16:00 **Registration and Poster Installation**

18:00 **Cocktail**

Session I Friday December 12

Moderator: Patrick Gulick

Symposium 1
19:00 – 19:30 **Genetic and Genomic Approaches to Vascular Tissue Patterning**
Enrico Scarpella,Wenzislava Ckurshumova, Naden Krogan, George Stamatiou, Ryan McKenzey, Rodger Beatson, CS Hardtke, Thomas Berleth.

Oral presentation 1
19:30 – 19:45 ***BREVIS RADIX (BRX)* gene, a major QTL for root growth in Arabidopsis, encodes a novel factor involved in root system morphology**
Céline F. Mouchel, Georgette C. Briggs and Christian S. Hardtke

Oral presentation 2
19:45 – 20:00 **Liquid-phase fluorescence *in situ* RT-PCR analysis for gene expression analysis in woody stems**
Madoka Gray-Mitsumune

Oral presentation 3
20:00 – 20:15 **Cellular architecture and cytomechanics in growing pollen tubes**
Anja Geitmann, Elodie Parre

Oral presentation 4
20:15 – 20:30 **The cuticle of soybean seeds controls their permeabilities to water**
Fengshan Ma, Ewa Cholewa, Tasneem Mohamed, Carol A. Peterson, Mark Gijzen

Session II Saturday December 13

Moderator: Luc Varin

Symposium 2
9:00 – 9:40 **Phosphite Blocks Phosphate Sensing in Plants and Yeast**
William C. Plaxton

Oral presentation 5
9:45 – 10:00 **Phosphate or phosphite addition promotes proteolytic turnover of intracellular and secreted phosphate-starvation inducible tomato purple acid phosphatase isozymes**
Gale G Bozzo, Vinay K Singh, and William C. Plaxton

Oral presentation 6
10:00 – 10:15 **Phosphate response of alfalfa overexpressing *Arabidopsis* phosphate-starvation responsive gene group 1**
M. F. Hétu, B. D. McKersie, L.J. Tremblay and D.D. Lefebvre

Oral presentation 7
10:15 - 10:30 **Low Ci induction of transcripts related to the CO_2-concentrating mechanism is not light dependent in the cyanobacterium *Synechococcus* PCC7942**
Patrick McGinn, Meaghan Jones and Douglas Campbell

10:30 – 11:00 **Coffee break**

Moderator: Bill Plaxton

Oral presentation 8
11:00 – 11:15 **The effect of PsbS on the redox characteristics of QA and QB in photosystem II reaction centres**
A. Balseris, M. Krol, L.V. Savitch, and N.P.A. Huner

Oral presentation 9
11:15 – 11:30 **Mechanically isolated *Asparagus sprengeri* cells perceive and respond to lipo-chitooligosachharides, a *Rhizobia*- secreted nodulation factors**
Ewa Cholewa and John McIver

Oral presentation 10
11:30 – 11:45 ***MUM4* encodes a putative pectin biosynthetic enzyme developmentally regulated by *AP2, TTG1* and *GL2* in the *Arabidopsis* seed coat**
Tamara L. Western, Diana S. Young, Gillian H. Dean, A. Lacey Samuels and George W. Haughn

Oral presentation 11
11:45 – 12:00 **Prokaryotic Orthologs of Plant Mitochondrial Alternative Oxidase and Plastid Terminal Oxidase**
A.E. McDonald, S. Amirsadeghi, and G.C. Vanlerberghe

Oral presentation 12
12:00 – 12:15 **Expression of non-symbiotic barley hemoglobin alters the phenotype of transgenic alfalfa**
K. Baron, C. Dordas and R.D. Hill

Oral presentation 13
12:15 – 12:30 **Altering hexokinase levels in transgenic potato (*Solanum tuberosum*) roots influences growth rate and branching**
E. Claeyssen, O. Wally, D. Matton and J. Rivoal

12 :30 – 14 :00 **Lunch and Posters**

Session III Saturday December 13

Moderator: Priti Krishna

Symposium III
14:00 – 14:40 **Genetics and Genomics of Cold Tolerance in Cereals**
Fathey Sarhan

Oral presentation 14
14:45 – 15:00 **Heat-activation of the MAP kinase, HAMK involves lipid signaling**
A. Mansour, S.S. Suri, A. Shawky and R.S. Dhindsa

Oral presentation 15
15:00 – 15:15 **Physiological and biochemical responses of *Thellungiella salsuginea* to osmotic stress**
David Guevara, Brian Golding, Brian McCarry, Paulo Nuin and Elizabeth Weretilnyk

Oral presentation 16
15:15 – 15:30 **Identification of plant hsp90 interactors by the yeast two-hybrid screen**
Z. Wang., Z. Zhang and P. Krishna

15:30 – 16:30 **Coffee break and Posters**

Moderator: Deep Saini

Oral presentation 17
16:30 – 16:45 **Regulatory Phosphorylation of PEP Carboxylase in Developing Castor Oil Seeds**
Karina E Tripodi and William C.Plaxton

Oral presentation 18
16:45 – 17:00 **Brassinosteroid-mediated stress tolerance**
S. Kagale and P. Krishna

Oral presentation 19
17:00 – 17:15 **Jasmonates induce nodulation *(nod)* genes of *Bradyrhizobium japonicum* in a strain specific manner**
Fazli Mabood, Wajahatullah. M. Khan, and Donald L. Smith

Oral presentation 20
17:15 – 17:30 **"Bioinformatics approaches: *In silico* discoveries in soybean expression data"**
Martina V. Stromvik, Anupama Q. Khanna, Lila O. Vodkin

Oral presentation 21
17:30 – 17:45 **Molecular Phylogeny of the tree genus Populus (Salicaceae)**
Mona Hamzeh and S. Dayanandan

Oral presentation 22
17:45 – 18:00 **Environmental Control of Exodermal and Endodermal Development in Maize**
Daryl E. Enstone and Carol A. Peterson

Oral presentation 23
18:00 – 18:15 **Effect of Hup Status on Rhizobacteria and Soil Fertility**
Cheryl Dean and Zhongmin Dong

Oral presentation 24
18:15 – 18:30 **Effects of exudates extracted from transformed-tomato-roots grown in bi-compartment Magenta boxes and colonized with or without *G. intraradices* on *Phytophthora parasitica* var. *nicotianae* zoospores.**
Laetitia Lioussanne, Mario Jolicoeur and Marc St-Arnaud

18:30 – 20:30 **Mixer + Posters**

Symposium papers

Symposium 1 Session 1

Genetic and Genomic Approaches to Vascular Tissue Patterning

Enrico Scarpella, Philip Francis and Thomas Berleth
University of Toronto, Department of Botany, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada

During leaf development, ground meristem cells along continuous lines undergo coordinated oriented cell divisions and differentiate to form procambial cells, the precursors of all vascular cells. The molecular genetic dissection of early procambial development suffers from the lack of easily identifiable markers, especially of cell states preceding procambium formation. We have identified and characterized reporter gene expression markers that reflect distinct preprocambial stages as well as one marker whose expression seems to be perfectly congruent with the appearance of procambial cells. All markers are invariably expressed in continuous domains connected to pre-existing vasculature and their expression profiles reveal a common spatial and temporal pattern of early vein formation. During development of all veins, cells acquire early preprocambial identity progressively, while subsequent procambium formation occurs simultaneously along entire veins. The progressive extension of vascular strands at the preprocambial stage suggests that veins are initiated as freely-ending preprocambial domains and that network formation occurs through subsequent fusion of these domains. Consistent with this interpretation, we demonstrate that veins are generally not programmed to become freely-ending or interconnected network elements. Instead, we found that the progressive extension of preprocambial domains can be interrupted experimentally and that this leads to less complex vein patterns consisting of fewer vein orders, in which even lower-order veins become freely-ending. Mesophyll differentiation turned out to be strictly correlated with the termination of preprocambial domain extension. Our findings suggest that Arabidopsis vein pattern is not inherently determinate, but arises through reiterative initiation of new preprocambial branches until this process becomes terminated by the differentiation of mesophyll.

Symposium 2 Session II

Phosphite Blocks Phosphate Sensing in Plants and Yeast

William C. Plaxton,
Dept. of Biology, Queen's Univ., Kingston, ON

Phosphite ($H_2PO_3^-$, Phi) is an important agricultural commodity that is being widely marketed both as a fungicide and as a superior P fertilizer. Phi-based fungicides effectively control pathogens that cause numerous crop diseases. Although plants rapidly absorb and translocate Phi, it does not appear to be oxidized or metabolized *in vivo*. While Phi has little influence on the growth of phosphate (HPO_4^{2-}, Pi) sufficient plants and yeast, it is extremely deleterious to their development under Pi deficient conditions. Phi negates the acclimation of plants and yeast to suboptimal [Pi] by specifically obstructing the derepression of genes encoding proteins characteristic of their Pi starvation response (*e.g.* acid phosphatases, etc). Phi treatment of Pi-deprived *Brassica napus* suspension cells also: (i) caused marked differential protein phosphorylation *in vivo*, and (ii) accelerated the onset of Pi-starvation-mediated programmed cell death by about 3 weeks. In *Saccharomyces cerevisiae*, Phi appears to target PHO84, a high-affinity Pi transporter and putative component of a Pi-sensor complex. Although Phi cannot replace Pi for fulfilling the nutritional P requirements of plants/yeast, Phi can substitute for Pi in repressing typical molecular and developmental responses to Pi deficiency. Phi thus represents a valuable tool for investigating the signaling pathway by which plants perceive and coordinate appropriate cellular responses to Pi stress. We also need to assess the long-term consequences of the significant input of Phi into food products that arises from its extensive use in agriculture.

Symposium 3 Session III

Genetics and Genomics of Cold Tolerance in Cereals

Fathey Sarhan
Département des Sciences Biologiques, Université du Québec à Montréal, C.P.8888, Succ.Centre-Ville, Montréal, Québec, Canada, H3C 3P8
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Low temperature stress is a major factor limiting plant productivity and survival. Overwintering plants, such as winter cereals, sense the upcoming winter and delay flowering by postponing the transition from the vegetative to the cold-sensitive reproductive growth phase. This delayed transition is associated with the development of a high degree of freezing tolerance that is required for winter survival. These two important biological processes are regulated by low temperature and are part of a complex interacting genetic system. To understand the nature of this system, identification and functional analysis of the complete network of genes that are differentially expressed in response to low temperature are required. Towards this goal, we are using combinations of approaches including: genome-wide expression studies, bioinformatic predictions, structural analyses, sub cellular- localization, protein-protein interactions, genetic mapping, reverse genetics and comparative genomics. These functional genomics analyses reveal the complexity of the low temperature responses in plants and facilitate a large-scale identification of novel low temperature regulated genes. In addition, they provide valuable information that improves our understanding of their function and regulation. Progress on the characterization of some of these genes and their potential use to improve low temperature tolerance in plants will be discussed with emphasis on the newly identified *Tavrt-1* gene. The gene encodes a putative transcription factor that belongs to the MADS-box family and mapped to the vrn-1 regions on the long arms of homologous group 5 chromosomes. Expression profiling and genetic analysis indicated that the accumulation of its transcript is associated with the vernalization response, the transition from vegetative to reproductive phase, the expression of COR genes, and the degree of freezing tolerance.

