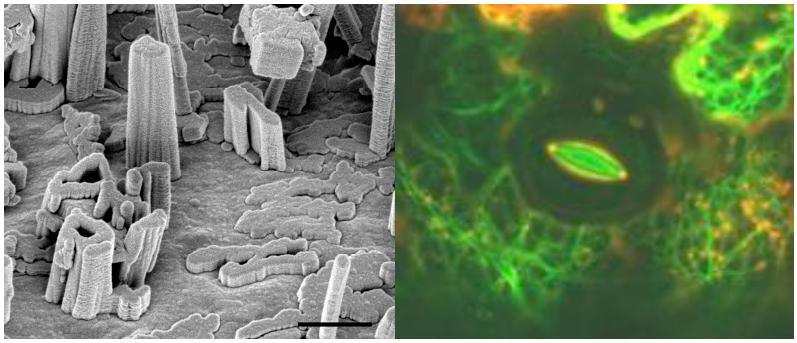

Western Regional Meeting of the Canadian Society of Plant Physiologists

University of Saskatchewan

December 9-10, 2004

Canadian Society of Plant Physiologists
Société Canadienne de Physiologie Végétale

GenomePrairie

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The Canadian Society of Plant Physiologists
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Schedule of Events

Thursday, December 9th, 2004

17:30 – 20:00 Registration / Mixer, Geology Building Atrium

Friday, December 10th, 2004

8:00 – 8:30 Registration / Coffee, Geology Building Atrium
8:30 – 8:45 Opening Remarks
8:45 – 9:30 Plenary Talk

Life inside a plant cell: dynamics of the early secretory pathway
Dr. Federica Brandizzi

9:30 – 10:30 Oral Presentations
10:30 – 10:45 Coffee Break
10:45 – 12:00 Oral Presentations

12:00 – 1:00 Lunch

1:00 – 1:45 Plenary Talk

Building a cell wall: new models for wax and monolignol export
Dr. Lacey Samuels

1:45 – 2:45 Oral Presentations
2:45 – 3:00 Coffee
3:00 – 4:00 Oral Presentations
4:30 – 6:00 Poster Session/ Reception
6:00 – 8:00 Dinner, Marquis Hall

Program

All Talks will be held in room 106 Biology. Coffee breaks, lunch and the poster session are in the 1st floor Atrium of the adjacent Geology building.

Thursday, December 9

Registration and Mixer, Atrium, Geology Building
5:30 - 8:00 pm

Friday, December 10

Registration/coffee
8:00 – 8:30 am

Welcoming Remarks
8:30 – 8:45 am

Session 1 – Chair: Troy Harkness

8:45 – 9:30	***Plenary Talk**: Life inside a plant cell: dynamics of the early secretory pathway* Federica Brandizzi, University of Saskatchewan
9:30 – 9:45	*Cytokinin-induced changes in CLAVATA1 expression are associated with clavata1-like phenotypes in Arabidopsis* Donna Lindsay, University of Saskatchewan
9:45 – 10:00	*The Role of APC10 in Plant Development* Troy Harkness, University of Saskatchewan
10:00 – 10:15	*Protein sorting to the peroxisome: a role for mRNA-cytoskeleton interactions?* Douglas Muench, University of Calgary
10:30 – 10:45	Coffee break

Session II – Chair: Steve Robinson

10:45 – 11:00	*Wfca genes from bread wheat resemble FCA, a prominent gene in the autonomous pathway of Arabidopsis thaliana.* Travis Hoffman, University of Saskatchewan
11:00 – 11:15	*The four members of the ribosomal protein S15A gene family are differentially transcribed in Arabidopsis thaliana.* J.L. Hulm, University of Saskatchewan
11:15 – 11:30	*Activation Tagged Arabidopsis Lines with Enhanced Freezing Tolerance in the Absence of Cold Acclimation* Steve Robinson, University of Saskatchewan
11:30 – 11:45	*Cold Acclimation of the Arabidopsis Immutans Mutant* Gordon Gray, University of Saskatchewan
12:00 – 1:00	Lunch

Session III – Chair: Soheil Mahmoud

1:00 – 1:45	***Plenary Talk**: Building a cell wall: new models for wax and monolignol export* Lacey Samuels, University of British Columbia
1:45 – 2:00	*Improving the Yield and Quality of Peppermint Essential Oil by Metabolic Engineering.* Soheil Mahmoud, Okanagan University College, Kelowna, BC
2:00 – 2:15	*Measuring non-photosynthetic carbon metabolic fluxes in Chlamydomonas reinhardtii* Hank Bestman, The King's University College, Edmonton, AB
2:15 – 2:30	*Photoinhibitory Responses in Wheat (Triticum aestivum L.) and Barley (Hordeum vulgare L.)* Gordon Gray, University of Saskatchewan
2:45 – 3:00	Coffee break

