Plant Biology Canada 2002

Annual Meeting of the Canadian Society of Plant Physiologists

June 8 to 12, 2002
University of Calgary, Calgary, Alberta

Conference Co-Chairs

Peter Facchini, *University of Calgary*
Doug Muench, *University of Calgary*

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Greg Moorhead, *University of Calgary*
David Reid, *University of Calgary*
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Conference Management Services

Margaret-Anne Stroh
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Conference at a Glance

Sat., June 8th

17:00 - 22:00
Registration and Welcoming Mixer

Sun., June 9th

8:30
Registration

8:45 - 9:00
Welcoming Addresses

9:00 - 12:00
Plenary Session I
Recent advances in cell and developmental biology

12:00 - 13:30
Executive Committee Meeting

13:30 - 16:30
Contributed Papers

18:30 - 20:00
Gold Medal Ceremony and Address

20:00 - 23:00
Poster Session

Mon., June 10th

8:30
Registration

9:00 - 12:00
Plenary Session II
Interactions between plants and their environment

12:00 - 13:30
General Business Meeting

13:30 - 16:30
Contributed Papers

17:30 - 23:00
Barbecue, Rodeo and Dance

Tues., June 11th

9:00 - 12:00
Plenary Session III
Metabolic control and engineering

12:00 - 13:30
Outgoing Executive Committee Meeting

13:30 - 16:30
Contributed Papers

16:50 - 17:30
Award Presentations and Closing Remarks

Wed., June 12th

9:00 - 17:00
Social Outings
Tyrrell Museum
Plateau Mountain
Banff/Lake Louise

Social and Administrative Program

Saturday, June 8th

Reception and Welcoming Mixer	**Location: University of Calgary Dining Centre** **17:00 - 22:00**

Sunday, June 9th

CSPP Executive Committee Meeting	**Location: ST 140** **12:00 - 13:30**

Monday, June 10th

CSPP General Business Meeting	**Location: ST 140** **12:00 - 13:30**

Barbeque, Rodeo and Dance	**Location: Balsac, Alberta** **17:30 - 23:00**

Tuesday, June 11th

CSPP Outgoing Executive Committee Meeting	**Location: ST 140** **12:00 - 13:30**

Wednesday, June 12th

Excursions	**Departure Times and Locations To Be Announced** **Banff/Lake Louise** **Plateau Mountion** **Tyrell Museum**

Scientific Program

Sunday, June 9th

Opening Remarks	**Location: ST 140** **08:45 – 09:00**

Welcoming addresses

P. Facchini and D. Muench - Co-Chairs, Plant Biology Canada 2002	08:45 - 08:50
M. Boorman - Dean, Faculty of Science, University of Calgary	08:50 - 08:55
N. Huner - President, Canadian Society of Plant Physiologists	08:55 - 09:00

Recent Advances in Cell & Development Biology	**Location: ST 140** **09:00 – 12:00**
A1 Plenary	**Chair – Robert Mullen**

A1-1	Axis formation at the cellular, tissue and organismal level C.S. Hardtke, J. Mattson, S. Singh, G. Stamatiou, N. Krogan, W. Ckurshumova, and T. Berleth	09:00 - 09:40
A1-2	The convergence of *knotted1* homeobox genes and hormone signaling in plant morphogenesis S. Hake, H. Smith, A. Hay, Y. Sato, N. Ori, and M. Tsiantis	09:40 - 10:20
	Coffee Break	10:20 - 10:40
A1-3	Signalling through receptor kinases in plants D. R. Goring, S.L. Stone, N.F. Silva, Y.Z. Haffani, and R.T. Mullen	10:40 - 11:20
A1-4	A genomics approach to cereal aleurone cell function A. Fath, P.C. Bethke, Y.S. Hwang, R. Wei, T. Zhu, and R.L. Jones	11:20 - 12:00

Plant Stress Responses I	**Location: ST 135** **13:30 – 16:30**
A2 Concurrent	**Chair – David Reid**

A2-1	Investigating the role of citrate synthase in detoxification of aluminum in yeast and *Brassica napus* V.M. Anoop, U. Basu, and G.Taylor	13:30 - 13:50
A2-2	Metallothionein-like (Mt-like) gene of *Typha latifolia* Y.W. Zhang, N.F.Y. Tam, and Y.S. Wong	13:50 - 14:10
A2-3	Molecular aspects of plant adaptation to ionizing radiation O. Kovalchuk	14:10 - 14:30
A2-4	Ion uptake and membrane permeability in *Pinus banksiana* treated with sodium chloride and sodium sulfate J.A. Franklin, and J.J. Zwiazek	14:30 - 14:50
	Coffee Break	14:50 - 15:10

Sunday, June 9th

Plant Stress Responses I (continued)	**Location: ST 135**
	13:30 – 16:30
A2 Concurrent	**Chair – David Reid**

A2-5	Remobilization of cadmium in maturing shoots of near isogenic lines of durum wheat that differ in grain cadmium accumulation N.S. Harris, and G.J.Taylor	15:10 - 15:30
A2-6	Cadmium association with the cell wall of *Chara corallina*: evidence for multiple binding sites within a single cell wall M.J. Bryman, and G.J. Taylor	15:30 - 15:50
A2-7	Identification and expression of low temperature responsive genes in winter wheat M. Frick, A. Laroche, R.Huel, B. Puchalski, T. Despins, S. Huang, and D. Gaudet	15:50 - 16:10
A2-8	HSP mRNA profiles in *Brassica napus* pollen, pistils and leaf disks L.W. Young, R. Wilen, and P.C. Bonham-Smith	16:10 - 16:30

Plant Cell and Developmental Biology	**Location: ST 145**
	13:30 – 16:30
A3 Concurrent	**Chair – David Bird**

A3-1	Plant cdk inhibitors: interactions with cell cycle regulators and functional comparisons in transgenic Arabidopsis plants Y. Zhou, L. C. Fowke, and H. Wang	13:30 - 13:50
A3-2	Characterization of the RNA and microtubule binding activities of the rice multifunctional protein (MFP) and its possible role in protein targeting to peroxisomes S.D.X. Chuong, M.A. Murphy, R.T. Mullen, and D.G. Muench	13:50 - 14:10
A3-3	The plastid translocon component toc36 exhibits an affinity for the bacterial protein translocation process B. Gordon, and K. Ko	14:10 - 14:30
A3-4	Subcellular localization of a RNA virus replicase protein in tobacco BY-2 cells: transient expression of the 33kDa protein from tomato barley stunt virus causes the disappearance of peroxisomes A.W. McCartney, and R.T. Mullen	14:30 - 14:50
	Coffee Break	14:50 - 15:10
A3-5	Expression and activity of β-mannosidase in tomato seeds during and following germination B. Mo, and J.D. Bewley	15:10 - 15:30
A3-6	A role for diacylglycerol acyltransferase during leaf senescence M.T. Kaup, C.D. Froese, and J.E. Thompson	15:30 - 15:50

Sunday, June 9th

Plant Cell and Developmental Biology (continued) — **Location: ST 145**
13:30 – 16:30
A3 Concurrent — **Chair – David Bird**

A3-7	What do nitrogen-fixing nodules and pollen have in common? L. Amyot, and K.Szczyglowski	15:50 - 16:10
A3-8	Alteration of growth response in transgenic aspen overexpressing an aspen expansin gene M. Gray-Mitsumune, E.J. Mellerowicz, H. Abe, K. Blomqvist, J. Schrader, S.J. McQueen-Mason, T.T. Teeri, and B. Sundberg	16:10 - 16:30

Plant Biotechnology — **Location: ST 140**
13:30 – 16:30
A4 Concurrent — **Chair – Randy Weselake**