Oral presentations

Oral presentation 1

BREVIS RADIX (BRX) gene, a major QTL for root growth in Arabidopsis, encodes a novel factor involved in root system morphology

Céline F. Mouchel, Georgette C. Briggs and Christian S. Hardtke
McGill University, Biology Department, 1205 Docteur Penfield Avenue, Montréal, Québec H3A 1B1, Canada

Arabidopsis thaliana accessions of diverse geographical origin offer an alternative genetic resource to identify novel loci or alleles that influence plant development. We sought to exploit this natural variation to identify genetic constitutions that affect root system morphology. In tissue culture, we identified one line with a shorter primary root and enhanced root branching as compared to average. This phenotype is heritable, stable over a range of conditions, reflected in the adult (soil grown) root system and not accompanied by apparent defects in the shoot structures. Using an accession with average morphology as control line, we analyzed this root phenotype in detail. In a number of physiological assays, such as sensitivity to different hormones and nutrients, we could not detect any defects in this line that would explain its unique root system morphology. Also, microscopic analysis indicated that the radial pattern of the primary root is intact. To test whether the root morphology in this line is due to genes not previously known to be involved in root system morphogenesis, we established various resources for genetic mapping. In an F2 population a locus that is principally responsible for the short primary root phenotype segregated in Mendelian fashion. We named this gene *BREVIS RADIX (BRX)* for 'short root'. In a map-based cloning approach we located *BRX* in an interval of ca. 40kb. The nine genes in this region were amplified from the line of interest, sequenced and compared to controls. One gene displayed a lesion that would result in a non-functional protein product, due to an early stop codon. Transgenic expression of the corresponding cDNA in a *brx* background rescues the short root phenotype, confirming that this gene encodes *BRX*. Sequence analysis of the corresponding conceptually translated protein indicates that it is part of a small new family of regulatory proteins. Further details will be presented.

Oral presentation 2

Liquid-phase fluorescence *in situ* RT-PCR analysis for gene expression analysis in woody stems

Madoka Gray-Mitsumune
UPSC, Swedish University of Agricultural Sciences, Sweden

I present a rapid *in situ* RT-PCR protocol for gene expression studies in woody stem tissues. *In situ* RT-PCR was performed using fluorescent dye-conjugated nucleic acid and the fluorescence signals were detected using confocal laser scanning microscopy. Strong gene-specific signals were obtained in secondary stem tissues. The signals were PCR-dependent, as shown by the lack of cytoplasmic signals in the tissue sections in which either DNA polymerase or primers were omitted from PCR reactions, and were RNA-dependent, as shown by great reduction of cytoplasmic signals when sections were treated with RNase before RT reactions. This protocol is straightforward and only takes two days from sectioning to observation. It is also amenable for high throughput application and may assist large scale gene expression studies in woody stems.

Oral presentation 3

Cellular architecture and cytomechanics in growing pollen tubes

Anja Geitmann, Elodie Parre
Institut de Recherche en Biologie Végétale, Université de Montréal, 4101, rue Sherbrooke est, Montréal, Québec, H1X 2B2, Canada

Tip growing cells such as pollen tubes have the capacity to rapidly elongate and to penetrate a semi-solid or solid substrate. Morphological studies of pollen tubes have shown that the configuration of structural cellular elements differs between the growing apex and the distal part of the cell thus reflecting the cell's highly anisotropic growth behavior. Correspondingly, it has frequently been postulated that the physical properties of pollen tubes such as cell wall plasticity should show anisotropic distribution, but no experimental evidence has been published hitherto. Using micro-indentation techniques we quantified the pollen tube's resistance to lateral deformation forces and we analyzed its visco-elasticity as a function of distance from the growing apex. We related these data to pollen tube architecture, analyzing the distribution of the cell wall components pectin, callose, cellulose as well as the actin cytoskeleton using fluorescence label. Our data revealed that in particular the degree of pectin methyl esterification and the configuration of the actin cytoskeleton correlate well with the distribution of the physical properties on the longitudinal axis of the cell. This confirms a role of these cellular components in the determination of the cytomechanics of pollen tubes.

Oral presentation 4

The cuticle of soybean seeds controls their permeabilities to water

Fengshan Ma[1,2], Ewa Cholewa[3], Tasneem Mohamed[1], Carol A. Peterson[1], Mark Gijzen[2]

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The permeability of soybean seed coats to water is a significant issue in both seed science and food industry. Soybean seeds range from highly permeable to impermeable. While a low permeability may be beneficial to long-term seed survival, a high permeability is desired in the food processing industry. But there has been no agreement as to what factor controls permeability. We compared water permeability of seeds to the structure of their coats using a variety of microscopic methods. Results suggest that the cuticle covering the palisade layer is the key to understanding the mechanism of initial water uptake. Impermeable seeds had an intact cuticle that was dense and strong, while permeable seeds had a cracked cuticle that was loose and weak. Cuticular cracks appeared to be the initial sites of water entry. These cracks normally occurred on the dorsal side of permeable seeds. We are now examining the effects of chemical and mechanical treatments on seed coat structure and permeability. Investigations of the chemical composition and assembly of the cuticle may provide new insights into soybean seed permeability. We will also carry out developmental and molecular studies of cuticle formation. These studies will eventually allow for selection of cuticle structures that give rise to high quality seeds for agriculture and industry.

Oral presentation 5

Phosphate or phosphite addition promotes proteolytic turnover of intracellular and secreted phosphate-starvation inducible tomato purple acid phosphatase isozymes

Gale G Bozzo[1], Vinay K Singh[2], and William C. Plaxton[1,2]
Depts. of Biology[1] and Biochemistry[2], Queen's Univ., Kingston, ON

The phosphate (HPO_4^{2-}, Pi) starvation response of tomato suspension cell cultures includes the marked induction of an intracellular purple acid phosphatase (IAP) and two secreted purple acid phosphatase isozymes (SAP1 and SAP2). Within 48 h of the addition of 2 mM of Pi or its analog, phosphite ($H_2PO_3^-$, Phi), to 8-d-old Pi-starved (-Pi) tomato cells: (i) IAP & SAP specific activities decreased by over 10-fold, and (ii) immunoreactive SAP and IAP polypeptides either disappeared (SAP1 & SAP2) or were greatly reduced (IAP). By contrast, no changes in IAP or SAP activity or immunoreactive polypeptides occurred in 10-d-old –Pi tomato cells, relative to 8-d-old –Pi cells. *In vitro* proteolysis of homogenous SAP protein occurred following its 24 h incubation in the presence of culture media filtrate from Phi-resupplied –Pi tomato cells. 'In gel' protease assays suggested that the turnover of SAP1 and SAP2 due to Pi or Phi addition to the 8-d-old –Pi cells was caused by the upregulation of two secreted serine proteases having M_rs of approx. 83 & 77 kDa. Studies are in progress to fully purify both proteases to allow thorough characterization of their molecular, immunological, and kinetic properties/substrate specificity.

Oral presentation 6

Phosphate response of alfalfa overexpressing *Arabidopsis* phosphate-starvation responsive gene group 1

M. F. Hétu, B. D. McKersie, L.J. Tremblay and D.D. Lefebvre
Department of Biology, Queen's University, Kingston, ON, K7L 3N6

The mechanisms of plant response to nutrient limitation are of great interest for agricultural reasons. AtPSR1 is a SnRK2 Ser/Thr protein kinase unique to plants. AtPSR1 contains a unique acidic C-terminus and transcription is induced by P_i-deprivation in roots. Sense, antisense, and four mutated versions of *Atpsr1* were introduced into *Medicago sativa* (alfalfa) under the control of the CaMV 35S promoter using *Agrobacterium*-mediated transformation. Plant cuttings from each transgenic line were grown hydroponically for 3 weeks in 0.1 x MS containing 0.125 mM P_i and then for 10 days in 0.1 x MS containing no P_i (P_i-starved), 0.125 mM P_i (control), or 12.5 mM P_i (high P_i). Root and shoot length, fresh weight, dry weight, and total P_i was analyzed. The transgenics had shorter and less biomass than non-transformed alfalfa, but had greater root to shoot ratios. Alfalfa expressing sense *Atpsr1* had a greater increase in root and shoot length and biomass than controls, non-transformed, and antisense *Atpsr1* alfalfa when starved of P_i for 10 days.

Oral presentation 7

Low Ci induction of transcripts related to the CO_2-concentrating mechanism is not light dependent in the cyanobacterium *Synechococcus* PCC7942.

Patrick McGinn*, Meaghan Jones and Douglas Campbell
Department of Biology and Coastal Wetlands Institute, Mount Allison University, Sackville, NB, Canada; * author for correspondence, pmcginn@mta.ca

Under inorganic carbon (Ci) limitation, many cyanobacteria induce a CO_2-concentrating mechanism (CCM) which drives the localised elevation of CO_2 around a catalytically inefficient Rubisco within a proteinaceous structure, the carboxysome, greatly improving the overall efficiency of photosynthesis. The primary signal which elicits CCM induction in cyanobacteria has not yet been identified. Candidates include photorespiratory or Calvin cycle intermediates, direct sensing of diminishing external or internal CO_2 and/or HCO_3^- levels and the reduction state of the photosynthetic and/or respiratory electron transport chains. Both photosynthetic CO_2 fixation and active CO_2 and HCO_3^- transport are light-dependent processes, so it is reasonable to expect that the induction of the CCM shares a similar light requirement and that light could be required for CCM induction. We tested this hypothesis in the cyanobacterium *Synechococcus* PCC7942 by shifting cultures grown under high Ci conditions (2.5-5.0 mM) to low Ci by aeration with CO_2-free air for up to 2 hours in the presence or absence of light. Infra-red gas analysis revealed that extracellular Ci levels decreased from 2.5-5.0 mM to 20-50 □M in both darkened and illuminated cultures flushed with CO_2-free air within 90 minutes and to near the instrument detection limit within 2 hours. Control cultures were maintained at high levels of Ci (2.5-5 mM) in the light and the dark over the same 2 hour interval. Real-time quantitative RT-PCR assays revealed that several key inducible CCM-related transcripts accumulated to high levels in both darkened and illuminated cells aerated with CO_2-free air for 2 hours, relative to control cells maintained in the light under high Ci. This suggests that light is not an obligate environmental signal for low Ci-mediated CCM induction in this strain. These results contrast with the pattern in *Synechocystis* PCC6803 where CCM induction is clearly light-dependent.

Oral presentation 8

The effect of PsbS on the redox characteristics of QA and QB in photosystem II reaction centres

[1]Balseris, A., [1]Krol, M., [2]Savitch, L.V., [1]Huner, N.P.A.
[1]University of Western Ontario, Dept. of Biology, London, Ontario, N6A 5B7 [2]ECORC, Agriculture and Agri-Food Canada, Ottawa, Ontario, K1A 0C6

A 22-kDa protein, PsbS, located between the light-harvesting complex and the core antenna of PSII plays a major role in non-photochemical quenching (NPQ), dissipation of excess energy as heat. It was shown that PsbS is directly involved in one of the mechanisms of NPQ, so-called qE mechanism which is associated with a xanthophyll cycle. The lack of PsbS decreases maximally NPQ capacity in antenna.
Analysis of the *npq4-1* mutant of *Arabidopsis thaliana*, which lacks PsbS in its antenna, showed that plants grown under different conditions are more sensitive to photoinhibition compared to wild type. The measurements of thermoluminescence showed that this protein influences the redox characteristics of Q_A and Q_B in photosystem II reaction centre. The functional relevance of these changes in photosystem II photochemistry to low temperature acclimation in *A. thaliana* will be discussed.

