We hypothesized that these structural rearrangements would cause nodules to have reduced rates of nitrogen fixation. We expanded the study to investigate the relationship in a symbiosis between *Glycine max* and *Bradyrhizobium japonicum* (due to the readily available HUP - *B. japonicum* strain USDA16). Altered positioning of amyloplasts and mitochondria again appeared when colchicine was applied to plants treated for 48 hours in liquid nutrient solution. Preliminary physiological studies indicated that levels of nitrogen fixation are impaired in nodules treated with the microtubule inhibiting agent, colchicine. Nitrogen fixation was measured prior to and after treatment for 48 hours in liquid nutrient solution. Those plants treated with colchicine did not show an increase in nitrogen fixation rate over the four days when they were treated and then replanted in silica sand, compared with a control group under the same protocol. These preliminary results suggest that the microtubule arrays within *G. max* root nodules are of key importance in the positioning of cellular organelles and are associated with decreased nitrogen fixation rates.

Poster 15



Response of conifer forests to climate change – Much more than simple temperature relations?

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We studied tree and forest photosynthesis in a boreal climate (Zotino, central Siberia), ranging from the cellular to the ecosystem level. Our aim was to characterise the physiological mechanisms underlying the onset of photosynthesis in spring and a downregulation of the photosynthetic capacity in autumn. Whole canopy gas exchange revealed a rapid onset of photosynthesis mainly triggered by above-zero air temperatures in spring. A slow but protracted increase followed. The subsequent decline in canopy photosynthesis in autumn was only partially attributable to less favourable environmental conditions. A detailed analysis on the needle level using controlled environmental facilities showed air temperature and light to be the main drivers of spring recovery processes, but that intermittent low temperature events have strong (but reversible) retarding effects on the reconstituting photosynthetic apparatus. Soil temperatures around zero degrees during spring do not inhibit the recovery process but do decrease the potential rate of recovery. For Scots pine exposed to different temperature and daylength treatments during autumn, photosynthetic capacity was largely retained by warm temperatures and seemed to be independent of daylength. However, endogenous factors were responsible for the further downregulation of photosynthetic capacity in late autumn in all experimental treatments, irrespective of daylength or temperature conditions. There was, however a decreased frost resistance of needles as assessed by chlorophyll fluorescence of Scots pine grown under simulated increased autumn temperature conditions. Our results suggest that boreal evergreen conifers might not be able to exploit the suggested increased length of the growing season due to global warming, because they might fail to properly time the processes of hardening in autumn and dehardening in spring.

Poster 16

Effects of altered leaf sucrose starch partitioning on diel patterns of leaf ^{14}C export and shoot growth of *Flaveria linearis*

Evangelos D. Leonardos, Barry J. Micallef, Malgre Micallef and Bernard Grodzinski

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Diel patterns of leaf photosynthesis and ^{14}C -export in two parental *F. linearis* lines [85-1: high activity of cytosolic Fructose-1,6-bisphosphatase (cytFBPase) and 84-9: low activity of cytFBPase] were estimated using steady-state $^{14}\text{CO}_2$ labelling. Using these lines, the proportion of recently fixed C incorporated into starch versus sucrose was quantified and the growth and extension rates of main branches were determined using linear voltage signal transducers. A higher proportion of ^{14}C was incorporated into starch rather than sucrose in 84-9. Photosynthesis and ^{14}C -export during daytime were higher in 85-1. ^{14}C -export as % of photosynthesis was about 40% higher in 85-1. ^{14}C -export rates were higher at the beginning of the night in 84-9, but similar in the two lines at the end of the night. At night, about 25% more ^{14}C was exported and 44% more ^{14}C was respired in 84-9. More ^{14}C remained in the leaves of 84-9 both at the end of day and end of night. The diel export patterns obtained using $^{14}\text{CO}_2$ correlated with estimates based on measurements of assimilate fluxes through leaf C-pools or dry mass during the daytime, but not during the night. Low starch accumulation and higher export during the daytime in 85-1 correlated to greater total biomass gain and increased main stem elongation and growth. In conclusion, a change in sucrose-starch partitioning can alter the diel patterns of export from the leaf that can influence the patterns of shoot growth in *F. linearis*.