A4-1	A new regeneration plant protocol for expression of genes in monocots A. Laroche, F. Eudes, S. Acharya, M.M. Frick, R. Huel, C.L. Nykiforuk, R.L. Conner, A. Kuzyk, B. Selinger, and K.J. Cheng	13:30 - 13:50
A4-2	Metabolic engineering of hydroxycinnamic acid amide biosynthesis in tobacco J. Hagel, Y. Zhan, and P.J. Facchini	13:50 - 14:10
A4-3	Manipulation of genes involved in carbon partitioning in transgenic potato R.S. McKibbin, S. Laurie, J.P. Freeman, M. Burrell, and N.G. Halford	14:10 - 14:30
A4-4	Metabolic engineering of benzophenanthridine alkaloid biosynthesis in California poppy S.U. Park, and P.J. Facchini	14:30 -14:50
	Coffee Break	14:50 - 15:10
A4-5	A small antimicrobial peptide protects transgenic potatoes against late blight and pink rot M. Osusky, L. Osuska, R.E. Hancock, W.W. Kay, and S. Misra	15:10 - 15:30
A4-6	Ectopic expression of oleosin in *Arabidopsis thaliana* shows the protein trafficking along the endoplasmic recticulum A.J. Reid, I.R. Moore, and M.M. Moloney	15:30 - 15:50
A4-7	Subcellular targeting of human interleukin-10 in plants R. Menassa, W. Kennette, V. Nguyen, and J. Brandle	15:50 - 16:10
A4-8	Photosynthetic control and wheat engineering O.I. Kershanskaya	16:10 - 16:30

Sunday, June 9th

Gold Medal Award and Address	**Location: ST 140** **18:30 – 20:00**

Metabolism by mutation — 19:00 - 20:00
J. King

Poster Session	**Location: Science Theatre Foyer** **20:00 - 23:00**

Monday, June 10th

Interactions Between Plants and Their Environment	**Location: ST 140**
	09:00 – 12:00
B1 Plenary	**Chair – Joerg Bohlmann**

B1-1	Plant ecology in the genomics era: lessons from native *Nicotiana* I.T. Baldwin	09:00 - 09:40
B1-2	Stress tolerance in Arabidopsis: mutants, genes and signalling pathways J.-K. Zhu	09:40 - 10:20
	Coffee Break	10:20 - 10:40
B1-3	The regulatory role of low temperature in winter cereals F. Sarhan	10:40 - 11:20
B1-4	SA- and NO-mediated signal transduction in plant disease resistance D.F. Klessig, D. Kumar, P. Kachroo, D. Slaymaker, R. Navarre, D. Clark, M. Chandok, K. Yoshioka	11:20 - 12:00

Plant Stress Responses II	**Location: ST 135**
	13:30 – 16:30
B2 Concurrent	**Chair – Tanya Hooker**

B2-1	Influence of abiotic stress on the plant genome O. Kovalchuk, and I. Kovalchuk	13:30 - 13:50
B2-2	Influence of pathogens on plant genome stability I. Kovalchuk	13:50 - 14:10
B2-3	Hypoxia and salinity stresses on jack pine (*Pinus banksiana* Lamb.) seedlings K.G. Apostol, and J.J. Zwiazek	14:10 - 14:30
B2-4	Reactive oxygen species mediated signaling in the acclimation process to stress: effects of copper and ultraviolet radiation on the aquatic plant *Lemna gibba* T.S.Babu, T.A. Akhtar, M.A. Lampi, S.Tripuranthakam, D.G. Dixon, and B.M.Greenberg	14:30 - 14:50
	Coffee Break	14:50 - 15:10
B2-5	Genomics and proteomics decoding of plant defense pathways T. Xing, C. Rampitsch, J. Stebbing, W. Mauthe, D. He, B. McCallum, K. Malik, B.L. Miki, and M. Jordan	15:10 - 15:30
B2-6	Insect footsteps on leaves initiate plant defence responses within twenty seconds D.E. Hall, K.B. MacGregor, and A.B. Bown	15:30 - 15:50
B2-7	Role of insect salivary glucose oxidase in suppression of plant defense responses J.C. Bede, and K.L. Korth	15:50 - 16:10
B2-8	Regulation of an oxygenase with homology to animal cyclo-oxygenases in salt and pathogen-challenged roots of tomato A.L. Plant, A. Tirajoh, T. Aung, and S. Diguistini	16:10 - 16:30

Monday, June 10th

Plant Biochemistry and Metabolism I — **Location: ST 140**
13:30 – 16:30
B3 Concurrent — **Chair – Alexandra Reid**

B3-1	Purification and characterization of two purple acid phosphatase isoforms from media of phosphate starved tomato suspension cells G.G. Bozzo, K.G. Raghothama, and W.C. Plaxton	13:30 - 13:50
B3-2	Purification to homogeneity and characterization of norcoclaurine synthase, the first committed enzyme in benzylisoquinoline alkaloid biosynthesis in plants N. Samanani, and P.J. Facchini	13:50 - 14:10
B3-3	Functional characterization of a novel ser/thr protein kinase, phosphate starvation-responsive gene group 1 (*Psr1*), of *Arabidopsis thaliana* M.F. Hétu, and D.D. Lefebvre	14:10 - 14:30
B3-4	Purification and characterization of enolase from the cyanobacterium *Synechococcus* pcc 6301 B.B. Weese, and W.C. Plaxton	14:30 - 14:50
	Coffee Break	14:50 - 15:10
B3-5	Characterization of the C-terminal extension of carboxysomal carbonic anhydrase from *Synechocystis* sp. PCC6803 A.K.C. So, S.S.W. Cot, and G.S. Espie	15:10 - 15:30
B3-6	Properties of the N-terminal region of recombinant diacylglycerol acyltransferase-I of oilseed rape M. Madhavji, S. Szarka, N. Patterson, W. Wiehler, C. Nykiforuk, F. Foroud, S. Mosimann, P. Boora, M. Moloney, A. Laroche, and R. Weselake	15:30 - 15:50
B3-7	The P23 surprise! P. Krishna, and Z. Zhang	15:50 - 16:10
B3-8	Purification and characterization of novel phosphoenolpyruvate carboxylase isoforms from endosperm of developing castor oil seeds J. D. Blonde, and W. C. Plaxton	16:10 - 16:30

Plant Physiology I — **Location: ST 145**
13:30 – 16:30
B4 Concurrent — **Chair – Kevin Rozwadowski**

B4-1	Novel in vitro induction of hyperphysiological levels of coniferyl alcohol in pine cambium V.J. Steeves, and R.A. Savidge	13:30 - 13:50
B4-2	Morphological and physiological responses of fiber hemp (*Cannabis sativa* l.) upon exposure to heavy metal stress N. Ahmed, and D. Hayden	13:50 - 14:10

Monday, June 10th

Plant Physiology I (continued)	**Location: ST 145**
	13:30 – 16:30
B4 Concurrent	**Chair – Kevin Rozwadowski**

B4-3	Ecophysiological adaptations of winter-hardened black spruce (*Picea mariana*) and tamarack (*Larix laricina*) seedlings to flooding A. Islam, and S.E. Macdonald	14:10 - 14:30
B4-4	Role of an *Arabidopsis thaliana* sulfotransferase (AtSt2) and its substrate (12-hydroxyjasmonate) in floral induction A. Tkatcheva, C. Wasternack, and L. Varin	14:30 - 14:50
	Coffee Break	14:50 - 15:10
B4-5	Putatively inactive *cis*-isomers of cytokinins predominate early in seed development among three genera of legumes P.E. Quesnelle, X.S. Miao, and R.J.N. Emery	15:10 - 15:30
B4-6	Interactive role of ethylene and polyamines on shoot morphogenesis in vitro E.C. Pua, W. Cheng, and S.H. Lim	15:30 - 15:50
B4-7	Ethylene production, as controlled by the expression of different ACC synthse genes, determines the time of year when japanese pear ripens A. Itai, K. Tanabe, and F. Tamura	15:50 - 16:10
B4-8	Gene expression profiles for Arabidopsis MAPKK/MAPK genes during plant growth and development S. Sritubtim, and B. Ellis	16:10 - 16:30