Oral presentation 9

Mechanically isolated *Asparagus sprengeri* cells perceive and respond to lipo-chitooligosachharides, a *Rhizobia*- secreted nodulation factors

Ewa Cholewa and John McIver
BiosAgriculture Inc. 21,111 Lakeshore Rd., P.O.Box 187, Ste-Anne-de-Bellevue, H9X 3V9 Quebec, Canada. e-mail: echolewa@biosagriculture.com

Lipo-chitooligosaccharides (LCOs) are part of the *Rhizobium*-legume molecular dialogue. Those powerful developmental regulators are produced by rhizobia in response to legume-root-secreted flavonoids. LCOs set in motion a whole range of plant responses that eventually are manifested in organogenesis and formation of the nodule in symbiotic legumes. The LCO perception at the root level and nodule formation seems to be very precise process requiring a specific strain of *Rhizobia* (and therefore specific LCO) and responsive legume. In this study we are investigating whether LCOs are perceived in non-legume plants using mechanically isolated *Asparagus sprengeri* cells as a model system. Addition of LCOs to the *A. sprengeri* cell suspensions resulted in number of physiological responses: alkalinization of the media, sustained production of hydrogen peroxide, inhibition of photosynthetic oxygen evolution and stimulation of phytoalexins synthesis. Oxidative burst was diminished by addition of La^{3+}, a calcium channel blocker, and by ascorbic acid. This indicates that LCO is activating a Ca-mediated signal transduction pathway that leads to the stimulation of membrane-associated NADPH oxidase responsible for H_2O_2 release. LCO-induced inhibition of photosynthetic oxygen evolution was not lessened by the presence of La^{3+} or ascorbic acid in the incubation media indicating that inactivation of PSII activity was not due to possible H_2O_2 damage. All of these responses were observed at relatively high LCO concentration (10^{-5} M), compared to the effective LCO level (10^{-9} M) needed for nodulation. Nevertheless, we can conclude that LCOs are perceived by non-legume plants and evoke a number of responses in *A. sprengeri* cells. The precise mechanism of LCO perception in non-legumes needs to be investigated.

Oral presentation 10

***MUM4* encodes a putative pectin biosynthetic enzyme developmentally regulated by *AP2, TTG1* and *GL2* in the *Arabidopsis* seed coat**

Tamara L. Western, Diana S. Young, Gillian H. Dean, A. Lacey Samuels and George W. Haughn

The *Arabidopsis* seed coat epidermis undergoes a complex process of differentiation that includes the biosynthesis and secretion of large quantities of pectinaceous mucilage, cytoplasmic rearrangement and secondary cell wall biosynthesis. Mutations in *MUM4* lead to a decrease in seed coat mucilage and incomplete cytoplasmic rearrangement. We show that *MUM4* encodes a putative NDP-L-rhamnose synthase, an enzyme required for the synthesis of the pectin rhamnogalacturonan I, the major component of *Arabidopsis* mucilage. In addition, this result establishes a causal link between mucilage production and cellular morphogenesis. The cellular phenotype seen in *mum4* mutants is similar to that of several transcription factors (*AP2, TTG1, TTG2, MYB61* and *GL2*). Expression studies suggest that *MUM4* is developmentally regulated in the seed coat by *AP2, TTG1* and *GL2*, while *TTG2* and *MYB61* appear to be regulating mucilage production through alternate pathway(s). Our results provide a framework for the regulation of mucilage production and secretory cell differentiation.

Oral presentation 11

Prokaryotic Orthologs of Plant Mitochondrial Alternative Oxidase and Plastid Terminal Oxidase.

A.E. McDonald, S. Amirsadeghi, and G.C. Vanlerberghe.
Depts. Of Botany and Life Sciences, University of Toronto at Scarborough, 1265 Military Trail, Scarborough, ON, Canada, M1C 1A4.

Higher plants and green algae contain two similar members of the membrane-bound diiron carboxylate proteins: the mitochondrial alternative oxidase (AOX) and the plastid terminal oxidase (PTOX). AOX is a ubiquinol oxidase that may serve to stabilize reactive oxygen species generation by the respiratory electron transport chain. PTOX is a plastoquinol oxidase required in carotenoid biosynthesis and may represent the elusive oxidase of chlororespiration. Given the endosymbiotic origin of mitochondria and plastids, we sought to find prokaryotic orthologs of AOX and PTOX proteins. PTOX orthologs are present in four different cyanobacteria and an AOX ortholog is present in an α-proteobacterium. We confirmed the presence and expression of the PTOX gene in *Anabaena variabilis* PCC 7120 and demonstrate that the expression of this gene is influenced by light intensity. Our results have implications for the photosynthetic and respiratory metabolism of these prokaryotes, as well as for the origin and evolution of eukaryotic plastid PTOX and mitochondrial AOX proteins.

Oral presentation 12

Expression of non-symbiotic barley hemoglobin alters the phenotype of transgenic alfalfa

K. Baron, C. Dordas and R.D. Hill
Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2 Canada

Our laboratory has shown that stress-induced hemoglobins modulate plant NO levels. NO is known to be an important signal molecule participating either directly or indirectly in various plant hormonal stimuli. We sought to determine the influence of hemoglobin expression on plant growth and development. Varying hemoglobin expression results in dramatic alterations to growth and development, producing plants with distinct phenotypic characteristics. Plants over-expressing hemoglobin have early vigorous growth, strong apical dominance of both root and shoot systems, and flower earlier than wild-type or lines under-expressing hemoglobin. Flower colour and chlorophyll content of transgenic lines are also modified. The morphological characteristics of these transgenic plants closely follow documented responses of alfalfa to exogenous application of GA, auxin or anti-auxin. Preliminary work has also been conducted to ascertain the ability of these transgenic lines to tolerate a flooding stress.

Oral presentation 13

Altering hexokinase levels in transgenic potato (*Solanum tuberosum*) roots influences growth rate and branching

E. Claeyssen, O. Wally, D. Matton and J. Rivoal
IRBV, Université de Montréal, 4101 rue Sherbrooke est, Montreal, QC H1X 2B2 Canada

The control of hexokinase (HK) over plant glycolysis is under investigation. Potato (*S. tuberosum*) roots have been transformed with a *S. chacoense* HK cDNA in sense and antisense orientations to over- and under-express HK, respectively. We have generated transgenic roots with HK activities ranging between 80 and 700% those of clones transformed with an empty vector. Among 18 enzymes of primary metabolism studied, only PFK, GAPDH, PEPC and PEP Phosphatase activities displayed marginal changes in transgenic roots. Root total length and tip number were quantified and inversely correlated to HK activity, suggesting a major regulatory role for HK. We hypothesize that changes in root HK catalytic activity may have perturbed glycolytic flux. Alternatively, growth inhibition may stem from an alteration of HK function as a sugar sensor. Studies of the control of hexokinase over glycolytic flux in normoxia and anoxia should enable us to clarify these issues. (Supported by NSERC)

Oral presentation 14

Heat-activation of the MAP kinas, HAMK, involves lipid signaling

Mansour, A., Suri, S.S., *Shawky, A. and Dhindsa, R.S.
Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Quebec H3A 1B1; * Department of Genetics, Zagazig University, Zagazig, Egypt.

The role of phospholipid signaling in heat shock response of BY-2 cells of tobacco (*Nicotiana tobaccum*) has been examined. Activation of HAMK (Heat-Activated MAP Kinase) and the accumulation of heat shock protein HSP70 were used as end-point markers. The inhibitors of phospholipase C (PLC) and protein kinase C (PKC) inhibited the HAMK activation and HSP70 accumulation during heat shock. The inhibitors of diacylglycerol (DAG) kinase that converts DAG to Phosphatidic acid (PA) also inhibit heat activation of HAMK and accumulation of HSP70. Treatment of cells with PA causes HAMK activation and HSP70 accumulation at 25°C. Treatment of cells with either IP_3 or cADPR, both known to release Ca^{2+} from intracellular stores, caused the activation of HAMK and accumulation of HSP70 at 25°C. We concluded that heat shock response as measured by HAMK activation and HSP70 accumulation requires phospholipid signaling.

Oral presentation 15

Physiological and biochemical responses of *Thellungiella salsuginea* to osmotic stress

David Guevara[1], Brian Golding[1], Brian McCarry[2], Paulo Nuin[1] and Elizabeth Weretilnyk[1].
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Thellungiella salsuginea is a subarctic crucifer closely related to *Arabidopsis thaliana*. In contrast to *Arabidopsis*, *Thellungiella* is highly tolerant of abiotic stresses and so provides an excellent model to study stress tolerance mechanisms. Rates of photosynthesis for *Thellungiella* watered with 300 mM NaCl were over 60% those measured for unsalinized controls. With drought, wilting occurs at a leaf relative water content (RWC) of 65%. When the plants reached a leaf RWC of 35-40%, a point beyond the critical RWC of many plants, re-watering restored full turgor to leaves and plants flowered and set seed. Leaf water and solute potential measurements of osmotically stressed plants show that *Thellungiella* accumulates solutes and so undergoes "osmotic adjustment". Metabolic profiling is being used to identify compatible organic solutes associated with osmotic stress tolerance in this plant. Over 200 compounds are detected in leaf extracts of *Thellungiella* with 4.3% and 7.6% unique to drought and salinized plants, respectively. Metabolites enriched or unique to tissue of stressed plants could provide bio-markers for traits underlying the abiotic-stress tolerant phenotype of *Thellungiella*.

Oral presentation 16

Identification of plant hsp90 interactors by the yeast two-hybrid screen

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The highly conserved and abundant molecular chaperone hsp90 plays a key role in signal transduction networks, cell-cycle control, protein degradation and protein trafficking. The number of identified client proteins whose functions are facilitated by hsp90 continues to grow, especially in animal systems. A critical dependence on hsp90 has been established for animal steroid hormone receptors and several serine/threonine and tyrosine kinases. Hsp90 is believed to serve as a capacitor for morphological evolution, and in recent years it has emerged as a promising drug target. The hsp90 protein family is largest in plants. Despite the availability of hsp90 genes from a variety of plants, the identities of client proteins for plant hsp90 remain undefined. We have identified potential interactors of plant hsp90 in a yeast two-hybrid screen. Current focus is on confirming the interaction of an ethylene receptor, and of two proteins containing tetratricopeptide repeat domain, with hsp90. These results will be discussed in the context of the hsp90 chaperone system in plants.

Oral presentation 17

Regulatory Phosphorylation of PEP Carboxylase in Developing Castor Oil Seeds

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Two PEPCase isoforms (PEPC1 & PEPC2) were recently purified and characterized from endosperm of developing castor oil seeds (COS) (Blonde & Plaxton 2003 J Biol Chem 278:11867). The association of a common 107 kDa catalytic subunit (p107) with an unrelated 64 kDa polypeptide (p64) leads to marked physical and kinetic differences between the PEPC1 p107 homotetramer and novel PEPC2 p107/p64 heterooctamer. Here we describe the production of anti-phospho-site specific IgG to the conserved *N*-terminal phosphorylation site (Ser5) of p107. This IgG was used to establish that p107 phosphorylation is: (i) maximal in full cotyledon (stage VII) developing COS, (ii) more pronounced in PEPC1 than in PEPC2, (iii) reversed following PEPC1 incubation with bovine PP2A, and (iv) not involved in a possible interconversion of PEPC1 and PEPC2. Enhanced p107 phosphorylation during COS development was correlated with an 8-fold increase in PEPC's I_{50}(malate). Ca^{2+}-independent PEPC protein kinase activity was detected throughout COS development. One day following decapitation of shoots containing intact developing COS, COS PEPC kinase activity was unaffected, whereas the p107 of stage VII COS was entirely dephosphorylated. Thus, p107 phosphorylation appears to be at least partially dependent upon the 'fine control' activation of COS PEPC kinase due to sucrose import from source tissue.