Session IV – Chair: Ken Wilson

3:00 - 3:15	*Nitric Oxide Metabolism In Barley Seedlings* A.U. Igamberdiev, University of Manitoba

3:15 – 3:30	*Nitric oxide emission from aerated and anoxic tobacco roots – origin and physiological significance.* Maria Stoimenova, University of Manitoba
3:30 – 3:45	*Uptake and partitioning of cadmium in durum wheat during grain filling*. Neil Harris, University of Alberta
3:45 – 4:00	*Determining seed water relations using an aquaporin inhibitor $HgCl_2$ and NMR spectroscopy in winterfat (Krascheninnikovia lanata (Pursh) A.D.J. Meeuse & Smit).* Ruojing Wang, University of Saskatchewan
4:00 – 6:00	Poster session/reception
6:00	Dinner, Marquis Hall

Abstracts – oral presentations

Life inside a plant cell: dynamics of the early secretory pathway

BRANDIZZI, F.
Department of Biology, University of Saskatchewan, Saskatoon, Canada

Our goal is to understand how secretion occurs in plant cells. Cells built and package secretory material in a series of compartments, collectively named the secretory pathway, comparable to an assembly line in a factory. The endoplasmic reticulum (ER) and Golgi apparatus are the first organelles of this assembly line; the mechanisms that control protein transport between them are poorly characterised in plants.
By using live cell imaging techniques, we have explored the dynamics of protein trafficking between the ER and Golgi, and their spatial and functional relationship with the cytoskeleton. It appears that plant cells contain multiple mobile Golgi stacks distributed over the entire cytoplasm. These stacks move over the ER by means of actin-myosin motors. The domains of the ER dedicated to the export of proteins, the ER export sites (ERES) form secretory units that move along the surface of the ER together with the Golgi. We also found that the integrity of Golgi and ERES is regulated by the activity of specific GTPases, such as Sar1p and Arf1p. Our results indicate that in plant cells the ER and Golgi form a dynamic membrane system whose components continuously cycle through the ER via a regulated membrane trafficking pathway.

Cytokinin-induced changes in *CLAVATA1* expression are associated with *clavata1*-like phenotypes in *Arabidopsis*

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Exogenous cytokinin 6-benzylaminopurine (BAP) induced aberrant flower development in *Arabidopsis thaliana*, including an increase in floral organ number, floral buds in the axils of sepals and intermediate floral organs resembling *clavata1* (*clv1*), *apetala1* (*ap1*), *ap2* and *ap3* mutant phenotypes. The BAP-altered phenotypes were most numerous in the first five flower positions of the raceme. Analysis of genetic responses using *Arabidopsis* 22k and 8k Affymetrix GeneChips® indicated that BAP-treatment significantly altered transcript levels of several genes associated with flowering processes and meristem function. In particular, there was a decrease in the transcript levels of *CLV1*, a gene encoding a receptor-like kinase involved in organ differentiation in shoot apical meristems. Subsequent time course analyses of *CLV1* transcript levels by RT-PCR showed an initial decline, and subsequent recovery, while the transcript levels of *WUSCHEL*, a gene encoding a homeodomain transcription factor required for shoot meristem proliferation and normally suppressed by CLV, showed an inverse pattern to *CLV1*. This temporal pattern of gene expression coincides with the changes in floral phenotype.

The Role of APC10 in Plant Development

HARKNESS, T. (1), YAWORSKY, L. (2) AND GRAY, G.R. (2)
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The purpose of this study was to test whether a molecular mechanism involved in promoting longevity in yeast was conserved in *Arabidopsis*. In yeast, we showed that the Anaphase Promoting Complex (APC) is required for prolonged longevity. The APC is an evolutionarily conserved ubiquitin protein ligase (E3) required for transit through, and exit from, mitosis. Disruption of a single gene encoding a non-essential APC subunit, *APC10*, resulted in rapid acceleration of aging. Overexpression of *APC10*, alternatively, extended yeast lifespan by approximately 50%. *APC10* is conserved from yeast to humans, as is the function of the APC. Thus, the APC could serve as a viable target for influencing lifespan. To test this hypothesis, we asked whether altering the expression of *Arabidopsis APC10* influenced lifespan of the plant. We obtained a knockout mutant of *APC10* which harboured a T-DNA insert in the promoter region. A growth analysis was performed and characterization of *APC10* transcripts in these plants is presently underway. We hope that this work will provide evidence that the molecular mechanisms discovered in yeast are indeed evolutionarily conserved.

Protein sorting to the peroxisome: a role for mRNA-cytoskeleton interactions?