Tuesday, June 11th

Metabolic Control and Engineering	**Location: ST 140**
	09:00 – 12:00
C1 Plenary	**Chair – Gregory Moorhead**

C1-1	The pivotal role of phosphoenolpyruvate in plant metabolism W.C. Plaxton	09:00 - 09:40
C1-2	Molecular genetic dissection of wax biosynthesis in Arabidopsis L. Kunst, T.S. Hooker, O. Rowland, A.L. Samuels, and H. Zheng	09:40 - 10:20
	Coffee Break	10:20 - 10:40
C1-3	One-carbon metabolism and its engineering A.D. Hanson	10:40 - 11:20
C1-4	Floral scent – from compounds to metabolic pathways and their regulation N. Dudareva, N. Kolosova, C.M. Kish, J.L. Boatright, G. Peel, and D. Rhodes	11:20 - 12:00

Plant Molecular Biology	**Location: ST 145**
	13:30 – 16:30
C2 Concurrent	**Chair – Simon Chuong**

C2-1	Sequence differences between the genes for endo-beta-mannanase in tomato fruit cultivars producing active and inactive forms of the enzyme R. Bourgault, and J.D. Bewley	13:30 - 13:50
C2-2	Isolation and characterization of phytochrome genes, *Phyb* and *Phyc* in *Stellaria longipes* W. Li, and C.C. Chinnappa	13:50 - 14:10
C2-3	Snrk1 interactions involved in signalling and regulation of carbon metabolism in wheat S. Laurie, R.S. McKibbin, N.G. Halford, M. Burrell and J.P. Freeman	14:10 - 14:30
C2-4	DNA recombination and repair processes in Arabidopsis: characterization and manipulation P. Fu, F. Ouellet, M. Wigness, T. Kreiser, W.Yang, D. Lydiate, and K. Rozwadowski	14:30 - 14:50
	Coffee Break	14:50 - 15:10
C2-5	Characterization of rapid alkalinization factors (RALFs) from hybrid poplar M. Haruta, and C.P. Constabel	15:10 - 15:30
C2-6	Development of an inventory of expressed sequence tags in spruce and poplar S. Ralph, N.Kolosova, C. Douglas, B. Ellis, S. Jones, K. Ritland, M. Marra, and J. Bohlmann	15:30 - 15:50
C2-7	The role of arginine in regulating loblolly pine arginase gene expression in vitro C.D. Todd, and D.J. Gifford	15:50 - 16:10

Tuesday, June 11th

Plant Molecular Biology (continued)	**Location: ST 145**
	13:30 – 16:30
C2 Concurrent	**Chair – Simon Chuong**

C2-8 Expression pattern comparison for the two gene copies encoding *Arabidopsis thaliana* ribosomal protein L23a — 16:10 - 16:30
K.B. McIntosh, and P.C. Bonham-Smith

Plant Biochemistry and Metabolism II	**Location: ST 140**
	13:30 – 16:30
C3 Concurrent	**Chair – Nailish Samanani**

C3-1 Biochemistry, molecular genetics, cell biology and genomics of induced terpenoid defenses in *Picea* spp. — 13:30 - 13:50
J. Bohlmann, J. Fäldt, D. Huber, N. Kolosova, D. Lippert, D. Martin, and B. Miller

C3-2 Substrate specificity of microsomal lysophosphatidylcholine acyltransferase from cultures of oilseed rape — 13:50 - 14:10
R. Weselake, T. Furukawa-Stoffer, R. Boyle, A. Thomson, and M. Sarna

C3-3 Triacylglycerol biosynthesis in developing flax seed — 14:10 - 14:30
B. Sorensen, E. Page, R. Forster, Z. Mir, and R. Weselake

C3-4 Immunolocalization of alkaloid-specific enzymes identifies alkaloid-synthesizing cells in *Papaver somniferum* — 14:30 - 14:50
D.A. Bird, V.R. Franceschi, and P.J. Facchini

Coffee Break — 14:50 - 15:10

C3-5 Regulation of condensed tannin in trefoil (*Lotus*) species — 15:10 - 15:30
H. Ray, M.Y. Gruber, and B. Skadhauge

C3-6 Cloning, characterization, and subcellular localization of plant 5-formyltetrahydrofolate cycloligase — 15:30 - 15:50
S. Roje, M.T. Janave, M.J. Ziemak, and A.D. Hanson

C3-7 Genetic engineering of canola with decreased palmitic acid content in the seed oil — 15:50 - 16:10
S. Davis, M. Bondaruk, A. Degefu, N. Foroud, P. Boora, W. Wiehler, R. Weselake, and S. Shah

C3-8 The biochemical characterization of a wound-induced plasma membrane NAD(P)H dependent oxidase — 16:10 - 16:30
F.A. Razem, and M.A. Bernards

C3-9 Evidence for single carbon metabolism in non-green pine tissues — 16:30 - 16:50
N.A. Forneris, and R.A. Savidge

Tuesday, June 11th

Plant Physiology II	**Location: ST 135**
	13:30 – 16:30
C4 Concurrent	**Chair – Priti Krishna**

C4-1	Effects of moderate light under low temperature on photochemical activity of photosystem II in intact leaves G. Sridharan, and R. Carpentier	13:30 - 13:50
C4-2	Photoinhibition and recovery in the antarctic green alga *Chlamydomonas subcaudata* T. Pocock, and N.P.A. Huner	13:50 - 14:10
C4-3	Photoinhibitory light induced alterations of chlorophyll-protein complexes and energy transfer in photosystem I submembrane particles S. Rajagopal, and R. Carpentier	14:10 - 14:30
C4-4	The role of alternative oxidase in regulation of high energy input into the photosynthetic metabolism J. Barker-Åström, O. Nilsson, and P. Gardeström	14:30 - 14:50
	Coffee Break	14:50 - 15:10
C4-5	Effects of water deficit stress on root water flow properties in trembling aspen J.A. Siemens, and Dr. J.J. Zwiazek	15:10 - 15:30
C4-6	Regulation of water flow properties in aspen (*Populus tremuloides*) seedlings by phosphorylation and dephosphorylation of aquaporins M.C. Voicu, J.J. Zwiazek, and M. Kamalludin	15:30 - 15:50
C4-7	Medicago Inc, manufacturing alfalfa-made pharmaceuticals L.P. Vézina, S. Aquin, P. Bilodeau, M. Couture, M.A. Daoust, D. Hamel, M. Martel, and S. Trépanier	15:50 - 16:10
C4-8	Plant-environment interaction in natural and agro-ecosystems and global climate change in Central Asia O. Kershanskaya, and H.G. Jones	16:10 - 16:30

Award Presentations and Closing Remarks	**Location: ST 140**
	16:50 – 17:30

A1-1

AXIS FORMATION AT THE CELLULAR, TISSUE AND ORGANISMAL LEVEL

C.S. Hardtke[1], J. Mattsson[2], S. Singh, G. Stamatiou, N. Krogan, W. Ckurshumova, T. Berleth

Dept. Botany, Univ. Toronto, Canada, [1]Present address- Biol. Dept., McGill Univ., Montreal; [2]Present address- Dept. Biol.Sci., Simon Fraser Univ., Burnaby

In most organisms, body axes are established at an early embryo stage and provide a framework for subsequent patterning processes. In plants, genetic and experimental evidence suggests common mechanisms underlying the initiation of the embryonic axis, the continuous organization of vascular tissues and the acquisition of cell polarity in general. Recent observations in many laboratories suggest apical-basal auxin transport as a basic orienting signal and define more precisely a developmental role of auxin in local patterning events. Mutations in the Arabidopsis gene MP strongly interfere with auxin perception, embryo axis formation and vascular tissue continuity. The MP gene encodes an “Auxin Response” transcription factor capable of recognizing functional control elements in auxin inducible promoters. Microarray data identify genes with MP dependent auxin inducibility, some of which are involved in auxin transport and early vascular differentiation. Double mutant and protein interaction studies identify redundant and non-redundant interaction of MP and the related transcription factor NPH4. Auxin response reporter gene expression patterns indicate auxin accumulation in provascular tissue prior to overt differentiation. Experimentally induced shifts in the position of these local auxin maxima result in correspondingly altered venation patterns, indicating an instrumental role of auxin in vascular differentiation during organogenesis.