Oral presentation 18

Brassinosteroid-mediated stress tolerance

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Brassinosteroids (BRs) are natural plant steroidal compounds that possess growth-promoting and stress response-modulating properties. Previously we have shown that *Brassica napus* seedlings grown in the presence of 24-epibrassinolide (EBR), a BR, are more tolerant to a heat treatment that is lethal to control seedlings grown in the absence of the compound. We now demonstrate that EBR-treated seedlings are also more resistant to dehydration and salt stress than are untreated seedlings. Preliminary studies indicate that during stress, EBR-treated seedlings express a subset of stress specific genes at much higher levels than untreated seedlings. To gather further evidence in support for a role of BRs in plant stress responses, the Arabidopsis *DWF4* gene encoding a cytochrome P450 enzyme that mediates the putative rate-limiting step in BR biosynthesis, was introduced into *B. napus* and Arabidopsis. Our approach, and results obtained thus far, will be discussed.

Oral presentation 19

Jasmonates induce nodulation *(nod)* genes of *Bradyrhizobium japonicum* in a strain specific manner

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Jasmonic acid and methyl jasmonate, collectively known as jasmonates, are naturally occurring in plants; they are important signal molecules involved in induced disease resistance and mediate many physiological activities in plants. We studied the effect of jasmonates (jasmonic acid – JA and its methyl ester, methyl jasmonate – MeJA), on the induction of *nod* genes in 6 strains of *Bradyrhizobium japonicum*. All strains harbored a plasmid with a translational fusion between *B. japonicum nodY* and *lacZ* of *Escherichia coli* and the expression activity was measured indirectly through the amount of β-galactosidase activity. Genistein induced *nod* genes in all the strains tested and this treatment was used as a positive control. Our β-galactosidase activity results showed that both JA and MeJA induced the expression of *nod* genes in GG4 (USDA3). However, GG1 (USDA 31), GG2 (USDA76), GG3 (USDA121), LB100 (USDA135) and ZB977 (USDA110) did not show any *nod* gene induction following exposure to JA or MeJA. Combined treatment with genistein and jasmonates resulted in synergistic effects in strain GG4. Taken together, these observations demonstrate unanticipated complexity of inducer molecule perception and transcriptional regulatory mechanisms of *nod* genes in *B. japonicum* in the form of interactions between strains and inducer compounds.

Oral presentation 20

"Bioinformatics approaches: *In silico* discoveries in soybean expression data"

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In plant species where the whole genome is not yet sequenced, large scale public data such as EST and SAGE data, is a valuable resource for finding genes. To date there are ca 3.6 million plant ESTs in dbEST of which ca 340,000 represent soybean genes. This soybean data was generated from ca 80 different tissue libraries and so represents a broad view of plant gene expression. To support new *in vitro* results, we screened this data collection computationally for potential homologues to the soybean seed lectin *Le1* gene. 304 ESTs were retrieved and subsequently contigged. The results from this experiment and other interesting discoveries in the contents of the large soybean EST collection will be discussed and also related to soybean microarray data.

Oral presentation 21

Molecular phylogeny of the tree genus Populus (Salicaceae)

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The species of *Populus*, collectively known as poplars, comprise some of the most commercially exploited, pioneer and riparian forest trees distributed throughout the northern-hemisphere. In order to understand the evolutionary relationships among poplars and to provide a framework for biosystematic classification, we reconstructed the phylogeny of the genus *Populus* based on inter simple sequence repeats (ISSR) data and nucleotide sequences of three non-coding regions of the chloroplast DNA (intron of *trnL*, and intergenic regions of *trnT-trnL* and *trnL-trnF*) and *ITS1* and *ITS2*of the nuclear *rDNA*. The resulting phylogenetic trees showed polyphyletic relationships among species in the sections *Tacamahaca* and *Aigeiros*, and provided evidence for a possible reticulate evolutionary origin for *P. nigra*, *P. tristis*, *P. szechuanica*. In contrast to the nucleotide sequence based phylogeny, the ISSR-based phylogenetic tree revealed a close relationship between *P. nigra* and species of section *Tacamahaca*. This relationship is in agreement with various phenotypic data including disease susceptibility, interfertility and bud exudates suggesting introgression between *P. nigra* and species of section *Tacamahaca*. Furthermore, the phylogenetic tree based on ISSR data is mostly congruent with phylogenetic trees based on other molecular and morphological data, and yielded a well resolved phylogenetic tree demonstrating the utility of ISSR as a tool to reconstruct phylogeny of closely related group of species.

Oral presentation 22

Environmental Control of Exodermal and Endodermal Development in Maize

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The development of the exodermis in maize is influenced by growing conditions. For example, development is inhibited by hydroponic culture, promoted by vermiculite culture, and is markedly advanced by exposure to moist air. The roots of many aquatic species have an exodermis that prevents radial oxygen loss from the root to the surrounding medium. Is exodermal maturation promoted by low oxygen levels? In maize, hypoxic conditions induce the formation of aerenchyma in the central cortex, and promote exodermal development (compared with controls grown in aerated conditions). The mature exodermis tends to be radially aligned with the cortical air lacunae. Regions of the cortex through which a lateral root will emerge lack both lacunae and a mature exodermis. In the endodermis, suberin lamella development is delayed, creating an environment conducive to aeration of the stele from the central cortex. Preliminary experiments with ethylene indicate a similar effect on the endodermis, but the effect of this growth regulator on the exodermis was variable and inconclusive.

Oral presentation 23

Effect of Hup Status on Rhizobacteria and Soil Fertility

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Through the selective release of exudates, plants activate and sustain specific rhizobacterial communities in the root zone. In turn, rhizobacteria are able to positively influence plant establishment, growth and development. This study focused on the hydrogen gas released by Hup^- nodules of some legume plants, through the process of nitrogen fixation. Two isogenetic strains of *B. japonicum*, Hup^- and Hup^+, were utilized in this study. Soybean plants evolving hydrogen from their Hup^- nodules are significantly larger then that of the control. The rhizpshperic soil samples of these Hup^- soybean plants have significantly higher hydrogen uptake rates when compared to that of Hup^+ soybean plants. Significant plant growth promotion was observed after barley was grown in rotation with Hup^- soybean plants. In this present study, molecular analysis showed that bacterial communities of both hydrogen treated soil and soil adjacent to Hup^- nodules are distinctively different from that the control. These results indicate that the rhizosphere bacterial population adjacent to Hup^+ and Hup^- nodules are different. The results of this study may help bridge the link between hydrogen fertilization and enhanced plant growth.

Oral presentation 24

Effects of exudates extracted from transformed-tomato-roots grown in bi-compartment Magenta boxes and colonized with or without *G. intraradices* on *Phytophthora parasitica* var. *nicotianae* zoospores.

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A modification in root exudation induced by Arbuscular Mycorrhiza Fungi (AMF) colonization has been reported. Furthermore zoospores liberated through the asexual reproduction of *Phytophthora parasitica* var. *nicotianae* have been shown to be attracted by plant-root-exudates which also promote their encystment and germination. Our objective is to elucidate the bioprotection (a reduction in the apparition of the disease symptoms) induced by AMF in symbiosis with tomato then infected by *P. parasitica*. Transformed tomato roots were grown with or without *Glomus intraradices* colonization, in Magentas boxes split into two compartments, *in vitro*. The roots and *G. intraradices* from the liquid compartment growth patterns were characterized. After 16 and 24 weeks of culture, the liquid medium containing the root-exudates was collected so that biotests could be carried out. Non-mycorrhizal-root-exudates attracted more the pathogen zoospores than exudates from mycorrhizal roots. These results indicate that *P. parasitica* development might be impacted in the mycorrhizosphere before it penetrates the mycorrhizal tomato roots through the modification of root exudation. As a consequence, the pathogen development would be limited to a few roots which would then be able to trigger resistance mechanisms. *P. parasitica* proliferation would in this manner be reduced. The composition of exudates (amino acids, sugars and second metabolites) liberated from non-mycorrhizal roots and from mycorrhizal-roots are being compared by HPLC analyses.

Poster presentations

Poster 1

Variability of photosynthesis in primary leaves of soybean and the effect of LCO

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Variability in photosynthesis of plant leaves is common due to factors such as age, position of leaf on the plant, and nutritional status. This variability may over shadow the effect of spraying photosynthesis stimulating compounds, particularly when the effect is small. Our objective was to evaluate the natural variability of photosynthesis in primary leaves, and the effect of LCOs on photosynthetic rate and plant growth. Soybean cv. OAC Bayfield seeds were germinated in vermiculate and as soon as seedlings emerged these were carefully transplanted into pots. Once the primary leaves were exposed photosynthesis and stomatal conductance was measured using a portable photosynthesis analyzer (LI-COR 6400). Also leaf area was determined using a digital camera and calculating the area through a sigma scan image analysis program. A group of photosynthetically uniform seedlings were selected and LCO was spraying directly onto the leaves to evaluate its effect. Photosynthesis in primary leaves ranged from 5 to 14 umoles CO_2 fixed/m^2/s. Regression analysis showed this variability to be associated with stomatal conductance variability (R^2=0.74) and leaf area variability (R^2=0.72). More photosynthetically uniform plants allowed a clear and significant effect of LCO. Increases in the photosynthetic activity caused by LCO were around 8%. As result plant dry matter accumulation growth was also increased.

Poster 2

Molecular Characterization of a Flavonol 6-Hydroxylase

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Chrysosplenium americanum, a semi-aquatic weed, accumulates a variety of highly methylated flavonoids through the action of a variety of regio-specific enzymes, most of which have been previously studied. One of these enzymes, flavonol 6-hydroxylase (F6H), an α-ketoflutarate-dependent dioxygenase introduces a hydroxyl group at position 6 of a trimethylated flavonol intermediate. The native enzyme has been purified and biochemically characterized. Internal amino acid sequence information from the native protein was obtained for the cloning of its gene. The near full-length fragment was isolated from a cDNA library, cloned and subsequently expressed heterologously in both prokaryotic and eukaryotic expression systems. Its activity was verified to catalyze the conversion of trimethylquercetin to trimethylquercetagetin, albeit at a reduced rate as compared to the native enzyme. At the molecular level, the F6H clone encodes the conserved sequence motifs for this class of enzymes along with the peptide microsequences obtained from the purified protein. F6H is present as a single copy in the *C. americanum* genome and contains two introns of approximate length and position comparable to a particular class of dioxygenases.

Poster 3

Implication potentielle de l'ascorbate oxydase dans la régulation de l'inhibition de croissance racinaire causée par l'aluminium

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En condition acide, l'aluminium (Al) des sols acquiert une spéciation phytotoxique (Al3+) affectant la croissance des plantes. L'Al agit principalement à la pointe des racines en induisant une panoplie de phénomènes biologiques menant à l'inhibition de la croissance racinaire. Normalement, l'auxine stimule la croissance à de faibles concentrations mais l'inhibe à plus fortes doses. Il a été démontré que l'auxine s'accumule anormalement à la pointe des racines en réponse à l'Al suggérant qu'elle pourrait être responsable des effets induits par l'Al. Afin de pouvoir détruire l'excès d'auxine, nous avons isolé un promoteur s'exprimant spécifiquement à la pointe des racines lorsque la concentration d'auxine augmente. L'ascorbate oxydase qui est capable de décarboxyler l'auxine a été placée sous le contrôle de ce promoteur et la construction utilisée pour transformer *Arabidopsis thaliana*. Cette affiche explique comment nous espérons combattre l'accumulation d'auxine en réponse à l'Al et fait état des progrès réalisés dans ce sens.