Poster 17

Excitation pressure dependent development of alternative non-radiative charge recombination pathways in wild type and F2 mutant of barley

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The estimated energy partitioning in wild type (WT) and F2 barley mutant plants grown at temperature/irradiance regimes corresponding to low (20/250, 5/50) and high (20/800, 5/250) excitation pressure (EP) indicated that the fraction utilized by photochemical electron transport (P) decreases in both WT and F2 mutant under high EP conditions. In parallel, the proportion of thermally dissipated energy (D) increased to 144% and 123% in WT plants acclimated to high EP (20/800 and 5/250) compared to 20/250 and 5/50 plants respectively. The fraction of excess energy (E) not utilized by the electron transport and not thermally dissipated was drastically increased by 2.6 and 3.7 fold in WT 20/800 and 5/250 plants compared to plants grown at low EP. Similar partitioning of the energy fluxes was also observed for the F2 mutant. The larger fraction of excess energy observed in high EP plants (20/800 and 5/250) was accompanied by lower capacity for state I – state II transitions compared to plants grown under low EP. Thermoluminescence measurements of PSII revealed that S2/S3QB- recombinations were shifted to lower temperatures in WT and F2 plants acclimated to high EP. This corresponded to lower activation energy of the S2QB- peak, thus narrowing the gap in the redox potentials between QA and QB electron acceptors and increasing the probability for reaction centre quenching of excess light. In addition, F2 mutant demonstrated a substantial shift of S2QA- to higher temperatures, thus again narrowing the gap between S2QB- and S2QA- compared to WT under all growth conditions. In this report we show that acclimation to high EP rather than low temperature per se increases the probability for non-radiative reaction center quenching of excess light via developmental changes in the redox properties of the acceptor site of PSII. The enhanced probability for development of this alternative pathway for non-radiative energy dissipation estimated by the temperature/energy gap between QA and QB positively correlates with the observed higher proportion of excess energy in plants acclimated to high EP.

Poster 18

Digalactosyl-diacylglycerol deficient mutant of arabidopsis exhibits altered psi function

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The photochemical performance of photosystem I (PSI) and photosystem II (PSII) as well as the capacity for intersystem electron transport, state transitions and the relative abundance of chlorophyll-protein complexes were assessed and compared in wild type and a *dgd1* lipid mutant (lacking 96% of levels of digalactosyl-diacylglycerol - DGDG) of *Arabidopsis thaliana* L. under control and photoinhibitory conditions. Results from the comparative analysis using chlorophyll fluorescence imaging and modulated room temperature chlorophyll fluorescence revealed lower photochemical efficiency of PSII, impaired linear electron transport, higher excitation pressure and increased thermal dissipation in *dgd1* mutant at the growth light intensities of 75  bl quanta m²/s. In addition, 77K fluorescence emission spectra and light induced absorbance transients of P700 revealed that the above results could be explained by an inhibition of PSI. Light dependent steady state oxidation level of P700 (P700+) was significantly lower, intersystem electron pool size higher and the rate of reduction of P700+, which indicates an increased capacity for PSI cyclic electron transfer was also higher in the *dgd1* mutant compared to wild type. The lower level of oxidizable P700 (P700+) in *dgd1* mutant corresponded with a lower amount of PSI related chlorophyll protein complexes (LHC1 and CP1) as revealed by non-denaturing SDS-PAGE measurements. Additionally, this mutant exhibited a limited ability to undergo state transitions and an up-regulation in the stromal reduction of the plastoquinone pool suggesting electron flux of a cyclic or chlororespiratory nature. Furthermore, *dgd1* mutant exhibited much higher sensitivity and incomplete recovery from high light stress of PSII and PSI. These data indicate an essential role of DGDG in the function, composition and/or assembly of the PSI complex.

Poster 19

Non-invasive Early Detection of Root Diseases in Plants: Net Carbon Gain, Chlorophyll Fluorescence and Plant Development of Green Pepper (*Capsicum annuum*) and Snapdragon (*Antirrhinum majus*) following root Infection by *Pythium aphanidermatum*

Noé Ortiz-Uribe¹, Renée R. Cloutier¹, Evangelos D. Leonardos¹, Weizhong Liu², Muhammad J. Iqbal¹, Jonathan Brockerville³, Adam DesLauriers³, Iain Christie³, John Sutton² and Bernard Grodzinski^{1, 2*}