CHUONG, S.D.X. (1), PARK, N-I (1), FREEMAN, M.C. (1), MULLEN, R.T. (2) AND MUENCH, D.G. (1)
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The subcellular localization of mRNA is important in facilitating the targeting of proteins to distinct regions of the cytoplasm and to the surface of some organelles. Using a biochemical screen aimed at identifying rice proteins that may function in mRNA/cytoskeleton interactions and possibly mRNA localization, we identified a protein that possessed both microtubule (MT) and RNA binding activity. This protein was purified to homogeneity and was identified as the multifunctional protein (MFP), a peroxisomal enzyme that possesses up to four enzymatic activities involved in the b-oxidation of fatty acids. The recombinant MFP possessed MT- and RNA-binding activities, in addition to its b-oxidation enzymatic activities. Expression of an MFP-GFP fusion in onion epidermal cells indicated that MFP is localized to both peroxisomes and MTs. Based on these findings, as well as recent reports demonstrating that plant peroxisomes move actively throughout the cell along actin filaments, we propose a working model that links mRNA/MT interactions to translation and peroxisomal protein import. Specifically, nascent, cytosolic MFP anchors to MTs the mRNAs that encode peroxisomal-destined proteins, and this MFP/mRNA/MT interaction functions to regulate translation of the associated mRNAs in response to the rate of protein import.

Wfca genes from bread wheat resemble _FCA_, a prominent gene in the autonomous pathway of _Arabidopsis thaliana_.

GAO, M. (1), HOFFMAN, T.L. (1), LIMIN, A.E. (2), FOWLER, D.B. (2), AND SELVARAJ, G. (1)
(1) Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, SK, Canada, S7N 0W9 (2) Crop Development Centre, University of Saskatchewan, Saskatoon, SK, Canada, S7N 5A8

Although hexaploid wheat (*Triticum aestivum*) was among the first plants studied for flowering physiology and genetics, we still have a limited understanding of the molecular details of its flowering transition. Vernalization-promoted flowering is agronomically important and attributed to recessive alleles at the orthologous *Vrn-A1*, *B1*, and *D1* loci on the group-5 chromosomes. We found expressed sequences potentially indicating numerous copies of *Wfca* (Wheat *FCA*), some acting as expressed pseudogenes. WFCA resembles FCA, a single-copy autoregulatory RNA-binding protein in Arabidopsis. Multiple *Wfca* copies represent a large arsenal of potentially functional and non-coding members. The latter gene products show various nucleotide substitutions, indels, and splice variants, including shorter transcripts (termed -forms, as opposed to potentially functional -forms). -forms were found in wheat lines of winter and spring habit, and in reciprocal near-isogenic lines (NILs) differing for vernalization requirement. Vernalization resulted in enhanced accumulation of only the -forms only in the winter habit NIL. We will discuss the multiple levels of *Wfca* regulation and their contribution to the phenology and adaptation of this important crop.

The four members of the ribosomal protein _S15A_ gene family are differentially transcribed in _Arabidopsis thaliana_.

HULM, J.L. and BONHAM-SMITH, P.C.
Department of Biology, University of Saskatchewan, Saskatoon, SK S7N 5E2

As a ribonucleoprotein complex, the plant ribosome consists of four ribosomal RNAs (rRNAs) and 75-92 ribosomal proteins (r-proteins), depending on the species. *Arabidopsis thaliana* r-protein genes exist in multi-gene families ranging in size from two to seven members. Unlike those in mammals more than one member of a plant r-protein gene family is usually transcriptionally active. The cytosolic *RPS15A* gene family consists of four members that, at the amino acid level, share 87-100% identity. Comparison among the 5´ regulatory regions shows that although the four genes contain many of the same putative regulatory elements they share only 46-49% nucleotide sequence identity. Semi-quantitative RT-PCR was used to determine *RPS15A* gene expression patterns and regulatory differences among genes. *RPS15A3* expression was not detected in any tissue while transcript abundance for *RPS15A1*, *-2* and *-4* was highest in mitotically active tissues including bud and flower. Hormone treated seedlings showed an increase in transcript abundance following treatment with BAP; ABA treatment induced a decrease; GA_3 had little effect; and IAA induced an increase in *RPS15A2* expression alone. While similar trends of expression have been established for *RPS15A1*, *-2* and *–4*, transcript abundance varies in temperature, mechanical and heavy metal stressed seedlings.

Activation Tagged *Arabidopsis* Lines with Enhanced Freezing Tolerance in the Absence of Cold Acclimation

ROBINSON, S., BEKKAOUI, D., LAURIE, J. AND PARKIN, I.
Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, S7N 0X2.