A1-2

THE CONVERGENCE OF *KNOTTED1* HOMEOBOX GENES AND HORMONE SIGNALING IN PLANT MORPHOGENESIS

Sarah Hake[1], Harley Smith[1], Angela Hay[1], Yutaka Sato[1], Naomi Ori[1], Miltos Tsiantis[2]

[1]Plant Gene Expression Center, Albany California, 94710, USA
[2]Oxford University, Oxford, U.K.

Shoot meristems produce lateral organs in repeating patterns. The term phytomer is used to describe the repeating unit and includes the leaf, the internode, and the axillary bud. *knotted1-like* homeobox (*knox*) genes are expressed in shoot meristems in distinct patterns in monocots (maize and rice) and Arabidopsis. *STM* in Arabidopsis and *kn1* in maize are expressed throughout the meristem, except in the group of cells that will produce the next leaf. In the absence of either of *STM* or *kn1*, the shoot meristem fails to progress. *KNAT1* in Arabidopsis and *OSH15* in rice are expressed in subdomains of the meristem and mutants in these genes are affected in internode development. We have used an inducible system to investigate the function of KNOX genes. Plants that carry the *kn1* gene fused to the glucocorticoid receptor are normal in the absence of the hormone, dexamethasone. Upon dexamethasone addition, the next few leaves to form are deeply lobed. We have followed the morphology of these plants at the same time examining repression and induction of genes in hormone pathways. Our data implicate both cytokinin and gibberellin as downstream of *KNOX* gene function. We are also using biochemical methods to find direct targets of the KN1 and STM transcription factors.

A1-3

SIGNALLING THROUGH RECEPTOR KINASES IN PLANTS

Goring DR[1], Stone SL[1, 2], Silva NF[1, 2], Haffani YZ[1], Mullen RT[3]

[1]Department of Botany, University of Toronto, Toronto, Ontario M5S 3B2; [2]Biology Department, York University, Toronto, Ontario M3J 1P3; Department of Botany, [3]University of Guelph, Guelph, Ontario N1G 2W1

Receptor kinases are one class of proteins through which plants are able to regulate growth and development as well as respond to their surrounding environment. These integral membrane proteins are designed to detect external stimuli and then to activate signalling pathways inside the cell. With the completion of the *Arabidopsis* genome, we now know that plants possess hundreds of putative receptor kinases, yet so little is known about how they function. We have been working with two receptor kinase systems. The first receptor kinase is the S Receptor Kinase, which controls the *Brassica* self-incompatibility response leading to the rejection of “self” pollen. ARC1 is another protein required for self-incompatibility and acts downstream of the S Receptor Kinase. Recently, we have focused efforts on determining the cellular function of ARC1 and have evidence suggesting that ARC1 may act in the proteasome degradation pathway. The second receptor kinase represents a novel class of receptor kinases, the PERK family, which share a unique proline rich extracellular domain. I will discuss our preliminary work on PERK1, which supports its role in plant development as well as in the wound response.

A1-4

A GENOMICS APPROACH TO CEREAL ALEURONE CELL FUNCTION

A Fath, PC Bethke, YS Hwang, R Wei, T Zhu, RL Jones

Department of Plant and Microbial Biology, University of California, Berkeley, California, 94720-3102

The cereal aleurone layer contains a uniform population of non-dividing, hormonally responsive cells. In imbibed grain, gibberellins (GA) released by the embryo trigger aleurone cells to synthesize and secrete hydrolytic enzymes. These enzymes hydrolyze stored reserves in the adjacent starchy endosperm and thereby provide nutrients for the growing embryo. Abscisic acid (ABA) antagonizes the effects of GA and prevents hydrolase secretion in developing and dormant grain. Because of its well-defined responses to GA and ABA, the cereal aleurone layer has been used extensively to investigate hormonal signaling. To obtain deeper insights into hormonal signaling we used the rice oligonucleotide microarray developed by our colleagues at the Torrey Mesa Research Institute. Oligonucleotides representing ~24,000 rice genes, or 50% of the genes in the rice genome, are present on the chip. Of these, 80% give a meaningful signal when hybridized with barley genomic DNA, indicting that the rice chip can be used successfully with both barley and rice RNA. RNA expression profiles of barley and rice aleurone layers treated with GA and ABA for up to 24 h have been obtained, and details of these experiments will be described. The data have confirmed previous work, and provide a roadmap for novel discoveries.

A2 Concurrent: Plant Stress Responses I

A2-1

INVESTIGATING THE ROLE OF CITRATE SYNTHASE IN DETOXIFICATION OF ALUMINUM IN YEAST AND *BRASSICA NAPUS*

Valarmathi M. Anoop , Urmila Basu and Gregory Taylor
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Aluminum (Al) toxicity is a major constraint for crop production in acid soils, although crop cultivars vary in their tolerance to Al. One mechanism of Al tolerance is chelation of Al by organic anions within root cells or in the rhizosphere. The role of citrate in mediating Al tolerance was investigated in Al-sensitive yeast (MMYO11) and canola (cv Westar). Yeast disruption mutants with defects in genes encoding TCA cycle enzymes, both upstream (citrate synthase, CS) and downstream (aconitase, aco) of citrate, showed altered levels of Al tolerance. Mutants defective in CS showed lower levels of citrate accumulation and reduced Al tolerance, while aconitase deficient mutants showed higher accumulation of citrate and increased levels of Al tolerance. A gene for *Arabidopsis* mitochondrial citrate synthase was overexpressed in canola using an *Agrobacterium*-mediated system. Increased levels of CS gene expression and enhanced CS activity were observed in transgenic lines compared to wild type. Root growth experiments revealed that transgenic lines have enhanced levels of Al tolerance. Citrate content in the tissues and root exudates have been measured to determine whether Al tolerance in these transgenic lines is due to internal detoxification of Al by cellular citrate or by enhanced exudation of citrate into rhizosphere.

A2-2

HSP mRNA PROFILES IN *BRASSICA NAPUS* POLLEN, PISTILS AND LEAF DISKS

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During Heat Stress (HS) of *Brassica napus* flowers, a reduction in gametophyte fertility is observed, resulting in reduced seed production. Our work has shown that both male and female gametophytes are adversely affected by HS in *B. napus*. The reductions in male gametophyte fertility are primarily due to a reduction in germinability. Heat Shock Proteins (HSPs) have been shown to protect cells from HS, thus the presence or absence of HSPs could indicate the ability of a cell to withstand HS. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was used to quantitatively determine levels of *HSP18*, *HSP70* and *HSP101* mRNA in both HS and control pollen, pistils and leaf disks. *HSP18* mRNA was only observed in pollen from plants developing under HS conditions. *HSP70* mRNA was observed in pistils and leaf disks, with higher levels observed in HS pistils than in control pistils. *HSP101* mRNA was observed in pollen from HS treated plants and in control and HS pistils and leaf disks. Higher levels of *HSP101* mRNA were observed in HS than control pistils. Given our observations, the question arises as to whether increased HSP mRNA levels in the two gametophytes are sufficient to improve thermotolerance.