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Pythium root rot is a major problem for greenhouse crops. Alterations in whole plant photosynthetic rate and carbon assimilation were characterized for pepper and snapdragon plants. Whole-plant net carbon exchange rate (NCER), total carbon accumulation, dark respiration rates, chlorophyll fluorescence, water loss and destructive growth parameters were examined in vegetative, hydroponically grown plants. Inoculated plants displayed lower whole-plant NCER. The cumulative carbon gain at 7 days after inoculation was 28 and 56% lower for infected pepper and snapdragon, respectively. Non-photochemical quenching (NPQ) was significantly higher in infected snapdragon 24 hours after infection, before shoot symptoms developed. However in the case of peppers the NPQ differences were not significant. Dry mass of the shoots and total leaf area (LA) were significantly decreased in infected plants. Consequently, expression of NCER and evapo-transpiration on LA basis, showed no differences for inoculated and control plants. Taken together these data indicate that although *Pythium* infection had an affect on NPQ in snapdragon, the reduced whole plant NCER was due primarily to reduced development of the leaf canopy in both plant species. Possible signals transported from infected roots to the developing canopy will be discussed.

Poster 20

Endogenous photosynthate translocation from shoot to roots may be blocked in common bean (*Phaseolus vulgaris* L.) dwarf plants.

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Crosses between certain bean genotypes result in a characteristic dwarfing of F1 plants, which reduces growth sufficiently to prevent the production of F2 seed. This dwarfing results from a dosage dependent effect of alleles at two complementary loci, DL1 and DL2. When a plant is heterozygous at both loci (DL1dl1DL2dl2) growth is severely reduced, and when a plant is homozygous (DL1DL1DL2DL2) lethal dwarfing occurs with failed root growth known as "F" plants. Grafting studies demonstrated that DL1 acts in the root system and DL2 in the shoot, making the DL system a unique example of two genes controlling a strong root-shoot interaction. Root growth can be achieved in both "F" plants and lethal graft combinations by adding sucrose to the growth medium. Restoration of root growth in "F" plant and lethal grafts may be because externally supplied sucrose acts as a triggering signal to restore the normal root shoot relationship or it may be an alternative to endogenous photosynthates. A series of $^{14}\text{CO}_2$ experiments were conducted on self-grafted parents and lethal graft combinations to further our understanding of the nature and causes of 'lethal dwarfing' in common beans.

Normal self grafted Ca/Ca and lethal grafted Pd/Ca plants grown on MS media with and without sucrose were fed with $^{14}\text{CO}_2$ and 24 hours later the allocation of ^{14}C was determined. In normal Ca/Ca plants growing without exogenous sucrose, 4% of ^{14}C was allocated to roots and 2% to the stem below the graft union. In the lethal graft Pd/Ca, 10% of ^{14}C was allocated to the stem below the graft union and none was recovered from the roots. For both the graft combinations when sucrose was added to the media, very little ^{14}C was recovered below the graft union and none was recovered from the roots. The data indicate that the physiological and biochemical basis of the lethal dwarfism may be the inability of the roots to receive endogenous assimilates.

Poster 21

Effects of altered leaf sucrose starch partitioning on diel patterns of leaf ^{14}C export and shoot growth of *Flaveria linearis*

Evangelos D. Leonardos, Barry J. Micallef, Malgre Micallef and Bernard Grodzinski

Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada, N1G 2W1

Diel patterns of leaf photosynthesis and ^{14}C -export in two parental *F. linearis* lines [85-1: high activity of cytosolic Fructose-1,6-bisphosphatase (cytFBPase) and 84-9: low activity of cytFBPase] were estimated using steady-state $^{14}\text{CO}_2$ labelling. Using these lines, the proportion of recently fixed C incorporated into starch versus sucrose was quantified and the growth and extension rates of main branches were determined using linear voltage signal transducers. A higher proportion of ^{14}C was incorporated into starch rather than sucrose in 84-9. Photosynthesis and ^{14}C -export during daytime were higher in 85-1. ^{14}C -export as % of photosynthesis was about 40% higher in 85-1. ^{14}C -export rates were higher at the beginning of the night in 84-9, but similar in the two lines at the end of the night. At night, about 25% more ^{14}C was exported and 44% more ^{14}C was respired in 84-9. More ^{14}C remained in the leaves of 84-9 both at the end of day and end of night. The diel export patterns obtained using $^{14}\text{CO}_2$ correlated with estimates based on measurements of assimilate fluxes through leaf C-pools or dry mass during the daytime, but not during the night. Low starch accumulation and higher export during the daytime in 85-1 correlated to greater total biomass gain and increased main stem elongation and growth. In conclusion, a change in sucrose-starch partitioning can alter the diel patterns of export from the leaf that can influence the patterns of shoot growth in *F. linearis*.