Fifty thousand *Arabidopsis* activation tagged lines were screened to identify those lines that, in the absence of cold acclimation, expressed an enhanced ability to withstand freezing temperatures compared to wild type. The mutant lines, named *Frigus* lines, were selected over four successive generations by assessing whole plant survival of two week old *Arabidopsis* lines after exposure to -5ºC. This has resulted in the identification of 17 lines that in comparison to wild type displayed a constitutively freezing tolerant phenotype. These lines are presently being analysed, and the sequence flanking the majority of the T-DNA insertion sites has been resolved. Homozygous insertion lines are being generated for each of the 17 lines to determine the precise level of freezing tolerance using electrolyte leakage and re-growth tests. Here we present the characterization of the *Frigu*s lines to date, including the response of the lines to freezing temperatures when grown under non-acclimating and acclimating conditions, the genomic location of the insertion sites and the identity of the associated genes.

Cold Acclimation of the *Arabidopsis Immutans* Mutant

BAERR, J.N. (1), MCINTOSH, K.B. (2), BONHAM-SMITH, P.C. (2), RODERMEL, S.R. (3) AND GRAY, G.R. (1)
(1) Department of Plant Sciences, University of Saskatchewan, Saskatchewan, SK, S7N 5A8; (2) Department of Biology, University of Saskatchewan, Saskatoon SK S7N 5E2; (3) Department of Botany and Interdepartmental Genetics Program, Iowa State University, Ames, IA 50011, USA.

The *immutans* mutant of *Arabidopsis* is characterized by variegated leaves containing both green and white sectors which lack the IMMUTANS (IM) protein. It has been suggested that IM may play an important role in carotenogenesis, functioning as a redox component of the phytoene desaturation pathway. Xanthophyll carotenoids play an important role in mediating non-photochemical quenching (NPQ) processes which are a major photoprotective mechanism. The exposure of plants to a combination of low temperature and high, or even moderate-to-low irradiance, predisposes them to photoinhibition of photosynthesis which has been defined as a light-dependent reduction in photosynthetic efficiency and/or capacity. However, the process of cold acclimation (the growth and development at low, non-freezing temperatures) is well documented to result in an increased tolerance to photoinhibition at low temperature. Cold acclimation also results in the expression of a regulon of cold-regulated (*COR*) genes that are thought to contribute to freezing tolerance. Our previous studies have characterized the photosynthetic and photoinhibitory responses of *immutans*. The present study indicates differential responses to photoinhibition based on either growth irradiance and/or temperature and question the role of *COR15a*, a gene thought to be responsible for maintaining chloroplast integrity at low temperatures.

Building a cell wall: new models for wax and monolignol export

LACEY SAMUELS, JAMIE PIGHIN, KIM RENSING
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The long term goal of my research is to understand how plant cells secrete their cell wall: both polysaccharides, such as pectins, as well as special components such as waxes and lignin. Current research focuses on two secretory products that encrust the cell wall: waxes that make up the plant cuticle and lignin in the wood of *Pinus contorta*. The hydrophobic wax precursors and phenylpropanoid lignin precursors are very different in chemical composition from "conventional" secretory products that are exported by Golgi-mediated exocytosis. The mechanisms for export of wax and lignin precursors are not well understood. In Arabidopsis, we have recently identified a plasma membrane protein, which is an ATP binding cassette (ABC) transporter, required for export of waxes to the stem cuticle. In developing wood, models of monolignol export are being tested with autoradiography paired with ultra-rapid freezing techniques. 3H-phenylalanine incorporation patterns into developing xylem do not provide evidence for Golgi mediated secretion of monolignols. However, treatment with inhibitors of ABC transporters led to decreased lignification.

Improving the Yield and Quality of Peppermint Essential Oil by Metabolic Engineering

MAHMOUD, S.S. (1) AND CROTEAU, R.B. (2)

(1) Okanagan University College, Kelowna, BC, Canada, V1V1V7; (2) Institute of Biological Chemistry, Washington State University, Pullman, WA, USA, 99164.

The essential oil of peppermint (*Mentha X piperita L*) is mainly composed of *p*-menthane type monoterpenes including (-)-menthol the most significant constituent of the oil. In optimal growth conditions, peppermint produces mostly menthol. However, poor environmental factors (e.g., low light intensity) can promote the accumulation of undesirable monoterpenes menthofuran and pulegone (which contribute off odors and are toxic to humans) in the oil, causing significant losses to farmers. Recently, a functional genomics approach facilitated the cloning of most of the monoterpene biosynthetic genes from oil glands of peppermint. We have altered the expression of a number of these genes in transgenic peppermint plants to elucidate regulation, and to improve the yield and composition of the essential oil in this agronomically important species. Some genes (e.g., pulegone reductase) are posttranscriptionally regulated, and their overexpression does not influence phenotype. However, the expression of other genes (e.g., menthofuran synthase) is primarily regulated at the level of transcription. In these cases, overexpression improves flux through the corresponding pathway steps. Metabolic engineering improved essential oil yield, increased production of menthol, and drastically reduced the accumulation of menthofuran and pulegone in peppermint plants.