Poster 4

Cytokinin-mediated up-regulation of the programmed cell death regulator Bax Inhibitor-1 in tobacco cells : hormonal regulation or Ca^{2+} signalling ?

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Programmed cell death (PCD) is a vital phenomenon for multicellular organisms because of its involvement in removing unnecessary or harmful cells during normal development or under pathological conditions. To date, biochemical processes involved in the control of plant PCD are only poorly understood. The protein Bax Inhibitor-1 (BI-1) is conserved between animals and plants and acts as a negative PCD regulator by a still unknown mechanism. To elucidate its mode of action, we used suspension cells of *Nicotiana tabacum* to study the effects of different molecules on the expression level of the protein NtBI-1 *via* Western analysis. We found that NtBI-1 is up-regulated following treatments with the cytokinin 6-benzylaminopurine (Bap) at concentrations inducing growth retardation or arrest, but not at lethal concentrations. Similar results were obtained with the other cytokinins tested. Moreover, we found that Bap treatment as short as 30 minutes was enough to induce the response. When intracellular activation of Bap was inhibited by the use of an adenosine kinase inhibitor, NtBI-1 up-regulation was still observed. Considering the rising effect of Bap on cytosolic Ca^{2+} fluxes (measured *via* aequorin-transformed tobacco cells), our results may indicate that NtBI-1 up-regulation could be a consequence of Ca^{2+} signalling instead of hormonal gene activation. This also suggests that NtBI-1 could be implicated in the control of cytosolic Ca^{2+}.

Poster 5

Isolation and Characterization of Nuclear Microsatellite Markers in Red Pine

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Red Pine (*Pinus resinosa*) is one of the most extensively planted trees in northeastern North America and is of great economic and ecological importance. Despite its wide geographic distribution, red pine is known to be one of the most genetically depauperate forest tree species in the region, possibly due to a series of population bottlenecks experienced during the Pleistocene glacial period. Further reduction in genetic variation may compromise the ability of this species to adapt to environmental change leading to increased risk of extinction. Therefore, it is crucial to protect as many genetically distinct populations as possible. Traditional genetic markers with low levels of polymorphism have been of limited use in identifying genetically distinct populations of red pine. We have isolated and characterized nuclear microsatellites, one of the highly polymorphic groups of genetic markers in order to assess the levels of genetic diversity in red pine. Out of the 25 microsatellites characterized, 14 showed single locus PCR amplification patterns, 4 of which were polymorphic. Sixty-eight individuals from various locations have been genotyped, and 41 (60%) individuals showed unique multilocus genotypes. A total of 25 alleles were detected, and the observed heterozygosity ranged from 0-0.32. The individuals from Newfoundland were genetically distinct from other populations sampled. These markers have revealed levels of polymorphism previously undetected by other marker systems and will be instrumental in assessing genetic diversity and structure, and for implementing conservation programs for red pine.

Poster 6

Cold-regulated plant lopocalins

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Lipocalins are a large and diverse group of small, mostly extracellular proteins implicated in many important functions such as modulation of cell growth and metabolism, binding of cell-surface receptors, membrane biogenesis and repair, induction of apoptosis and environmental stress response. Two novel lipocalin family members were identified from wheat and *Arabidopsis*. They were designated TIL (temperature-induced lipocalin) and CHL (chloroplastic lipocalin). Northern analyses demonstrated that *til* transcripts are upregulated during cold acclimation, heat-shock, high-light, and rose-bengal treatments while *chl* transcripts are specifically upregulated by cold acclimation. Structure analyses indicated the presence in both TIL and CHL of the three structurally conserved regions that characterize lipocalins. Sequence analyses revealed that TILs share homology with three evolutionarily related lipocalins: the mammalian apolipoprotein D, the bacterial lipocalin and the insect Lazarillo protein. The comparison of the putative tertiary structures of the human apolipoprotein D and the wheat TIL suggests that the two proteins differ in membrane attachment and ligand interaction. Transient expression of GFP::TIL fusion in onion epidermis cells showed that the protein accumulates at the plasma membrane. The putative functions of these novel plant lipocalin members during cold acclimation and oxidative stress are discussed.

Poster 7

Characterization of three pathogen-induced calmodulin-like proteins from *Arabidopsis* and tomato

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Calmodulin (CaM) is a conserved Ca^{2+}-binding eukaryotic protein that regulates many cellular proteins. We identified two *CaM-like* (*CML)* genes (*CML42, CML43*) from *Arabidopsis* and a tomato gene (*LeA31R*) that share homology with a pathogen-induced *CML* tobacco gene. Based on chromatography, gel-shift assays, and NMR, it was determined that all three proteins bind Ca^{2+}. RT-PCR and GUS-reporters show that *CML42* is expressed in all tissues while *CML43* expression was only detected in the root tip. *CML42, CML43*, and *LeA31R* were upregulated in response to a bacterial pathogen, *Pseudomonas syringae*. Salicylic acid induced a GUS reporter of *CML43*. Overexpression of either *CML42* or *CML43* resulted in an accelerated hypersensitive response to pathogen infection. Viral-induced gene silencing of *LeA31R* resulted in loss of pathogen resistance. Collectively, our data suggest these proteins likely play an important role during the plant immune response.

Poster 8

Nitrogen availability affests lignin biosynthesis in poplar wood

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Nitrogen fertilization induces dramatic changes in the growth and development of plants. In this project, we are investigating how nitrogen availability impacts wood formation in *Populus* at the cellular and molecular level. Biochemical analyses by pyrolysis molecular beam mass spectrometry revealed quantitative differences in the chemical composition of wood between plants treated with low vs. high levels of nitrogen. Specifically, lignin content is reduced in wood from high N-treated trees. These results were corroborated by stem girdling experiments, in which phloem transport is disrupted to cause an accumulation of glutamine below the girdle. Gene expression analyses indicated that transcript levels of genes associated with one-carbon metabolism and the lignin biosynthetic pathway decrease in response to elevated nitrogen concentrations. These results suggest that partitioning of carbon resources into cell wall components is altered in response to nitrogen availability.

Poster 9

***In silico* analysis of *Arabidopsis* prephenate dehydratases**

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To date, two pathways have been described for the synthesis of phenylalanine, the classical and the arogenate pathway. The classical pathway is widely used by microorganisms, however biochemical evidence in plants suggests that phenylalanine is synthesized via arogenate. A distinguishing enzymatic feature is the presence of a prephenate or an arogenate dehydratase, respectively. Based on the biochemical evidence we expected to identify coding sequences for arogenate dehydratases in the *Arabidopsis thaliana* genome. Instead, we have found six genes which share a high degree of sequence similarity with microbial prephenate dehydratases. These six prephenate dehydratase-like (*PDL*) genes encode proteins which have two highly conserved domains, one being very similar to the microbial catalytic prephenate dehydratase sequences, the other a ligand binding domain known as ACT domain. Although there is currently no sequence information on arogenate dehydratases available in public databases, the sequence analysis of PDLs has provided a starting point for molecular examination and serves as the basis for further experiments.

Poster 10
Characterization of potato cytosolic triosephosphate isomerase and its developmental regulation in leaves
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Cytosolic triosephosphate isomerase (cTPI) was cloned from *Solanum chacoense* and an isoform-specific antiserum was raised against recombinant cTPI. Immunoblot analysis of cTPI in potato tissues showed large variations in cTPI expression levels. In particular, there was a gradient of TPI activity and cTPI protein in leaves along the shoot axis with the highest levels found in the youngest tissues. Analysis of TPI isoforms profiles by anion exchange chromatography demonstrated that (i) all photosynthetic and non-photosynthetic tissues express 2 TPI isoforms and (ii) cTPI always represents the bulk of extractable TPI activity. Of all tissues surveyed, the ratio of cytosolic to plastidic TPI was the highest in expanding leaves. These results will be discussed in relation with the hypothesis that cTPI is important in growing leaf tissues, where glycolysis and respiration fulfill a key function in energy production and the production of C skeletons for biosynthetic purposes. (Supported by NSERC)

Poster 11
Characterization of a cytosolic nucleoside diphosphate kinase expressed in a cell-specific manner in potato roots.
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Nucleoside diphosphate kinase (NDPK) is widely distributed and may play a role in the homeostasis of cellular nucleoside triphosphate pools. A cytosolic NDPK was cloned from *S. chacoense* and expressed as an epitope-tagged recombinant protein in *E. coli*. The recombinant enzyme is strongly regulated by NDP/NTP ratios. A specific anti-NDPK polyclonal antibody was generated against recombinant NDPK. NDPK isoform analysis demonstrates that, in potato, cytosolic NDPK is the predominant isoform in all non photosynthetic tissues. Immunolocalization performed in roots demonstrated that NDPK was predominantly localized in the meristem region, the endoderm and in the apical epidermal layer. These and other data on NDPK expression in cell cultures suggest a specific role of cytosolic NDPK during cell division, synthesis of cell walls or production of mucilage. These hypotheses will be tested in transgenic roots with altered NDPK levels. (Supported by NSERC)

Poster 12

Gene expression profile comparison between freezing tolerant winter wheat and freezing sensitive spring wheat during cold acclimation

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Development of freezing tolerance in plants relies on biochemical and genetic changes involving multiple gene loci. To begin dissecting the wheat gene regulating system, we undertook a transcriptome analysis of cold stress. We used microarray analysis to compare gene expression profiles for 1200 genes over a 36 day cold acclimation time course between a cold tolerant winter wheat variety, Norstar and a cold sensitive spring wheat variety Glenlea. By this comparison we identified a subset of candidate genes whose regulation is correlated with the acquisition of freezing tolerance. In addition, by cluster analysis we grouped genes into patterns of expression which are distinctive in each of the two genotypes.

Poster 13

Small Upstream ORF controls Abscisic acid and Methyl Jasmonate responsiveness of Stress-Regulated Protein Kinases

Damiano Ferraro, Blerta Bashari, Frederic Piot, Patrick J. Gulick

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Protein kinases play a key role in signal transduction. The early salt-inducible Ser/Thr-like protein kinase *Esi47* from *Lophopyrum elongatum* has been shown to be induced by treatments of 250mM salt and of100µM ABA. A small open reading frame in the 5'-untranslated region was found to control ABA responsiveness in transient gene expression assays of barley aleurone. Three *Esi47* homologues were identified in *Arabidopsis thaliana*, F8A24.12, F12E4.50, and T7F6.28. All three homologues were found to have identical intro/exon structures and the F8A24.12 was found to have a conserved small upstream ORF (suORF) in the 5'UTR region of the gene. F8A24.12 is induced by ABA and by Methyl Jasmonate. Transgenic *A. thaliana* were produced which express the GUS reporter gene under the control of an intact F8A24.12 promoter + 5'UTR and with a mutated version in which the suORF was obliterated by a changing the ATG to a TTG. Tissue localization by histochemical staining revealed high levels of expression in abscission zones at the base of siliques, trichomes, and root primordia. Fluorometric analysis showed Methyl Jasmonate response is mediated by the suORF in the 5'UTR.