Poster 22

Effect of Nod factors on gas exchange and growth of soybean under greenhouse conditions

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Nod factors (Lipo-chitooligosaccharides, LCO) are part of a signal exchange between rhizobia and legumes that leads to the formation of root nodules, in which the bacteria fix nitrogen. These signals are produced by rhizobia and induce a series of responses in the host plant such as root hair deformation, and formation of pre-infection threads and nodule primordia. Recently we have been demonstrated that they can stimulate other physiological processes, such as seed germination, plant growth, and photosynthesis. Our objective was to evaluate the effect of LCOs on gas exchange characteristics, and plant growth of soybean grown under greenhouse conditions. Several concentrations and types of Nod factors were sprayed on soybean plants at the second trifoliolate leaf stage. Soybean responded positively to LCOs having the greatest response to a 10^{-7} M solution. Photosynthesis was enhanced up to 13% over the control; stomatal conductance and transpiration were also increased while intercellular CO₂ did not change. Plant dry biomass was enhanced 11% compared with the control. Nearly expanded leaves responded more strongly to LCOs than fully expanded leaves. Of the four LCOs tested, soybean respond to most of them, but Nod Bj (C18:1, MeFuc) had the strongest effects on photosynthesis and plant biomass.

Poster 23

Excitation energy partitioning and quenching during cold acclimation in Jack pine.

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Temperature and light are important factors driving physiological changes in boreal evergreen conifers since they determine the length of the growing season. Climate change and the raise of air temperature in northern latitudes are likely to increase the length of the growing season. Evergreen trees might benefit from warmer air temperatures in early spring or late autumn, thus increasing the photosynthetically active period and net carbon gain. However, our understanding of the cold hardening process in evergreen trees is still rather poor and trees might fail the right timing of this important developmental change. Our aim is to characterize the physiological mechanisms that are responsible for the downregulation of photosynthesis in Jack pine. Here we present first results from a factorial experiment using controlled environment facilities to dissect the effects of day length and temperature on the autumn downregulation of photosynthesis. For this purpose we used four different growth conditions (20°C/16h day length; 20°C/8h; 5°C/16h; 5°C/8h). Fluorescence measurements revealed changes in Fv/Fm values and excitation energy partitioning in response to day length and temperature. Photosynthetic capacity was generally lower in plants grown at 5°C. Day length did not have any effect on plants grown at 20°C, but in the cold treatments 16 h day length resulted in a decrease of Fv/Fm values. In addition, at low temperatures an increased fraction of the incident light was dissipated non photochemically via heat. In contrast to the autumn cessation of growth, which is supposed to be triggered by a critical day length, photosynthetic capacity in autumn is rather controlled by temperature. This suggests that conifers might be able to maximize photosynthetic gain during warm autumn temperatures.

Poster 24

Compartmentation of de novo NADP(H) biosynthesis: Subcellular Localization of NAD(H) Kinases in *Arabidopsis thaliana*

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In the model higher plant *Arabidopsis thaliana*, the de novo biosynthesis of the vital phosphopyridine nucleotide NADP is catalyzed solely by two NAD⁺ kinases (NADKs) and one NADH kinase (NADHK) that use MgATP to phosphorylate either oxidized or reduced NAD, respectively. Calmodulin, a ubiquitous eukaryotic Ca²⁺-binding protein, responds to intracellular [Ca²⁺] cytosolic fluxes or "signals" by activating numerous target enzymes. Interestingly, plants possess a Ca²⁺ and calmodulin (CaM)-dependent NADK that has been implicated in stress response and pathogen defence. As the chloroplast and peroxisome are considered to be impermeable to pyridine nucleotides produced in the cytosol, NADP(H) de novo synthesis by NAD(H)K isoforms is likely important for the supply of NADP. Unlike other eukaryotic mitochondria, plant mitochondria possess a NAD(P)⁺-transporter that allows passive diffusion of NAD(P)⁺ between the cytosol and mitochondrial matrix which would appear to negate the requirement for a NADP-synthesizing enzyme that is present in, for example, yeast mitochondria. We recently described the cloning and biochemical characterization of two novel NADKs and an NADHK from *Arabidopsis*. Determining the subcellular localizations of these NAD(H)Ks is key to elucidating their in vivo roles. We have sought to determine the subcellular distribution of each NAD(H)K isoform by a combination of transient expression of NAD(H)K-GFP fusion proteins in vivo and immunological analysis of subcellular fractions. Preliminary findings and possible roles of NAD(H)Ks in higher plants are presented.