Measuring non-photosynthetic carbon metabolic fluxes in *Chlamydomonas reinhardtii*

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The objective of our research program is to develop flux maps of the central carbon metabolic network in *Chlamydomonas reinhardtii* under varying light regimes and nutritional conditions. The initial focus is on the transition from using CO_2 as the primary carbon source to using acetate via the glyoxylate pathway. Using ^{13}C-labeling experiments we have shown that when dark-adapted algae use acetate as the carbon source, the carbon flux through the glyoxylate pathway is 5 times greater compared to algae grown in the light. Exposure of *Chlamydomonas* to acetate increases the activity of the enzyme isocitrate lyase (EC 4.1.3.1) 6- or 20-fold, in the dark or light, respectively. We have begun to generate carbon metabolic flux maps on the basis of the isotope distribution ratios of the proteinogenic amino acids (as determined by GC-MS) following exposure of *Chlamydomonas* to ^{13}C-acetate. Initial results suggest a significant effect on the rate of ^{13}C-label incorporation into several amino acids. We have developed MatLab® based software to extract the isotopomer distribution ratios from the GC-MS and NMR output, and convert it to a form readable by the ^{13}C-Flux software (W. Wiechert *et al.*, 2001, Metabolic Engineering 3:265-283) used to generate the metabolic flux maps.

Photoinhibitory Responses in Wheat (*Triticum aestivum* L.) and Barley (*Hordeum vulgare* L.)

SHABAGA, C. (1), PROPP, M.K. (1), BAERR, J.N. (1), FOWLER, D.B. (2), LIMIN, A.E. (2) AND GRAY, G.R. (1)
(1) Department of Plant Sciences, University of Saskatchewan, Saskatchewan, SK, S7N 5A8; (2) Crop Development Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5A8.

The exposure of plants to a combination of low temperature and high, or even moderate-to-low irradiance, predisposes them to photoinhibition of photosynthesis which has been defined as a light-dependent reduction in photosynthetic efficiency and/or capacity. The process of cold acclimation (the growth and development at low, non-freezing temperatures) is well documented to result in an increased tolerance to photoinhibition at low temperature. In wheat (*Triticum aestivum* L.), this is attributable to a cultivar-dependent increase in carbon dioxide assimilation rates as a result of a selective stimulation of the sucrose biosynthetic pathway. A distinguishing factor between winter and spring wheat is that winter cultivars possess a vernalization requirement (exposure to low temperature) in order to move from the vegetative to the reproductive phase whereas spring cultivars do not. To further understand the role of development in the photosynthetic acclimation process we have examined photoinhibitory responses in winter and spring cultivars of wheat and corresponding near-isogenic lines for the vernalization locus *Vrn-A1*. We have also initiated studies in barely (*Hordeum vulgare* L. cv Gateway) and its virescens mutant. This mutant is characterized by a non-lethal developmental mutation which exhibits a lag in chlorophyll biosynthesis that is affected by light and temperature.

Nitric Oxide Metabolism In Barley Seedlings

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Germinating barley seedlings exhibit high nitric oxide (NO) scavenging activity in all tested organs (root, shoot, aleurone, scutellum) with NADH as a cofactor. The highest specific activity was observed in roots (~0.4 μmol min^{-1} mg^{-1} protein), while in other organs it was 2-3 times lower. The activity decreased after the third day of germination. Putative proteins responsible for this activity were fractionated by ammonium sulfate and subjected to further separation on Phenyl Superose column. In eluted fractions the activity disappeared and could be recovered by adding purified barley hemoglobin to certain fractions. These fractions contained the protein of ~43 kDa measured both by size exclusion chromatography and by SDS-PAGE. The protein was further purified and sequenced showing a high similarity to the rice cytosolic monodehydroascorbate reductase. The protein uses NADH as a cofactor (NADPH with ~40% efficiency) and transfers electrons to metHb, cytochrome c and monodehydroascorbate. Together with Hb, it effectively catalyses NAD(P)H dependent NO scavenging. We conclude that the NO scavenging activity in barley is attributed to the operation of two protein components, one being Hb and the other being a flavoprotein such as a cytosolic monodehydroascorbate reductase utilizing NAD(P)H as a reducing agent.