A2-3

MOLECULAR ASPECTS OF PLANT ADAPTATION TO IONIZING RADIATION

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The industrial developments of recent decades, nuclear power accidents, continued nuclear tests, transport and leakage of radioactive wastes confront scientists with the problem of the effects of chronic radiation exposure on plants. Plants deserve special attention relative to their susceptibility to ionizing radiation due to their stationary life style they have to adapt to harsh conditions. The molecular mechanisms of plant adaptation as well as the molecular basis of different sensitivity of plant species to radiation still remained unexplained. I study the fundamental biological processes that govern plant responses and adaptations to chronic radiation exposure and radiation sensitivity, the molecular players and communication involved in such responses. I use molecular, genetic and physiological approaches with a strong emphasis on the analysis of epigenetic changes to understand the molecular mechanisms of plant adaptation to radiation. I used several generations of Arabidopsis and wheat grown in Chernobyl to analyze changes in plant genome stability by looking at homologous recombination levels in several generations of plants. In parallel I have analyzed the germline microsatellite mutation rates in exposed plant populations. Detailed analyses of several generations of plants exposed to radiation revealed that the rates of homologous recombination, somatic and germline mutation in plants were strongly elevated.

A2-4

ION UPTAKE AND MEMBRANE PERMEABILITY IN PINUS BANKSIANA TREATED WITH SODIUM CHLORIDE AND SODIUM SULFATE

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Soil salinization due to agricultural and industrial land use is of increasing importance worldwide. Although NaCl is most commonly used in salinity studies, plant ion uptake and physiological effects may differ greatly between plants treated NaCl and with Na_2SO_4, a common salt in terrestrial soils. This study tested the hypothesis that a greater Na uptake and translocation in the presence of Cl is related to increased membrane permeability. One-year-old jack pine (*Pinus banksiana*) seedlings were grown in sand culture and treated for 5 weeks with NaCl or Na_2SO_4 solution. In a second experiment, transpiration of treated plants was measured as the loss of water from a sealed system. Sodium uptake and root-to-shoot transport rates were greater in treatments containing Cl. A delay in root-to-shoot transport of both Na and Cl indicate retention of these ions in the roots. Uptake of Ca and Mg were more reduced by Na_2SO_4 treatment than by NaCl treatment. Electrolyte leakage of needles was more closely related to treatment chloride concentrations than treatment sodium concentrations. The flux of sodium and chloride ions to the shoot was related to differences in permeability of the root sytem to these ions, and was not related to transpiration rate.

A2-5

REMOBILIZATION OF CADMIUM IN MATURING SHOOTS OF NEAR ISOGENIC LINES OF DURUM WHEAT THAT DIFFER IN GRAIN CADMIUM ACCUMULATION

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Cadmium (Cd) concentrations in Canadian durum wheat (*Triticum turgidum* L. var *durum*) grain often exceed proposed international trade standards. In an effort to understand regulation of Cd accumulation in maturing grain, we examined remobilization of ^{109}Cd applied to stem and flag leaves in two near-isogenic lines that differ in grain Cd accumulation. Absorbed ^{109}Cd was primarily retained in the labelling flap. Cadmium exported from the stem flap initially accumulated in the stem in a declining gradient towards the head. Subsequent remobilization of Cd deposited in the stem was associated with Cd accumulation in the grain. Cadmium exported from the flag leaf flap was primarily directed to the grain. Little (<1%) Cd accumulated in the glumes or rachis, and transport of Cd to shoot tissues below the flag leaf node was low (<1%). The largest difference between isolines in grain Cd-accumulation (2.3-fold) coincided with the period of most rapid Cd remobilization from the flag leaf (2 wk post-anthesis). Cadmium accumulation in the grain strongly declined after the grain reached maximum dry weight. These results show that elevated remobilization of Cd from the leaves to the maturing grain may be partially responsible for high accumulation of Cd in durum wheat grain.

A2-6

CADMIUM ASSOCIATION WITH THE CELL WALL OF CHARA CORALLINA: EVIDENCE FOR MULTIPLE BINDING SITES WITHIN A SINGLE CELL WALL

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Compartmental efflux analysis has been used to estimate amounts and location of cations in subcellular compartments (cell wall, cytoplasm, vacuole) of plant cells. Originally work was done with monovalent cations using giant algal cells (Nitellopsis), where pool sizes could be verified directly. Similar profiles were found for desorption of monovalent cations from root tissues. This analytical tool has recently been used to study localization of divalent cations (Cd^{2+}, Zn^{2+}) in plant roots without additional effort to verify the subcellular location of desorbing pools. In this study, Cd absorption by and desorption from single cells was examined using the giant algae Chara corallina. The cell wall was the dominant location for Cd accumulation (85%, 1nM exposure). When internodal cells were exposed to 1 nM $CdCl_2$, then desorbed in 100 fold excess Cd, Cd desorption was adequately explained by a two component model. The size of each of these components was larger than total protoplasmic Cd accumulation. Desorption from isolated cell walls mirrored profiles from intact cells, suggesting the two pools originated from the cell wall. Observation of two cell wall pools contradicts current interpretation of profiles containing a single cell wall, cytoplasmic, and vacuolar pool.

A2-7

IDENTIFICATION AND EXPRESSION OF LOW TEMPERATURE RESPONSIVE GENES IN WINTER WHEAT

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A low temperature-induced subtracted cDNA library was constructed from a winter wheat doubled haploid line exhibiting moderate levels of freezing tolerance and snow mold resistance. RNA was isolated from crowns of plants grown at 20°C for 2 weeks and cold hardened for 7 or 21 days at 2°C. cDNA from the two hardening treatments was subtracted against cDNA from the non-hardened treatment. A library of 1600 clones was arrayed on nylon membranes and differentially hybridized with subtracted and reverse subtracted cDNA probes. Approximately 320 clones were differentially expressed in hardened and non-hardened tissue. Clones included homologs to sequences known to be induce at low temperature, sequences not previously known to be cold regulated and those with no significant homology to known sequences. The low temperature expression of specific clones was determined in winter wheat cultivars in the growth cabinet and in the field throughout winter. Expression in the field was also determined in winter wheat lines exhibiting varying degrees of cold tolerance. Results will be discussed in relation to identification and expression of novel sequences regulated by low temperature in winter wheat and their possible relationship to cold tolerance and snow mold resistance.

A3 Concurrent: Plant Cell and Developmental Biology

A3-1

PLANT CDK INHIBITORS: INTERACTIONS WITH CELL CYCLE REGULATORS AND FUNCTIONAL COMPARISONS IN TRANSGENIC *ARABIDOPSIS* PLANTS

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The eukaryotic cell cycle is regulated by the cyclin-dependent kinase (CDK). Recently, a family of novel CDK inhibitor genes were identified in *Arabidopsis* and the first one, *ICK1*, has been characterized in several aspects (1-3). To characterize other *ICK1*-related inhibitor genes, the interactions of plant CDK inhibitors with cell cycle regulators were analysed by the yeast two-hybrid system and their functions were compared in transgenic *Arabidopsis* plants. Yeast two-hybrid results indicate two likely groups of plant CDK inhibitors. The A-group inhibitors ICK1, ICK2, ICK6 and ICK7 interacted with *Arabidopsis* Cdc2a and D-type cyclins (D1, D2 and D3), while the B-group inhibitors ICK4, ICK5 and ICKCr interacted only with D-type cyclins. Overexpression of *ICK1* (A-group), and *ICK4* or *ICKCr* (B-group) resulted in transgenic *Arabidopsis* plants with a smaller size, serrated leaves and modified flowers. These plants also had reduced nuclear ploidy levels, suggesting that endoreduplication was inhibited. Furthermore, there were apparent differences among the inhibitors. These results provide the first evidence for the function of ICK4 and ICKCr, and suggest important roles for these CDK inhibitors in the cell cycle and plant growth. 1. Wang H et al. (1997) *Nature* 386: 451; 2. Wang H et al. (1998) *Plant J* 15: 501; 3. Wang H et al. (2000) *Plant J* 24: 613