Poster 14

Biological iron availability and uptake by the cyanobacterium *Anabaena flos-aquae*

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Iron acquisition from various ferric chelates was studied using iron-limited cells of *Anabaena flos-aquae* (Lyng.) Brèb UTEX 1444, a cyanobacterial strain that produces high levels of siderophores under iron limitation. Various chelators of greatly varying stability constants (HEDTA, EDDHA, desferrioxamine mesylate, HBED) were assayed for the degree of iron acquisition by iron-limited cyanobacterial cells. Iron uptake rates (measured by GFAAS) varied inversely with chelator stability constant, and decreased with increasing chelator to iron ratio. No iron uptake was observed when Fe(III) was chelated with HBED, the strongest of the tested chelators. Iron-limited cells of *A. flos-aquae* were able to take up iron from purified humic acid, and also from 8-hydroxyquinoline ("oxine"), a compound frequently used to preconcentrate iron in aquatic samples. These results suggest that for cyanobacteria, even tightly bound iron is biologically available, including iron bound to humic acids. However, iron bound to chelators with a stability constant greater than 38 is likely to be unavailable.

Poster 15

Partial Purification, Kinetic Analysis and Amino Acid Sequence Information of a Flavonol 3-*O*-Methyltransferase from *Serratula tinctoria*

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Serratula tinctoria (Asteraceae) accumulates mainly 3,3´-dimethylquercetin and small amounts of 3-methylquercetin (3-MeQ) as an intermediate. The fact that 3-MeQ rarely accumulates in plants, and given its importance as an antiviral and antiinflammatory agent, prompted us to purify and characterize the enzyme involved in its methylation. The flavonol 3-*O*-methyltransferase (3-OMT) was partially purified by ammonium sulfate precipitation and successive chromatography on Superose-12, Mono-Q and adenosine-agarose columns, resulting in a 194-fold increase of its specific activity. The enzyme protein exhibited an expressed specificity for the methylation of position 3 of the flavonol, quercetin; although it also utilized kaempferol, myricetin and some monomethylated quercetin derivatives as substrates. The physico-chemical properties of this enzyme and its kinetic mechanism will be discussed. Furthermore, in-gel trypsin digestion of the purified protein yielded several peptides, two of which exhibited strong amino acid sequence homology to several Group II plant OMTs. The availability of peptide sequences will allow the design of specific nucleotide probes for future cloning of the gene encoding this *novel* enzyme for its use in metabolic engineering.

Poster 16

Data Mining for Na^+ transporters in *Triticum aestivum* Genome Quebec EST data set

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Salt tolerant plants are able to regulate internal Na^+ ion concentrations. Salinity challenges growth in plants by both ion toxicity and reduced soil water-potential, leading to dehydration stress. In the presence of NaCl, tolerant plants have limited Na^+ uptake and can sequester Na^+ in vacuoles and older tissue. SOS1 is a plasma-membrane Na^+/H^+ antiporter that exports Na^+ from the cytoplasm to extracellular spaces. Plants over-expressing SOS1 have been shown to accumulate less Na^+ and to be salt tolerant (Zhu *et al*, 2003). AtNHX is a vacuolar Na^+/H^+ antiporter that sequester Na^+ to vacuoles also leading to salt tolerance (Blumwald *et al*, 2003). There is evidence for both transcriptional and post-transcriptional regulation of Na^+ transporters that lead to salt tolerance in plants. New genomic sequence data mining identified 9 EST sequences corresponding to Na^+ transporters in our Genome Canada/ Genome Prairie/ Genome Quebec wheat EST database. Six clones are potentially full-length and a structural analysis of them will be presented.

Poster 17

Indirect activation tagging mutagenesis in *Arabidopsis*

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Activation-tag mutations are typically dominant and transferable to other species. However, dominant mutations with detrimental effects on plant growth or fertility are usually lost in one of the first generations. Here we describe a large-scale activation tag mutagenesis based on the transactivation system as designed by I. Moore, University of Oxford, Oxford, UK (Moore et al. 1998). In the background of a ubiquitously expressed chimeric LhG4- transcription activator (TA), a specific response element is randomly inserted into the Arabidopsis genome resulting in the over- and mis-expression of flanking genes. Transformed populations are screened for desired traits and crossed out of the TA background, if detrimental side effects occur. A variety of tissue specific expression variants of TA allow for the targeting of a trait to a particular organ or tissue. An optimized combination can then be transferred to crop plants.
We report phenotype spectra from the first 56,000 transformants, their stability and strategies to clone the activated genes. Mutant phenotypes include altered plant architecture, leaf and flower morphology.
Moore, I., Galweiler, L., Groskopf, D. Schell, J. and Palme, K. (1998) A transcription activation system for regulated gene expression in transgenic plants. PNAS, 376-381.

Poster 18

Biochemical and molecular characterization of biosynthesis of volatile sulfur compounds from plants, and their implications for plant-pest interactions

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Members of the family Brassicales emit a number of sulfur-containing volatiles, including methylthiocyanate (CH_3SCN), methylisothiocyanate, and methanethiol (CH_3SH). These and other species also accumulate sulfur-containing thioglucosides—glucosinolates—along with a separately compartmentalized enzyme, myrosinase. The latter comes in contact with glucosinolates upon tissue-disruption through herbivory or infection, and degrades them into thiocyanate ion (^-SCN), isothiocyanates (R–NCS), biosulfide ion (HS^-) and organic thiolates ($R–S^-$). We recently demonstrated that glucosinolate-containing species possess enzymes that use S-adenosyl methionine to methylate ^-SCN, HS^- and several $R–S^-$ to CH_3SCN, CH_3SH and $R–SCH_3$ (Plant Cell Environ 2000, 23:165). Five distinct isoforms of this novel plant enzyme—thiol methyltransferase—were purified to homogeneity from *Brassica oleracea* (Archiv Biochem Biophys 2000, 380:257). Their molecular masses ranged from 26 to 31 kDa. Each isoforms could methylate all of the above substrates but with significantly different kinetic properties. They also had distinct pH optima ranging from 5 to 8. Working from the partial internal amino acid sequence of one of these proteins, we isolated two full-length cabbage cDNAs encoding proteins of identical lengths (226 aa) but differing in 13 positions (Plant Mol Biol 2002, in press). When expressed in *E. coli,* the recombinant proteins displayed properties characteristic of the native thiol methyltransferases. Their transcript levels were high in the younger plant tissues, paralleling the known distribution of glucosinolates. Although these cDNAs, and a corresponding genomic clone, contained the typical conserved motifs for methyltransferases, the thiol methyltransferases are distinct from conventionally known N– or O–methyltransferases as they share neither much sequence similarity nor any substrates with the latter classes of enzymes. Volatile methylated sulfur compounds produced upon herbivory are believed to protect plants against insect attack and fungal infection. By providing the first insight into the metabolic origins of these volatiles, and by furnishing the molecular tools to manipulate the pathways involved, our work would facilitate further dissection of the relationship of this metabolic sector with insect- and pathogen-resistance. This could eventually open the door for metabolic engineering to enhance biotic stress tolerance.

Poster 19

APETALA2 is able to activate transcription in a yeast system

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APETALA2 (AP2) is a major regulator of developmental events in *Arabidopsis thaliana*. AP2 has a role in establishing the floral meristem, specifying floral organ identity, regulating floral homeotic gene expression, and it is required for proper seed and ovule development. Based on sequence comparison, AP2 is a member of a large family of transcription factors and computer analyses have revealed that AP2 contains several putative amino acid domains that are predicted to mediate protein binding, DNA recognition, nuclear localization, and transcriptional activation. However, none of these putative domains have been functionally tested or confirmed. We have now the first evidence that AP2 is able to activate transcription in a yeast system. This ability is consistent with AP2's predicted role as a transcription factor.

Poster 20

Chlorophyll Synthesis and Antioxidant Enzymes of Hot Pepper under Combined Excess Boron and Salinity

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This study was conducted to investigate chlorophyll synthesis and antioxidant enzymes of hot pepper (*Capsicum annuum* L.) under different levels of salinity and boron (SB). Boron treatments included 4, 8 and 16 mg kg^{-1}. Salinity levels were created adding NaCl at 3 and 6 dS m^{-1}. Plants treated with boron, 4 mg kg^{-1}, showed visible injuries such as chlorosis and necrosis. Further, increases in combined SB limited serious plant growth responses. B, Na and Cl accumulations in intracellular tissue were accelerated with increasing SB levels. As a result, increases in combined SB levels decreased of plant height, dry weight and chlorophyll content. In addition, a reduction in the efficiency of photosynthetic energy conversion (F_m/F_v) in photosystem □ occurred, compared to control plants. Aminolevulinic acid (ALA) activity increased 4-7 fold in all treatments after 3 days. Increasing SB levels increased ascorbate peroxidase (APX) and glutathione reductase (GR) activities by two-fold, compare to control plants. The maximum activity of APX and GR was achieved at combined 3 dS m^{-1} salinity and 8 mg kg^{-1} boron levels at 1, 3, 5 and 7 days. Increases in combined excess SB levels caused serious physiological disruption in chlorophyll synthesis and induced production of secondary toxic substances leading to chlorosis, and suppression of growth in hot pepper.

Poster 21

Phylogenomics of Plant *O*-Methyltransferases

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Plant *O*-methyltransferases (OMTs) play an important role in the biosynthesis of many secondary metabolites involved in structural support, chemical defence and cellular signalling, as well as possess antiviral/antimicrobial activities. The identification of numerous methyltransferase genes based on sequence homology studies assumes that sequence similarity is indicative of enzyme function. However, sequence homology alone may not necessarily reflect similarity in function, as it is not a reliable indicator of specific motifs involved in substrate preference and evolutionary relatedness. We are applying phylogenomic techniques to improve OMT substrate prediction and to study their evolution. This approach takes into account the concepts of paralogy and orthology of putative OMTs and infers their relatedness by reconstructing their evolutionary history through molecular phylogenetic analyses. We reconstructed phylogenetic trees based on sequences of 49 biochemically characterized OMTs and demonstrated the presence of five distinct groups of OMTs with respect to their substrate utilization in seed plants. The results obtained will allow the prediction of the functions of putative enzymes and the characterization of novel OMTs genes from various plant species.

Poster 22

***Arabidopsis thaliana sulfotransferase 2* (*AtST2*) and its substrate (12-hydroxy-jasmonate) are components of the photoperiod dependent flower induction pathway**

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Initiation of flowering occurs through the action of a network of genetic pathways. We have recently characterized a new member of the photoperiod dependent flower induction pathway from *Arabidobsis thaliana*. This gene (*AtST2*) encodes a sulfotransferase, whose function is to reduce the endogenous levels of a hydroxylated derivative of jasmonic acid, 12-hydroxyjasmonate (12-OHJA). Through the analysis of *AtST2* transgenic lines as well as various mutants, AtST2 was shown to act as a negative regulator in the photoperiod control of flowering time. The level of AtST2 protein in *A. thaliana* was found to be directly proportional to the timing of flowering: elevated or reduced levels of this protein lead to late or early flowering, respectively. We also demonstrated that the accumulation of the AtST2 protein is repressed by *CONSTANS*, a putative transcription factor that accelerates flowering in response to long photoperiod. These results suggest that *CONSTANS* promotes flowering through the direct or indirect repression of *AtST2* expression, allowing 12-OHJA accumulation to take place. We propose that 12-OHJA acts as a signal that promotes the transition from vegetative to reproductive growth when *A. thaliana* is exposed to an inductive photoperiod. In addition, we propose that *AtST2* plays a critical role at the interface between the photoperiod dependent flower promotion pathway and the flower inducing activity of 12-OHJA. Results of studies on the regulation of *AtST2* expression in other flowering mutants will also be presented.