Nitric oxide emission from aerated and anoxic tobacco roots – origin and physiological significance

STOIMENOVA M.[1], JAGADISH GUPTA K.[2] AND KAISER W.M.[2*]
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Under anoxia, nitrate-grown whole tobacco root systems or root segments in buffered solutions emitted nitric oxide (NO) into NO-free air at rates of 5 to 20 nmol g^{-1} FW h^{-1}. In air, NO emission was ≤ 0.1 nmol g^{-1} FW h^{-1}. Roots of nitrate reductase (NR)-free plants did not emit NO even under anoxia; however, when supplied with nitrite (0.5 mM), NR-free roots emitted NO at rates similar or exceeding those mentioned above.
Anoxic NO emission from both, NR-containing and NR-free roots was sensitive to mitochondrial electron transport inhibitors, suggesting that a large part of NO was produced by mitochondria. Additionally purified root mitochondria, produced NO from nitrite and NADH under anoxia, but not in air. The apparent Km nitrite (300 μM) of mitochondria was within the range of Km's reported for nitrite-to-NO reduction by NR. A comparison of NO scavenging by roots and by mitochondrial preparations in air or nitrogen indicated that the very low NO emission of both systems in air was only partly due to oxidative NO scavenging. Rates of 'scavenging corrected' mitochondrial NO emission would have been 2 % of the respiratory electron transport. A role for NO production in the anoxic metabolism of roots is discussed.

Uptake and partitioning of cadmium in durum wheat during grain filling.

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Cadmium (Cd) concentrations in durum wheat grain grown in prairie soils often exceed proposed international trade standards. To understand the physiological processes responsible for elevated Cd accumulation in grain, Cd uptake and partitioning were compared in a pair of near-isogenic durum wheat lines that differ three-fold in grain Cd accumulation. The Cd distribution among plant organs was determined during grain filling in plants grown under chelator-buffered hydroponic culture. Grain Cd concentration of the low-Cd isoline remained constant during grain filling, whereas it increased 2.5-fold in the high-Cd isoline. Cadmium accumulation in the grain was 2 to 5-fold higher in the high-Cd isoline. Similarly, Cd accumulation in the shoot tissues was at least 2-fold higher in the high-Cd isoline at all harvests, but the pattern of Cd accumulation was similar in both isolines. Cadmium accumulation per plant increased 4-fold during grain filling, but there was no difference between the isolines in whole-plant Cd accumulation. The low-Cd isoline retained more Cd in the roots than the high-Cd isoline. Labelling the roots with an enriched Cd isotope (^{111}Cd) during the period of peak Cd influx into grain confirmed differential root-to-shoot Cd translocation as the main factor regulating Cd accumulation in shoots and grain.

Determining seed water relations using an aquaporin inhibitor $HgCl_2$ and NMR spectroscopy in winterfat (*Krascheninnikovia lanata* (Pursh) A.D.J. Meeuse & Smit)

WANG, R. (1), BAI, Y. (1), KENDALL, E.J. (2) AND TANINO, K. (1)
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(2) Department of Medical Imaging, University of Saskatchewan, Saskatoon, SK, S7N 0W8.

Water relations and the function of aquaporins were investigated using $HgCl_2$ inhibition during germination and seedling growth in winterfat. A series of concentrations of $HgCl_2$, 0, 30, 35, 40, 50, 100 mM, was used to test the inhibition effects. Water uptake, water distribution among seed tissues, and water mobility were studied using NMR spectroscopy. Water first entered the embryo then expanded to the perisperm. $HgCl_2$ significantly restricted water flux to the perisperm. The embryo had higher seed water content, but lower proton density with inhibition by $HgCl_2$. The $HgCl_2$ reduced germination rate and final germination percentage. The LD_{50} of $HgCl_2$ for seed viability after 7 days of imbibition ranged from 30 to 60 mM. The $HgCl_2$ reduced seedling water content of both root and cotyledons. The reduction, however, was reversible at the lower concentrations of $HgCl_2$ when seeds were transferred to distilled water. The root length was 2 to 4 times shorter with $HgCl_2$ inhibition. Dry matter was allocated more to cotyledons than to roots during seedling growth with inhibition, showing higher impact on root growth than on cotyledon growth. This study indicates aquaporins play a major role in water relations from seed germination to seedling elongation in winterfat.

Abstracts – poster presentations

***Agrobacterium* –mediated Transformation of Chickpea (*Cicer arietinum*); Gene Integration, Expression and Inheritance.**

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Chickpea (*Cicer arietinum*) is a legume rich in protein for both human and animal consumption. Typical of legumes, tissue culture and transformation has been difficult. This report describes a method of transformation, recovery of transgenic plants and testing of expression and inheritance of the introduced gene. Initial explants were longitudinal slices from embryonic axes of imbibed, mature seed. The plasmid contained a 35S35SAMV promoter and a bi-functional fusion gene, conferring both -glucuronidase (GUS) and neomycin phosphotransferase (NPTII) activities. The average success rate through co-cultivation, shoot-induction and rooting media was 1.4%. Addition of a shoot-elongation medium increased the tissue culture phase, from co-cultivation to soil, from 160 to 217 days. However, the frequency of recovery also increased to a mean of 3.1%. Southern hybridization analysis confirmed the incorporation of the inserted genes, indicated the copy number and confirmed the clonal nature of plants arising from a single explant. *Gus* gene expression, quantified by MUG assays, was variable but not reduced by the increased period of tissue culture. Recovered plants were fertile. Inheritance of the *gus* gene was followed beyond four generations. This *Agrobacterium* -mediated chickpea transformation method is reliable and reproducible with Kabuli and Desi chickpea cultivars.