A3-2

CHARACTERIZATION OF THE RNA AND MICROTUBULE BINDING ACTIVITIES OF THE RICE MULTIFUNCTIONAL PROTEIN (MFP) AND ITS POSSIBLE ROLE IN PROTEIN TARGETING TO PEROXISOMES

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RNA localization is known to be an important mechanism involved in the establishment of subcellular asymmetries and the trafficking of proteins to organelles. In a screen for RNA- and microtubule-binding proteins, we identified a rice seed protein possessing both of these activities. The protein was purified to homogeneity using a two-step procedure, and amino acid sequencing identified it as the multifunctional protein (MFP), a peroxisomal enzyme with up to four enzymatic activities involved in the β-oxidation of long-chain fatty acids. The recombinant version of this MFP isoform binds with high affinity to RNA, co-sediments with microtubules (MTs), and contains at least two enzymatic activities involved β-oxidation. GFP-MFP fusion constructs demonstrated that MFP is targeted exclusively to peroxisomes, and additional targeting experiments indicated that MFP is imported (and functions) as a monomer and that its carboxy-terminal tripeptide -SRM functions as a type 1 peroxisomal targeting signal. The targeting of GFP-MFP to peroxisomes was disrupted by treatment with MT-destabilizing agents, suggesting that MTs are required for MFP import into peroxisomes. We propose that the RNA- and MT-binding activities of MFP may play a role in regulating the localization and/or translation of its own mRNA on MTs, followed by the efficient MT-mediated import of MFP into peroxisomes.

A3-3

THE PLASTID TRANSLOCON COMPONENT TOC36 EXHIBITS AN AFFINITY FOR THE BACTERIAL PROTEIN TRANSLOCATION PROCESS

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The 44 kDa envelope polypeptides are active components of the plastid translocon, but their role in protein transport remains elusive. One form, bnToc36B from *Brassica napus* seeds, was observed to exert a significant overall effect on bacterial protein translocation, but the nature of the influence requires further characterization. This study thus focuses specifically on the nature of the relationship between bnToc36B and the bacterial Sec translocon to gain a further understanding of Toc36's function. The presence of bnToc36B in bacteria gave rise to a number of features related to the protein transport process that together point to functional interactions with the bacterial Sec translocon. The effects related to protein transport are: 1) Reduced sensitivity to the inhibitor sodium azide as measured by a higher recovery rate from azide treatment, 2) Reduced sensitivity to sub-optimal temperatures manifesting as sustained levels of protein synthesis and translocation, 3) Sustained levels of growth and β-lactamase transport in high ampicillin concentrations, and 4) Evidence for a physical association with the bacterial translocon. A reduction in overall SecA levels and a more stable SecA profile, when subjected to azide treatment, was observed in bnToc36B-containing bacteria. The implications of the bacterial data will be discussed.

A3-4

SUBCELLULAR LOCALIZATION OF A RNA VIRUS REPLICASE PROTEIN IN TOBACCO BY-2 CELLS: TRANSIENT EXPRESSION OF THE 33KDA PROTEIN FROM TOMATO BUSHY STUNT VIRUS CAUSES THE DISAPPEARANCE OF PEROXISOMES

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Tomato Bushy Stunt virus (TBSV) is a member of a single-stranded RNA virus family (Tombusviridae) in which the product of the first open reading frame encodes a 33 kDa replicase protein (p33). Previous electron microscopic analysis of tissues infected with a modified form of TBSV implicated p33 in multivesicular body formation (the site of viral RNA replication) from the boundary membrane of peroxisomes. To examine the molecular mechanisms involved in sorting p33 to peroxisomes, an epitope-tagged version of the protein was expressed transiently in tobacco BY-2 suspension cultured cells. Our results showed that within 4 hours after biolistic bombardment p33 localized exclusively to peroxisomes. Remarkably, however, all cells expressing p33 displayed dramatic alterations in the distribution and morphology of peroxisomes, whereby individual organelles aggregated into discrete globular structures. Even more surprising observations were at later stages following bombardment (e.g. 12 hours) when p33 localized to the endoplasmic reticulum (ER) concomitant with the disappearance of all peroxisomes within the cell. These data suggest that p33 affects the integrity of pre-existing peroxisomes as well as the formation of nascent peroxisomes, possibly at the site of their synthesis on the ER.

A3-5

EXPRESSION AND ACTIVITY OF BETA-MANNOSIDASE IN TOMATO SEEDS DURING AND FOLLOWING GERMINATION.

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Beta-mannosidase activity increases in seeds of tomato prior to the completion of germination, in both the micropylar and lateral endosperm, and increases further following germination. Tomato seed beta-mannosidase was purified to homogeneity and its cDNA (*LeMSide1*) obtained by 3'-RACE-PCR using oligonucleotide sequences based on peptide sequences obtained from the purified enzyme. One gene (*LeMSide2*) encodes the protein in tomato. Beta-mannosidase is a high-salt-soluble enzyme associated with the cell wall, and is a member of the Glycosyl Hydrolases Family 1 (GHF1). It has a low sequence identity with that of beta-mannosidase from non-plant sources; there are no other plant sequences known for this enzyme. Using northern hybridization and tissue prints we have determined that beta-mannosidase gene expression occurs first in the micropylar endosperm, and this is followed by an increase in expression in the lateral endosperm. Expression of the beta-mannosidase gene is mainly in the endosperm, but this requires signals from the embryo for induction. Six hours of contact with the embryo is sufficient to induce a maximum increase in activity of the enzyme in the endosperm. Since activity of beta-mannosidase of the gibberellin-deficient *gib-1* mutant seeds can be induced by gibberellin, the signal from the embryo may be this hormone.

A3-6

A ROLE FOR DIACYLGLYCEROL ACYLTRANSFERASE DURING LEAF SENESCENCE

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Lipid analysis of rosette leaves from *Arabidopsis thaliana* has revealed an accumulation of triacylglycerol (TAG) with advancing leaf senescence coincident with an increase in the abundance and size of plastoglobuli. The terminal step in the biosynthesis of TAG in *Arabidopsis* is catalyzed by DGAT1 (diacylglycerol acyltransferase; EC 2.3.1.20). When gel blots of RNA isolated from rosette leaves at various stages of development were probed with the *Arabidopsis* EST clone, E6B2T7, which has been annotated as DGAT1, a steep increase in DGAT1 transcript levels was evident in the senescing leaves coincident with the accumulation of TAG. The increase in DGAT1 transcript correlated temporally with enhanced levels of DGAT1 protein detected immunologically. Two lines of evidence indicated that the TAG of senescing leaves is synthesized in chloroplasts and sequesters fatty acids released from the catabolism of thylakoid galactolipids. First, TAG isolated from senescing leaves proved to be enriched in hexadecatrienoic acid (16:3) and linolenic acid (18:3), which are normally present in thylakoid galactolipids. Second, DGAT1 protein in senescing leaves was found to be associated with chloroplast membranes. These findings collectively indicate that diacylglycerol acyltransferase plays a role in senescence by sequestering fatty acids de-esterified from galactolipids into TAG.

A3-7

WHAT DO NITROGEN-FIXING NODULES AND POLLEN HAVE IN COMMON?