Poster 23

Carbon status constrains light acclimation in cyanobacteria by altering the fate of photosynthetic electron transport.

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Cyanobacteria in shallow fresh water habitats are subject to rapid changes in light coincident with a variable availability of inorganic carbon (Ci). We tested how inorganic carbon (Ci) availability (high at c. 4 mM or low at < 0.1 mM) affects acclimatory changes in Synechococcus PCC7942 in response to increased light. Cells grown at 50 □mol photons m^{-2} s^{-1} showed marked differences in cellular pigment contents and PSI:PSII. Despite these differences in macromolecular organisation, the low and high Ci cells achieved similar electron transport and growth rates. Six hours after a shift to 500 □mol photons m^{-2} s^{-1}, high Ci cells nearly doubled their growth rate, while low Ci cells maintained a pre-light shift growth rate, although both high and low Ci cells increased their electron transport capacity to a similar degree. After the light shift the high Ci cells maintained high electron transport rates into a practically unlimited Ci sink by increasing the turnover rate of a smaller number of photosystems. In contrast, low Ci cells supported a similar rate of electron transport, but with more photosystems turning over slowly. Despite their similar rates of electron transport, in low Ci cells only about half of the photosynthetically generated electrons were invested in growth after the light shift, in contrast to c. 100% of electrons generated by the high Ci cells. We postulate that the large photosynthetic electron transport capacity of the low Ci cells is maintained to power carbon concentrating mechanisms, or to serve as a large potential reductant generator to fix transiently available Ci.

Poster 24

Jasmonates induce Nod factor production in *Bradyrhizobium japonicum*

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Jasmonates are signaling molecules involved in induced systemic resistance, wounding and stress responses of plants. They can also act as signaling molecules in rhizobia-legume symbioses by inducing nodulation (*nod*) genes. We have previously demonstrated that jasmonates can induce *nod* genes in *Bradyrhizobium japonicum* when measured by β-galactosidase activity. In order to test whether jasmonates can effectively induce the production and secretion of LCOs from rhizobia, we used two *B. japonicum* strains, 532C and USDA3, induced with JA, MeJA and genistein. As genistein is well characterized as an inducer of *nod* genes it was used a positive control. Our HPLC profile of LCOs isolated following treatment with jasmonates or genistein showed that both JA and MeJA effectively induced *nod* genes and caused production of LCOs from bacterial cultures. JA and MeJA are more efficacious inducers of LCO production than genistein. When added together genistein and JA or MeJA, resulted in greater LCO production than with either added alone. This enhanced LCO production demonstrates the complexity of inducer molecule perception and transcriptional regulatory mechanisms of *nod* genes in *B. japonicum*. The LCOs produced by jasmonates were equally effective at soybean root hair deformation. This is the first report demonstrating that jasmonates induce Nod factor production by *B. japonicum,* and thus this report establishes the role of jasmonates as a class of signaling molecules in rhizobia-legume symbioses.

Abbreviations: JA, jasmonic acid; MeJA, jasmonic acid, methyl ester; HPLC, high-performance liquid chromatography; LCO, lipo-chitooligosaccharide.

Poster 25

Identifying molecular players in cell shape development in higher plants

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The initiation, fixation and maintenance of polarized growth form the basis for the myriad shapes of plant cells. The molecular players involved in plant cell morphogenesis are only beginning to be identified. They include ROPs (Rho-like proteins of plants), Actin-related proteins (ARPs), BRICK and SPIKE genes as well as a plethora of proteins that regulate the activity of the actin and microtubule cytoskeletons. Information gathered through the phenotypic, molecular and cell biological analysis of these genes shall be presented in a comprehensive view of how a round, apparently non-polar plant cell can achieve differential growth to evolve into a precise, recognizable and functionally relevant form.
Pertinent References: Mathur et al. Plant Cell 5(7):1632-45,2003
Development 130(14):3137-46,2003; Current Biology13, 1991-1997, 2003.

Poster 26

Enhancer-trap Lines for Indirect Activation-tagging

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The plant vascular system is a cellular network that is responsible for the long-distance transport of substances throughout the plant. Economically important plant products, such as wood and fibres, are derived from vascular tissue, and consequently an understanding of the molecular cues underlying vascular differentiation will eventually allow for the wood and fibre properties in plants and trees to be manipulated. However, an essential prerequisite for this is the identification and functional characterization of genes that are preferentially expressed in specific stages of vascular development. Making use of an enhancer-trap strategy, we have generated a collection of 6000 transgenic *Arabidopsis thaliana* enhancer-trap lines that express a green fluorescent protein (GFP) reporter and a modified form of the yeast GAL4 transcriptional activator in different temporal and spatial patterns. Here, we show and describe the expression patterns that we have observed. We also show how these enhancer-trap lines can be used to identify genes that are expressed in vascular tissues, and a means by which the function of other genes can be studied through their mis-expression in the specific vascular cell types marked in each of these enhancer-trap lines.

Poster 27

The Whirlies : A New Family of Plant Transcription Factors Involved in Defence Responses

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Transcriptional activation of the potato defence gene *PR-10a* involves the single-stranded DNA binding protein PBF-2. This protein displays sequence specificity for the single-stranded form of the Elicitor Response Element (ERE) required for elicitor induced *PR-10a* expression. PBF-2 is a homotetramer of four 24 kD subunits (p24), homologs of which are present in species throughout the plant kingdom. We have solved the crystal structure of PBF-2 to 2.3 Å resolution, revealing that the p24 protomers assemble to form a tetramer with cyclic C4 symmetry. Assembly of the protomers in PBF-2 produces a quaternary structure with a whirligig-like appearance, which inspired the name 'Whirly' for this family of plant proteins. The Whirlies also show structural similarities to other ssDNA binding proteins, which suggest an interesting example of structural convergence. Three genes with homology to p24 are present in the genome of *Arabidopsis thaliana*. Functional analysis of TILLING mutations in the *Arabidopsis* gene most similar to potato p24 indicates that the encoded protein plays a significant role in defence against the oomycete Pernospora parasitica.

Poster 28

Cytomechanical properties of *Solanum chacoense* pollen tubes are strongly influenced by the distribution of pectins in the cell wall

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Pollen tubes are tip growing cells that exert penetration and resistance forces while growing through the receptive stigma *in vivo* (to perform fertilization) or through an artificial semi-solid medium *in vitro*. These cells are surrounded by a cell wall and therefore require the continuous synthesis and deposition of an extracellular layer that is rigid enough to withstand substantial internal turgor pressure, yet flexible enough to permit the cell to grow. Pectin, a major component of the cell wall, is supposed to exhibit a gradient of rigidity along the longitudinal pollen tube axis due to the activity of the enzyme pectin methyl esterase (PME) in the maturing distal cell wall. We altered this gradient using the enzymes pectinase and PME, which affected the apical expansion rate to different degrees depending on the stiffness of the surrounding growth matrix (i.e. the concentration of agarose). This indicates that pectins play an important role in the control of pollen tube penetration growth. Furthermore, micro-indentation experiments revealed that the local cytomechanical properties of the pollen tube were affected by the enzyme treatment thus indicating an important role for the configuration of pectins in the determination of cellular rigidity and visco-elasticity upon lateral deformation.

Poster 29

Stage-specific markers define early steps of procambium development in Arabidopsis leaves and correlate termination of vein formation with mesophyll differentiation

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During leaf development, ground meristem cells along continuous lines undergo coordinated oriented cell divisions and differentiate to form procambial cells, the precursors of all vascular cells. The molecular genetic dissection of early procambial development suffers from the lack of easily identifiable markers, especially of cell states preceding procambium formation. We have identified and characterized reporter gene expression markers that reflect distinct preprocambial stages as well as one marker whose expression seems to be perfectly congruent with the appearance of procambial cells. All markers are invariably expressed in continuous domains connected to pre-existing vasculature and their expression profiles reveal a common spatial and temporal pattern of early vein formation. During development of all veins, cells acquire early preprocambial identity progressively, while subsequent procambium formation occurs simultaneously along entire veins. The progressive extension of vascular strands at the preprocambial stage suggests that veins are initiated as freely-ending preprocambial domains and that network formation occurs through subsequent fusion of these domains. Consistent with this interpretation, we demonstrate that veins are generally not programmed to become freely-ending or interconnected network elements. Instead, we found that the progressive extension of preprocambial domains can be interrupted experimentally and that this leads to less complex vein patterns consisting of fewer vein orders, in which even lower-order veins become freely-ending. Mesophyll differentiation turned out to be strictly correlated with the termination of preprocambial domain extension. Our findings suggest that Arabidopsis vein pattern is not inherently determinate, but arises through reiterative initiation of new preprocambial branches until this process becomes terminated by the differentiation of mesophyll.

Poster 30

Analysis of the *CAD* gene family in *Arabidopsis* and characterization of each member corresponding mutants give a genome-wide view of the last step of monolignol biosynthesis

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Cinnamyl alcohol dehydrogenase (CAD), the last enzyme of the monolignol-specific pathway, is responsible for the reduction of hydroxycinnamaldehydes into hydroxycinnamyl alcohols which are the main units of the lignin polymer. Nine CAD encoding genes are present in the genome of *Arabidopsis thaliana*. These genes belong to three classes. One class (2 members) is related to *CAD* genes already identified in plants and used in an antisense strategy in tobacco (Halpin et al., 1994) and in poplar (Baucher et al., 1996). Another class (6 members) is related to the sinapyl alcohol dehydrogenase (*SAD*) gene identified in poplar by Li et al. (2001) and in alfalfa by Brill et al. (1999). The last class has only one representative in *Arabidopsis*. Using different methods, we have characterized the expression pattern of members of this gene family in *Arabidopsis* and observed that all the genes are expressed in the floral stem, an organ with substantial lignin levels varying between 10 and 18 % depending on culture conditions.
Knockout mutants for each expressed gene were identified and analyzed. Two mutants showed different phenotypes in regard to enzymatic activity and lignin composition (Sibout et al. 2003). The corresponding double mutant showing a drastically decreased of lignin content and sinapyl units has been produced. Quantitative RT-PCR analysis shows that expression of different genes from this family and other families are deregulated in this line. The relative role of CAD and SAD genes in plant kingdom will be discussed.

Baucher et al. (1996) Plant Physiol. 112: 1479-1490.
Brill et al. (1999) Plant Mol Biol 41: 279-291.
Halpin et al. (1994) Plant J. 6: 339-350.
Li et al. (2001) Plant Cell 13: 1567-1586.
Sibout et al. (2003) Plant Physiol. 132: 848-860.

Poster 31

Genetic complexity of cellulose synthase A gene function in Arabidopsis embryogenesis

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The product of the cellulose synthase A (*CESA*) gene family are though to function as isoforms of the cellulose synthase catalytic subunit, but for most *CESA* genes, the exact role in plant growth is still unknown. Assessing the function of individual CESA genes will require the identification of the null-mutant phenotypes and of the gene expression profiles for each gene. Here, we report that only four of 10 *CESA* genes, *CESA1*, *CESA2*, *CESA3* and *CESA* 9 are significantly expressed in the Arabidopsis embryo. We further identified two new mutations in the *RADIALLY SWOLLEN 1* (*RSW1/CESA1*) gene of Arabidopsis that obstruct organized growth in both shoot and root and interfere with cell division and cell expansion already in embryogenesis. One mutation is expected to completely abolish the enzymatic activity of *RSW1*(*CESA1*) because it eliminated one of three conserved Asp residues, which are considered essential for B-glycosyltransferase activity. In this presumed null mutant, primary cell walls are still formed, but are thin, highly undulated, and frequently interrupted. From the heart-stage onward, cell elongation in the embryo axis is severely impaired, and cell width is disproportionally increased. In the embryo, *CESA1*, *CESA2*, *CESA3* and *CESA9* are expressed in largely overlapping domains and may act cooperatively in higher order complexes. The embryonic phenotype of the presumed *rsw1* null mutant indicates that the RSW1(CESA1) product has a critical, nonredundant function, but is nevertheless not strictly required for primary cell wall formation.