One Carbon (C-1) Metabolism in Response to Biotic and Abiotic Stresses

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In plants, the generation and supply of methyl units is important in one-carbon (C-1) metabolism, which is essential to all organisms. We have identified a series of cDNA sequences encoding N^5, N^{10}-methylenetetrahydrofolate reductase (MTHFR), cobalamin-independent methionine synthase (Met Syn), *S*-adenosylmethionine synthetase (AdoMet Syn605 and AdoMet Syn2661), *S*-adenosylmethionine decarboxylase (SAMDC), serine hydroxymethyltransferase (SHMT) and N^5, N^{10}-methenyltetrahydrofolate cyclo- hydrolase / N^5, N^{10}-methylenetetrahydrofolate dehydrogenase (THFC/THFD) in the pathways of generation and supply of methyl units from a cDNA library constructed from mRNA of a susceptible wheat (*Triticum monococcum*) line 441 epidermis, 24 h after inoculation with powdery mildew fungus (*Blumeria graminis* f. sp. *tritici*). Phylogenetic tree cluster analysis and subcellular localization predicted by TargetP revealed that MTHFR, Met Syn, AdoMet Syn605, AdoMet Syn2661, SAMDC, and THFC/THFD may be localized in the cytosol; SHMT may be localized in mitochondria. Northern blot analysis indicated that *MTHFR, Met Syn, AdoMet Syn605, AdoMet Syn2661 and SAMDC* were up-regulated by powdery mildew infection, abiotic stresses and treatment with stress signal molecules; *SHMT and THFC/THFD* were either constitutively expressed or down-regulated. These results suggest a close metabolic link between stresses and the pathways of generation and supply of methyl units in this wheat variety.

The interaction between the Arabidopsis disease resistance protein NPR1 and the bZIP transcription factor TGA1 is regulated by redox

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The Arabidopsis NPR1 protein is essential for regulating salicylic acid-dependent gene expression during systemic acquired resistance. NPR1 differentially interacts with members of the TGA class of bZIP transcription factors and regulates their DNA binding activity. Although TGA1 does not interact with NPR1 in yeast two hybrid assays, treatment with salicylic acid induced the interaction between these proteins in Arabidopsis leaves. This phenomenon is correlated with a reduction of TGA1 Cys residues. Using chimeric genes between TGA2 (which interacts with NPR1 in yeast) and TGA1, a 30 amino acid region NPR1-interacting domain was identified. Site-directed mutagenesis of TGA1 cysteines within this domain enables the interaction with NPR1 in yeast and Arabidopsis. Together, these results indicate that TGA1 relies on the oxidation state of cysteine residues to mediate the interaction with NPR1. An intramolecular disulfide bridge in TGA1 precludes interaction with NPR1, and NPR1 can only stimulate the DNA binding activity of the reduced form of TGA1. Unlike its animal and yeast counterparts, the DNA binding activity of TGA1 is not redox regulated; however, this property is conferred by interaction with the NPR1 cofactor.

Microspore Embryogenesis in *Brassica napus*

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We are interested in the identification and isolation of the factors and genes necessary and sufficient to induce embryogenesis in microspores of *Brassica napus*. We have constructed both subtractive libraries and full-length cDNA libraries from microspores cultured for 72 hours, 5 days or 7 days under embryogenesis-inducing conditions. EST analyses of the 72 hour and 5 day cultures indicated that most sequences were related to pollen-specific genes; for example, a large number coded for pectinesterases, arabinogalactan-like proteins and polygalacturonases, and 20% were unknown proteins. Genes upregulated at 5 days included BURP domain-containing genes, a cytochrome P450, *B napus* seed-specific protein (Bn15D18B) and napin. The cDNA library made from 7 day microspore cultures contained embryo-specific genes such as ABI3, ATS1, LEC1, LEC2, FUSCA. However, semi-quantitative RT-PCR analyses revealed that the transcripts of these embryo-specific genes were detectable first at 48h to 72h of microspore culture. Our results suggest that pollen developmental pathways are operating simultaneously, and are predominant, within induced microspores that also expressed embryogenesis-related genes like BBM, LEC1 and LEC2. Subsequently, embryogenesis is established in some of these microspores by 7 days of culture.