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The importance of phosphatiylinositol transfer proteins (PITPs) in membrane trafficking and cellular signalling has been well-documented in mammalian and yeast systems. However, relatively little is known about PITPs in plants. We recently identified a novel family of plant PITP-like proteins in the model legume, *Lotus japonicus*. One of the genes in this family, LjPLPIV, was found to be of particular interest because of its unique regulatory features: in addition to an upstream promoter (UPP), the LjPLPIV gene also contains an intron-born bi-directional promoter (INT10P). Previous work has demonstrated that three types of products result from the transcriptional activities of this gene: (i) PITP with a C-terminal extension representing a plasma membrane targeting module, (ii) antisense transcripts of the PITP domain, and (iii) nodulin 16 (Nlj16). Functional analysis of the LjPLPIV gene has revealed a fascinating regulatory relationship between the promoter regions and has lead us to ask the questions: what do nitrogen-fixing nodules and pollen have in common and what potential role(s) do PITPs play in the development of these two different plant organs.

A3-8

ALTERATION OF GROWTH RESPONSE IN TRANSGENIC ASPEN OVEREXPRESSING AN ASPEN EXPANSIN GENE

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Expansins are cell wall proteins that promote cell enlargement by altering rheological properties of plant cell walls. Since the plasticity of cell walls determine plant cell size and shape, expansins are believed to play a key role in plant morphogenesis. We have isolated two alpha-expansin genes, *PttExp1* and *PttExp2*, from an EST library obtained from wood forming tissues of hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx). Strong *PttExp1* expression in the cambium region was confirmed using RNA blot analysis. However, *PttExp2* transcripts were detected only weakly in stem tissues and more strongly in young leaves. Cell-type specific expression *PttExp1* was studied using high-resolution Northern and *in situ* RT-PCR, which revealed strong signals in cambium cells and expanding xylem cells. This pattern is consistent with proposed roles of expansins in cell enlargement, tip growth and cell wall disassembly. In order to elucidate the exact role of expansin in xylem development, we generated transgenic aspen overexpressing the *PttExp1* gene. Transgenic plants exhibited changes in growth response (longer internodes and larger leaves) in a manner dependent on the *PttExp1* expression. Transgenic plants are being characterised in further detail and the significance of the transgene effect will be discussed.

A4 Concurrent: Plant Biotechnology

A4-1

A NEW REGENERATION PLANT PROTOCOL FOR EXPRESSION OF GENES IN MONOCOTS

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The major limiting factor to obtaining transgenic monocots is the efficiency in regenerating monocot cells into green and fertile plants. We have developed a novel regeneration protocol using recurrent embryogenesis for cereals. The protocol includes a rapid induction of direct somatic embryogenesis of scutellum cells followed by secondary embryogenesis, germination of primary and/or secondary embryos and regeneration of normally growing green and fertile plants. A critical element of this protocol is to transfer the tissues to the subsequent medium only when tissues reach the appropriate developmental stage. This protocol is applicable to all monocots tested so far and is also cultivar independent following testing on winter and spring hexaploid wheat, durum wheat, *Triticum monococum*, wheat amphiploids, barley, oat, sorghum and corn. The regeneration efficiency is 10-12 primary embryos per excised scutellum and up to 10 secondary embryos per primary embryo. An obvious application of a cultivar independent rapid regeneration technique in monocot is toward genetic transformation. This eliminates the difficulties of genotype-dependent regeneration encountered by many research groups around the world. An example demonstrating the functional activity of a stripe rust candidate gene will be provided.

A4-2

METABOLIC ENGINEERING OF HYDROXYCINNAMIC ACID AMIDE BIOSYNTHESIS IN TOBACCO

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Hydroxycinnamic acid amides (HCAAs) are a widely distributed group of plant secondary metabolites involved in defense responses to pathogen challenge and mechanical wounding. The biosynthesis of tyramine-derived HCAAs involves the decarboxylation of tyrosine to tyramine, followed by a condensation reaction with a hydroxycinnamoyl-CoA thioester. The enzymes that catalyze these reactions are TYDC (tyrosine decarboxylase) and THT (hydroxycinnamoyl-CoA: tyramine *N*-(hydroxycinnamoyl)transferase). *Nicotiana tabacum* cv. Xanthi was independently transformed with a construct encoding tobacco THT (35S::THT) or with the corresponding antisense construct (35S::antiTHT). Both constructs were under the transcriptional control of the cauliflower mosaic virus 35S promoter. RNA gel blot analysis showed elevated levels of THT mRNA in plants expressing the sense construct. Most T_o plants expressing 35S::THT exhibited increased THT activity. In contrast, THT activity was suppressed in T_o transformants expressing 35S::antiTHT. RNA gel blot analysis showed elevated levels of THT mRNA in plants expressing the sense construct. Independent upregulation of TYDC and THT activities in transgenic tobacco was found to increase [^{14}C]tyramine incorporation into the cell wall following *in vivo* feeding by petiolar uptake. Mechanical wounding of whole leaves prior to feeding and followed by autoradiography indicated increased [^{14}C]tyramine incorporation around the wound sites. Insight provided by these data on the regulation of HCAA biosynthesis in plant defense responses will be discussed.

A4-3

MANIPULATION OF GENES INVOLVED IN CARBON PARTITIONING IN TRANSGENIC POTATO

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Plant SNF1-related protein kinase (SnRK1) is believed to function as a metabolic sensor like it's yeast and animal counterparts, enabling plants to respond to nutritional stresses. To investigate this we are using a variety of approaches including stable and transient plant transformation. Transgenic potatoes containing sense and antisense *SnRK*1 tuber-specific constructs show up- and down-regulation, respectively, of *SnRK*1 expression. Accompanying the changes in *SnRK*1 expression are changes in the transcript levels of other genes involved in carbon partitioning and resource mobilisation, such as sucrose synthase, sucrose phosphate synthase and α-amylase. The activity of enzymes encoded by these genes is currently under investigation. Tubers expressing antisense *SnRK*1 are not as susceptible to sprouting in cold-storage as wildtype tubers, but will sprout and grow normally when planted. The potato homologue of a putative EREBP-type transcription factor has also been cloned (*St-EREBP*1). This transcription factor interacts with barley SnRK1 in 2-hybrid assays and *in vitro*. Potato plants have been transformed with *St-EREBP*1 in the antisense orientation and expression levels of the above genes are being monitored. *In vitro* experiments are also underway to test the interaction and substrate specificity of SnRK1 and St-EREBP1.

A4-4

METABOLIC ENGINEERING OF BENZOPHENANTHRIDINE ALKALOID BIOSYNTHESIS IN CALIFORNIA POPPY

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California poppy (*Eschscholzia californica*) cell and hairy root cultures produce several benzophenanthridine alkaloids with potent pharmacological activity. Antisense constructs of genes encoding two enzymes involved in benzophenanthridine alkaloid biosynthesis, the berberine bridge enzyme (BBE) and *N*-methylcoclaurine 3'-hydroxylase (CYP80B1), were introduced separately into California poppy cell cultures. Transformed cell lines expressing antisense-BBE or antisense-CYP80B1 constructs and displaying low levels of BBE or CYP80B1 mRNAs, respectively, showed reduced accumulation of benzophenanthridine alkaloids compared to control cultures transformed with a β-glucuronidase (GUS) gene. Sense and antisense constructs of genes encoding BBE were introduced into California poppy root cultures. Transgenic roots expressing BBE from opium poppy (*Papaver somniferum*) displayed higher levels of BBE mRNA, protein and enzyme activity, and increased accumulation of benzophenanthridine alkaloids compared to control roots transformed with the GUS gene. In contrast, roots transformed with an antisense-BBE construct from California poppy had lower levels of BBE mRNA and enzyme activity, and reduced benzophenanthridine alkaloid accumulation, relative to controls. Pathway intermediates were not detected in antisense-suppressed transgenic cell or root lines. Altering the accumulation of benzophenanthridine alkaloids caused changes in the growth rate of the cultures and the abundance of cellular amino acid pools. These data provide insight into the metabolic engineering of benzophenanthridine alkaloid pathways.