Poster 32

Production of Nod Factor metabolites by *Bradyrhizobium japonicum* with the addition of Casein Hydrolizate on Yeast Extract medium

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Increase in casein hydrolizate (C) or yeast extract (Y) improve bacterial growth and mixture of them support better growth, but high concentrations of C and Y (3.5 g L^{-1}) cause cell deformation of *Bradyrhizobium japonicum* and decrease nodulation. Our objective was to examine the addition of C in YEM medium on bacterial growth and the production of nod factor metabolites (lipo-chito oligosaccharides or LCOs) by B. japonicum. A factorial experiment with combinations of Y (0.4 or 0.8 g L^{-1}) and C (0, 0.8, 1.6 or 3.2 g L^{-1}) was established. Result showed that bacterial growth increased with increasing levels of C addition; however it was inhibited at the highest C addition level. From four considered-LCOs (NodBj-V(C16:0 MeFuc), NodBj-V(C18:1 MeFuc), NodBj-IV(Cb,C18:1 MeFuc) and NodBj-V(Ac,C16:0 MeFuc), in volume basis, differential production responses were observed. The production of three LCOs decreased at the highest level of C addition, NodBj-V(Ac,C16:0 MeFuc) production still increased. At low level of Y, total LCO production per liter increased with the increase of C addition, but at high level of Y, it decreased at the highest level of C addition. In cell basis production, however, higher concentration of Y and addition of C decreased total LCO production, and the total production leveled off with C addition of 1.6 g L^{-1} or more. Similar trend occurred with the individual LCO production, except the production of NodBj-V(Ac,C16:0 MeFuc), which recovered at the highest concentration.

Poster 33

Effect of Nitrogen Sources on Bacterial Growth, Lipo-chitooligosaccharide Production, Nodulation and Photosynthetic Activity in Soybean

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Culture with medium for high bacterial growth is a common practice to produce Rhizobial inoculant for biological nitrogen fertilization in legume crops. However, there is a concern that it might reduce nodulation efficiency due to the existence of quorum sensing in the biosynthesis of nod factors (lipo-chitooligosaccharides or LCOs). Experiments were conducted to evaluate two levels (0.044% and 1.56%) of nitrogen from yeast extract (Y), casein hydrolizate (C), or inorganic nitrogen Ammonium nitrate (N) in culture medium on *Bradyrhizobium japonicum* growth, LCO production, nodulation and photosynthetic activity of soybean plants. Y medium with 0.044% N (Vincent 1970) was set as a standard. Result showed that N reduce both bacterial growth and volume-basis LCO production; in contrast, C or higher level of Y or mixture of Y and C significantly improves cell growth, with the highest cell growth (more than 14 times of the standard) obtained in high-C-low-Y in the mixture. This mixture also produced highest total LCO in volume basis. However, since its high cell density, cell-basis LCO production in this mixture treatment was also only 1/14 of those in the standard. Experiment in the green house with observation at 14, 21 and 28 days did not reveal the difference in number of nodule, average and total nodule weight, amount of fixed N, and SPAD reading and photosynthetic rate in soybean plants.

Poster 34

Rhizobial Nod factor promotes calcium uptake into soybean

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Legume root hairs respond to inoculation with rhizobia or application of lipo-chitooligosaccharides (LCOs or Nod factor) through transient increases in cytosolic calcium concentration. We examined whether LCOs also enhance calcium uptake into soybean plants. Soybean seedlings were placed in liquid Murashige and Skoog basal medium containing LCO, rhizobial innoculum, or various other compounds. Trifoliolate leaf samples were harvested 24 hours following radiotracer addition, and the $^{45}Ca^{2+}$ radioactivities were measured. Incubation with NodBj-V(C19:1 MeFuc) prior to testing increased the $^{45}Ca^{2+}$ uptake into seedling leaves in a concentration manner. Similarly, incubation with *Bradyrhizobium japonicum* strains 532C and USDA3 also increase $^{45}Ca^{2+}$ uptake into trifoliolate leaves. No increased $^{45}Ca^{2+}$ uptake occurred into seedling leaves following incubation with strain Bj-168, a *nodC*- mutant incompetent to produce LCO or either *Rhizobium leguminosarum* and *Sinorhizobium meliloti*, two *Rhizobia* that do not normally nodulate soybean. The tetramer or pentamer of chitosan and lumichrome also did not affect $^{45}Ca^{2+}$ uptake. This work suggests that rhizobial symbiosis, in addition to its known role in provision of nitrogen, also improves calcium uptake into soybean plants.

Poster 35

Expression of heat shock-inducible protein HSP70 requires the activity of heat-activated MAP kinase (HAMK)

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A heat-activated, ERK-related MAP Kinase (HAMK) has been identified in BY2 cells of tobacco. The activation of HAMK at 37°C was transient and detected within 2 minutes and reached a maximum level within 5 minutes. Ca^{2+} chelators and channel blockers, and the known inhibitors of MEK, a MAP kinase kinase, prevented the heat-activation of HAMK. This suggests that HAMK activation is part of a heat-triggered MAP kinase cascade that requires Ca^{2+} influx. The heat-shock protein HSP70 accumulated at 37°C, but not when HAMK activation was prevented with the inhibitors of MEK or with Ca^{2+} chelators or channel blockers. As previously shown for heat-activation of HAMK (Sangwan *et al.*, 2002. Plant Journal 31: 629-638), heat-induced accumulation of HSP70 requires membrane fluidization and reorganization of cytoskeleton. We concluded that heat-triggered HAMK cascade might play an essential role in the launching of heat-shock response and *hsp* gene expression in tobacco cells.

Poster 36

Heat-responsive homologues of mammalian HSP70 and MAPKAPK2 are present in tobacco cells and are regulated by an ERK-related MAP kinase

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Mammalian heat shock protein HSP27 is associated with cytoskeleton and is phosphorylated by MAPK-activated protein kinase2 (MAPKAPK2). HSP27 and MAPKAPK2 have not been demonstrated in plants. Here we show that i) BY2 cells of tobacco contain heat-responsive homologues of HSP27 and MAPKAPK2; ii) the phosphorylation of tobacco HSP27 is prevented by rottlerin, a potent inhibitor of mammalian MAPKAPK2; and iii) the activation of tobacco MAPKAPK2 during heat-shock requires the activity of a heat-activated MAP kinase (HAMK). Possible roles of HSP27 and MAPKAPK2 in high temperature signaling and heat shock response in plants are discussed.

Poster 37

Isolation of Al-tolerance-associated genes by a new improved suppression subtractive hybridization (SSH) procedure

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The search for rare differentially expressed genes is a very difficult task. Several molecular biology techniques (Differential Display (DD), Serial Analysis of Gene Expression (SAGE), Suppression Subtractive Hybridization (SSH)) are currently used with variable success. We suggest here an improved Suppression Subtractive Hybridization procedure which reduces the number of false positives and increases the presence of rare genes. This new SSH was tested in parallel with the standard technique to prove its effectiveness. Each protocol was achieved between two populations of mRNA with similar expression patterns: apical roots mRNA of Aluminum-sensitive wheat exposed to 5 µM Al and apical roots mRNA of Aluminum-tolerant wheat exposed to 50 µM Al. At these Al concentrations, both varieties are subjected to the same level of stress: a similar inhibition of root growth and the same expression profiles of several stress-related genes were observed. The improved SSH showed nested-PCR products containing a smear with minor bands rather than major bands corresponding to common genes as found in the standard procedure. In the nested-PCR products of the two SSH conditions, two common stress-related genes were subtracted as expected. However, a new Al-tolerance-associated gene was abundant in the nested-PCR products of the improved SSH contrary to the standard SSH. The libraries obtained are being compared and analyzed by differential screening using 32P-labelled full length (CAP-selected) cDNA libraries from both mRNA populations. Preliminary results confirm the great reduction in common abundant genes in the improved SSH library. This work allowed us to isolate a new Al-tolerance-associated gene and a new stress-related gene. Further analyses by RT-PCR with mRNA from different tolerant and sensitive wheat near-isogenic lines and their respective parents confirmed that the Al-tolerance-associated gene was highly expressed only in the tolerant lines.

Poster 38

Characterization of *amp1:* a genetic suppressor of the *mp* mutant and its role in Arabidopsis development

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The MONOPTEROS (MP) transcription factor, a member of the Auxin Response Factor (ARF) family, plays a central role in Arabidopsis development (Hardtke and Berleth, 1998). Genetic analysis reveals that the loss-of-function *amp1* mutant suppresses the seedling lethal phenotype of the *mp* mutant. Remarkably, the *mp;amp1* double mutant results in a viable and fertile plant whereas the *mp* single mutant lacks all basal structures and is completely sterile. The mutual suppression of the two loss-of-function mutations suggests that their wild type protein products have opposing roles during plant development. AMP1 (ALTERED MERISTEM PROGRAM1) encodes a putative glutamate carboxypeptidase protein whose function is not understood (Helliwell et al, 2000). *AMP1-GUS* reporter gene shows expression in the vasculature of the cotyledons and leaves, shoot meristem and hypocotyl-root junction and expression of this reporter gene is enhanced upon application of sugar. Here we show that *amp1* and *mp* have opposing phenotypes in the presence of high concentrations of glucose; *amp1* acts glucose-insensitive and *mp* acts glucose-oversensitive. The glucose-insensitive phenotype of *amp1* can be rescued by the addition of ABA. These mutants provide important new insights into a novel link between auxin signaling and sugar responses. Further studies are necessary to determine how the each of the MP and AMP1 functions influence each other in Arabidopsis development.

Poster 39

Cold regulated genes from wheat that are differentially regulated between cold tolerant and cold sensitive genotypes

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More than 300 cold-regulated genes were identified from wheat cDNA microarray data. Among them, 192 genes are differentially regulated by cold between winter and spring wheat. A shortlist of candidate genes which are potentially regulatory genes were chosen for further characterization. A total of 20 genes were selected, 4 of them are potentially full length clones. Among the candidate genes, F29 encodes a receptor like kinase, which shows 78% similarity with wheat Lrk10 receptor-like kinase, J822 encodes a translation elongation factor -1-alpha-related GTP-binding protein. The microarray data showed that F29 transcript was more strongly induced in winter wheat than in spring wheat after 36 days of cold acclimation. J822 had a higher expression level in winter wheat than in spring wheat after 1 day cold acclimation. F29 and J822 were cloned from a cDNA library constructed from cold treated wheat tissues by a PCR strategy.

Poster 40

Translocation of ^{14}C- assimilates in tomato plants as affected by severe water stress

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The effects of polyethylene glycol (PEG)-induced water deficit were studied using tomato plants. The $^{14}CO_2$ incorporation of source leaves and subsequent ^{14}C distribution in the plants were investigated. Plants were forced to allocate scare resources to facilitate all life activity. This process allowed the plant to maintain a carbon-nutrient balance near the optimum for plant function. Our data showed that severe water stress enhanced the transport of ^{14}C-assimilates from the pulse leaf to allocate more resources in building root tissue, increasing root surface area and nutrient absorption.