Poster 25

Biophysical and biochemical characterization of pathogen-induced calmodulin-like proteins in Arabidopsis and tomato

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Calcium is a key second messenger in multicellular organisms where it regulates a diverse array of cellular processes in response to external stimuli. During calcium signaling, complex fluctuations in intracellular calcium levels are thought to encode specific information about a perceived stimulus. Decoding these signatures involves calcium-binding proteins (calcium sensors) of which calmodulin (CaM) is the most extensively studied. CaM is a noncatalytic protein that regulates the activity of more than fifty different targets such as kinases, channels, transcription factors, and metabolic proteins. In plants, calcium and CaM play critical roles in cellular responses to environmental stresses such as drought, soil salinity, and pathogenic assault. In contrast to other eukaryotes, plants possess a unique, extended family (~50 in Arabidopsis) of CaM-like proteins (CMLs) in addition to the evolutionarily conserved CaM. We recently demonstrated that two closely related CMLs in Arabidopsis, CML42 and CML43, and their putative ortholog in tomato, Apr143, are important in the plant immune response to microbial pathogens. Our current work focuses on elucidating the cellular roles of these proteins through biophysical characterization, tertiary structure determination, and identification of their downstream targets. Analysis of overall secondary structure was conducted on recombinant CML42 and CML43 in the presence or absence Ca^{2+} using circular dichroism spectroscopy. Two-dimensional NMR spectroscopy was used to probe ^{15}N -labeled CML42 and CML43 structural changes during Ca^{2+} titration. Preliminary results indicate that, like CaM, significant conformational changes accompany high affinity Ca^{2+} binding in these CMLs, perhaps to accommodate target interaction. Target identification studies are in progress and a putative target of CML42 has been isolated by yeast two-hybrid screening but remains to be corroborated by a second method. Determining the roles of these calcium sensors through structural and functional analyses will aid in providing a fuller understanding of calcium signaling and stress physiology in plants.

Poster 26

Legume Production in Central Asia: Effects of Deficit and Alternate Furrow Irrigation on Common Bean (*Phaseolus vulgaris*) and Green Gram (*Vigna radiata*)

Maryse Bourgault, Heidi Webber, Chandra A. Madramootoo, Mikhail Horst, Galina Stulina, Donald L. Smith



Global water scarcity is a growing problem, exacerbated by population growth and climate change. The agriculture sector uses more water than any other. In dryland areas, such as Central Asia, there is little crop production without irrigation, and much of the crop production is cotton. There is a need for better water use and food self sufficiency in the area. The objective of the reported work was to evaluate physiologic and agronomic variables of legumes grown under a range of irrigation regimes, to determine best practices for production in the Aral Sea basin (Uzbekistan).

The experiment was organized following a split-plot completely randomized block design four blocks and with irrigation scheduling as main treatments. The treatments consisted of factorial combinations of three factors: depletion levels (levels to which we would let the soil dry - recommended schedule, intermediate stress level, and severe stress level, based on FAO recommendations), crop (common bean - *Phaseolus vulgaris* and green gram - *Vigna radiata*), and irrigation strategy (every and alternate furrow).

Phenological characteristics were monitored every week. Destructive sampling was performed at four development stages to assess biomass dry weight, leaf area, root depth, number and dry weight of nodules. Stomatal conductance and leaf water potential were recorded before and after each irrigation. At harvest, the number of seeds per pod, 100-seed weight and yield were determined. Amount of nitrogen supplied by nitrogen fixation will be determined from the nitrogen content of biomass samples of soybean and non-fixing reference crops. Oil and protein content of seeds were also determined. Both stomatal function and crop development indicate that water applications can be reduced below recommended levels, particularly with the correct choice of crop. Application of these technologies in the Canadian context will also be discussed.

Poster 27

A novel bacteriocin, Thuricin 17, produced by PGPR strain *Bacillus thuringiensis* NEB17: isolation and classification.