Abscisic Acid Binding Site Mapping And Protein Interactions Of ABAP1

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The abscisic acid-binding protein ABAP1 is a 52 kDa protein cloned and purified from barley aleurones (Razem et al. 2004, J.Biol.Chem. 279: 9922). It optimally binds (+)-ABA at neutral pH with maximum specific binding of 0.8 mol ABA mol^{-1} protein and a K_d of 28 X 10^{-9} M. The 472-amino acid protein shares sequence homology and conserved domains at the 3' end of the FCA flowering time control proteins, particularly from wheat, rice and Arabidopsis. The highly conserved domains include a WW protein:protein interaction domain and hydrophobic regions flanked by highly hydrophilic platforms. By applying truncated versions and mutational analysis of ABAP1 and pull-down assays, we have preliminary data to suggest that ABA binding occurs in the hydrophobic domain and that ABAP1 interacts with an ABA-induced and plasma membrane-associated protein known as ABA45. Here we describe our progress to date on the mapping of ABA binding site and ABAP1 interactions through its WW domain.

Functional genomics of the phenylpropanoid pathway in *Arabidopsis*

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In crucifers, sinapoyl choline (sinapine) and sinapoyl malate are the main storage forms of phenolic compounds in seeds and leaves, respectively. In *Arabidopsis* seeds, sinapine accounts for more than 50% of total phenolics. To develop strategies to reduce levels of the anti-nutritional factor, sinapine, in *Brassica* oilseeds, *Arabidopsis* was used as a model system to identify rate limiting steps in the phenylpropanoid pathway leading to the biosynthesis of sinapine from phenylalanine. A total of 19 *Arabidopsis* mutant lines (Fig. 1) with knockouts in genes affecting metabolic steps in the phenylpropanoid pathway were identified either by searching public databases, such as TAIR (SALK lines) or by screening AAFC's knockout population. Chemical analysis of seed phenolic extracts from homozygous lines of 10 of these mutants revealed that knockouts in genes affecting later, rather than earlier, steps in the pathway caused significant alterations in the composition of phenylpropanoids and the level of sinapine. These results provide insight into critical steps in phenylpropanoid biosynthesis, and suggest strategies that could be used to modify the profiles of phenylpropanoids in related *Brassica* oilseeds.

Algal Iron Acquisition: Competition between a Cyanobacterium and Two Species of Eukaryotic Alga

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Photosynthetic organisms utilize two different and for the most part mutually exclusive iron acquisition mechanisms, termed Strategy I and Strategy II. Strategy I species exhibit elevated plasma membrane ferric chelate reductase activity under iron limitation, while Strategy II species release siderophores. Among algae, the cyanobacteria use Strategy II iron acquisition, while many eukaryotic algal species use Strategy I. Co-cultivation experiments using iron-limited algal cells were performed in order to examine whether the acquisition mechanism, and the nature of the iron source, will determine the outcome of interspecific competition. When Fe^{3+}-HEDTA (a relatively weak chelate) was provided as the iron source, growth of *Anabaena* sp. (Strategy II cyanobacterium) was suppressed by co-cultivation with *Chlorella kessleri* (Strategy I). C*hlamydomonas reinhardtii* growth was suppressed by co-cultivation with *Anabaena* or *Chlorella*. When Fe^{3+}-HBED (a very strong chelate) was provided as the iron source, *Anabaena* growth was stimulated by co-cultivation with eukaryotic algae. *Chlorella* and *Chlamydomonas* growth were suppressed by *Anabaena* co-cultivation. These results suggest that the cyanobacterium *Anabaena* is a strong competitor for aquatic iron compared with two species of eukaryotic algae, and that cyanobacterial iron acquisition from strong chelates may be facilitated by the presence of algae that use a reductive (Strategy I) iron acquisition.

Charaterization of plant Mago Nashi homolog and its possible role in subcellular mRNA localization

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mRNA localization is widely identified as an essential process and common way of targeting proteins to their site of function of many cell types. Localized mRNAs normally move in the form of large ribonucleoprotein (RNP) particles that may contain many RNAs and proteins. One protein that is a component of RNP complexes that exit from the nucleus is Mago Nashi (Mago). In *Drosophila*, Mago and the RNA binding protein Y14 interact with several other proteins to form a complex that assembles near exon-exon junction complex. This protein complex is required for *oskar* mRNA localization. Mago is a protein that is highly conserved across kingdoms, including plants. Using a Mago-GFP expression analysis, plant Mago protein is predominantly localized to the nucleus. We are currently characterizing the effect of over- and under-expression of Mago on plant development and identifying the proteins and mRNAs that are associated with the Mago complex in plants. Root hairs are a potentially good model for studying RNA localization in plants. Profilin, a low molecular weight actin binding protein, and *profilin* mRNA both accumulate within outgrowing buldges at locations where root hairs form. This region is assembled by F-actin meshworks. We are identifying zipcode sequences responsible for localization and proteins that profiling mRNA localization in maize root hairs.