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A novel bacteriocin, thuricin 17, has been isolated and characterized. It is produced by the plant growth promoting rhizobacterium (PGPR), *Bacillus thuringiensis* NEB17. Previous studies (unpublished data) have shown that this compound effects nodulation on soybean, providing the initial motivation for its characterization. All bacterial compounds were analyzed and purified via preparatory and analytical HPLC. The molecular weight estimate of thuricin 17 was determined via SDS PAGE and confirmed via MALDI-QTOF, to be 3.1kDa. This bacteriocin maintained its biological activity up to 100 °C for 15 min and within the pH range, 1.50-9.25. The biological activity of thuricin 17 disappeared when treated with proteinase K and protease, but not with α -amylase. Characteristic of all bacteriocins, this peptide inhibited the growth of other *bacillus* members including: 6/19 *B. thuringiensis* strains, 4/4 *B. cereus* strains, 2/2 *B. megatarium* strains and 2/3 *B. licheniformis* strains. It also inhibited the growth of additional strains, including, *Brevibacillus brevis* ATCC 8246, *Geobacillus stearothermophilus* 10, as well as *Escherichia coli* MM294 (pBS42). We conclude that this is the first bacteriocin reported from a *Bacillus thuringiensis* strain known to be a PGPR.

Poster 28

When a luminal protein interferes with stromal side affairs: *Synechocystis* PCC 6803 mutants lacking PsbU

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The luminal side structural protein psbU present in cyanobacteria and red algae PSII complexes enhances the structural stability of PSII and has been implicated in the regulation of S-state transitions of the oxygen evolution complex. The present study was undertaken to investigate the alterations in energy transfer in the *psbU*- mutant. We have used a combination of fluorescence spectroscopy, pulse amplitude modulation and picosecond time-resolved fluorescence spectroscopy to characterize energy transfer in the *Synechocystis* PCC 6803 *psbU*- cells. The absolute 77K fluorescence spectra with 590nm excitation shows a marked increase in the 680nm peak in the *psbU*- mutant as compared to the wild-type, while with 435nm excitation the *psbU*- mutant and wild-type absolute 77K fluorescence spectra are not significantly different. Gaussian deconvolution of the absolute 77K fluorescence spectra with 590nm excitation reveals that the APC terminal emitter/PSII component at 680nm increases out of proportion with the PSII component at 690nm in the *psbU*- mutant cells. In addition, the absolute value of F_0 is greater in the *psbU*- mutant and this increase is a good deal less pronounced when using a 450nm measuring light as opposed to a 665nm measuring light. These results indicate that the phycobilisome/PSII energy coupling is less efficient in the *psbU*- mutant. Picosecond time-resolved fluorescence spectroscopy was undertaken to corroborate the idea of decreased PSII/phycobilisome energy coupling in the *psbU*- mutant and has yielded slower decay kinetics for the *psbU*- mutant cells as compared to the wild-type cells at F_0 using both a 407nm and 650nm laser. The global lifetime analyses of the fluorescence decay data suggests that PSII possesses altered decay kinetics in the *psbU*- mutant. Therefore, the existing data indicates that the *psbU*- mutant has both a relatively decoupled phycobilisome/PSII connection and a PSII complex with altered excitation transfer and/or primary photochemistry.

Poster 29

Identification and characterization of O-glucosyltransferases from *Vitis* sp. Involved in anthocyanin biosynthesis

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The terminal steps of anthocyanin biosynthesis involve different hydroxylation reactions that may be substituted with sugars, phenolics and/or methyl groups. Since each of these substitutions modify the color and/or stability of anthocyanins, they are important for breeding and biotechnological production of novel flower colors in horticultural crop species. In grapes the amount of anthocyanin pigments and the particular pigmentation that they contribute to red wine are very important for its visual attractiveness and antioxidant properties. As part of our program to identify and functionally characterize new genes in anthocyanin biosynthesis in grape, the UDP-glucose: anthocyanin 5-O glucosyltransferase (5gt) responsible for producing the dark blue 5-O-glucosylated anthocyanins found in many North American grape and some hybrid wine grape species has been studied. Molecular cloning of the 5gt from *Vitis labrusca* yielded a putative 448 amino acid protein exhibiting 30-50% identity to previously characterized 5gts from several other horticultural species. Developmental studies indicate that this gene is expressed in grape peels shortly before veraison (8 weeks after flowering) and it increases to a maximum at 16 weeks just before the grapes are harvested. The properties of this gene in relation to the biochemical production and accumulation of 5-O-glucosylated anthocyanins will be described.

Poster 30

Enhancement of Soybean Photosynthesis by Treatment with a Compound Produced by a *Bacillus thuringiensis* strain

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