A4-5

A SMALL ANTIMICROBIAL PEPTIDE PROTECTS TRANSGENIC POTATOES AGAINST LATE BLIGHT AND PINK ROT

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Potato late blight disease caused by *Phytophthora infestans*, is considered the most serious potato disease. Today the pathogen costs the developing world alone up to US $3 billion per year. The disease was effectively controlled by multiple applications of fungicides. However, it is reemerging as a serious threat to continued potato production all around the world. Pink rot disease, also caused by *Phytophthora* species, causes the tubers to become soft and gradually changing their color from off-white to salmon pink to black. Unfortunately, resistance to mefanoxam, the only fungicide available for this disease, has developed in Phytophthora erythroseptica, chief cause of pink rot. It is imperative to find alternate strategies for disease control. Here we describe a strategy for engineering potato plants with enhanced protection against these economically important pathogens. We expressed a naturally occurring smallest (13 a.a.) antimicrobial peptide MsrA3 that was modified at the N-terminus to increase its antimicrobial activity while retaining the low cytotoxicity. Highly stringent challenges demonstrated powerful resistance to several diseases. This is the first report of engineering broad-spectrum disease resistance encompassing the late blight and the pink rot of potato. Research supported by the National Centers of Excellence-Canadian Bacterial Diseases Network grant to SM.

A4-6

ECTOPIC EXPRESSION OF OLEOSIN IN ARABIDOPSIS THALIANA SHOWS THE PROTEIN TRAFFICKING ALONG THE ENDOPLASMIC RECTICULUM

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Fusions of green fluorescent protein (GFP) and *Brassica napus* oleosin were ectopically expressed in transgenic *Arabidopsis thaliana* plants. A transient expression system was designed to observe real-time movement of the oleosin-GFP bodies moving along the endoplasmic reticulum (ER) in plant epidermal cells. This is the first time that oleosin has been shown to be a dynamic protein, trafficking along the ER at rates comparable to other organelles such as Golgi bodies and mitochondria. Oleosin-GFP bodies were observed to range in size and speed from fast-moving small bodies (0.5 to 2 μm) to slow-moving large clusters (5 to 10 μm). These larger bodies appear to be stabilized within the cortical ER membrane, with the smaller bodies moving rapidly along the ER, and at times passing through the large clusters. When compared to the seed-specific expression of oleosin-GFP, the constitutively expressing oleosin-GFP embryos displayed identical localization of oleosin. The size and shape of oil bodies were also identical in the heart, torpedo and mature stages. However, prior to triacylglycerol (TAG) accumulation, the constitutive oleosin-GFP embryos possessed irregularly shaped oleosin-GFP bodies, similar to those seen in non-oil tissues. This provides an interesting clue to the timing of oleosin accumulation on oil bodies.

A4-7

SUBCELLULAR TARGETING OF HUMAN INTERLEUKIN-10 IN PLANTS

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It is now clear that plants can be used to produce a wide range of mammalian proteins. However, the that challenge remains is to produce recombinant proteins at concentrations sufficient to allow economic extraction. Furthermore, cumbersome tags and signals could create new polypeptides that misfold or possess altered biological properties. To address these issues, we have investigated the ability of plant cells to accumulate human interleukin-10 (hIL-10) targeted to chloroplasts and/or mitochondria. Both organelles have the ability to import and specifically process precursor polypeptides. We have previously shown that IL-10 retained in the ER assembles into its homodimeric form and is biologically active when expressed in tobacco. In this study, we found that IL-10 accumulates in chloroplasts to levels similar to those obtained with ER-targeted IL-10. Conversely, the IL-10 protein does not accumulate in mitochondria despite the presence of appreciable levels of IL-10 transcript. Immunolocalisation by TEM showed that the chloroplast-targeted IL-10 is indeed localized to chloroplasts, while ER-retained IL-10 is present mostly in protein bodies inside the vacuole. Analysis of the chloroplast-targeted IL-10 protein revealed that the homodimer does not assemble in the chloroplast, but that partial biological activity is present in *in vitro* cell assays.

A4-8

MOLECULAR REGULATION OF ESSENTIAL OIL COMPOSITION IN PEPPERMINT: METABOLIC FATE OF (+)-PULEGONE.

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(+)-Pulegone is a key intermediate in the biosynthesis of the monoterpene constituents of peppermint (*Mentha X piperita L*) essential oil. This branch point metabolite may be reduced to (-)-menthone by pulegone reductase (PR), or oxidized to the hepatotoxin (+)-menthofuran by menthofuran synthase (MFS). To elucidate regulation at this branch point, we altered the expressions of *mfs* and *pr* in independently transformed peppermint plants. Overexpression of *pr* failed to improve overall PR enzyme activity levels in transgenic plants, suggesting that the expression of this gene is post-transcriptionally regulated. Overexpression of *mfs* led to increased menthofuran production, and downregulation of expression of this gene resulted in reduced menthofuran biosynthesis. Surprisingly, the reduction of pulegone to menthone was inhibited in *mfs*-overexpressors, and was enhanced in *mfs*-knockouts. This outcome was a consequence of transcriptional downregulation of *pr* by menthofuran. The *mfs*-knockouts also expressed a monoterpene epoxidase (EPO) that catalyzed the formation of (-)-*trans*-piperitone oxide in these plants. These findings demonstrate that menthofuran acts as a negative transcriptional regulator of at least two monoterpene biosynthetic genes, including *pr* and *epo*, in peppermint.

Gold Medal Address

GMA-1

METABOLISM BY MUTATION

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Genetic variants are often key tools for advancing knowledge in many areas of plant physiology. Plant tissue, cell and protoplast cultures can be excellent sources of many classes of such variants. We isolated two useful classes of biochemical variants from mutagenized suspensions of cells of the true haploid (n = x =12), *Datura innoxia* P. Mill. A few complete auxotrophic cell lines were isolated by negative selection and used to gain knowledge of plant metabolism, in genetic complementation, and for genetic engineering. Most notably, greater definition of the steps in the metabolic pathways for pantothenate and branched-chain amino acid (BCAA) biosynthesis in plants was achieved.A second class of variants had resistance to herbicides that inhibit acetolactate synthase (ALS) in the BCAA pathway. These variants were exploited to investigate binding of substrates, pathway end-products, and herbicides to ALS. Later, more stringent investigations of ALS and the BCAA pathway were made possible through the isolation and use of mutant plants of *Arabidopsis thaliana* (L.) Heyn. Columbia wild type, in place of *Datura* cell variants. The search for resistance mutants in pathways other than BCAA biosynthesis in *Arabidopsis* led us to folates and one-carbon (C1) metabolism. We are now using NMR spectroscopy and molecular biological techniques to learn more about C1 metabolism, continuing a career-long search for greater understanding of how plants work, biochemically.

B1 Plenary: Interactions Between Plans and Their Environment

B1-1

PLANT ECOLOGY IN THE GENOMICS ERA: LESSONS FROM NATIVE *NICOTIANA*

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The quip that "ecology is the study of the incomprehensible by the incompetent" reflects not only the difficulties that scientists from more reductionistic disciplines of biology have in appreciating progress in ecology, but also the recognition that ecological interactions are intrinsically complicated. Plant ecologists have not fully participated in the genomics revolution in part due to the difficulties of transforming or otherwise manipulating the expression of genes in plants with a rich suite of ecological interactions. This talk will present progress with *Nicotiana attenuata*, a model ecological expression system native to the southwestern USA, which can be been transformed with *Agrobacterium*-based techniques or silenced with VIGS to manipulate the expression of genes involved in plant-insect interactions. These plants 'recognize' attack from one of their natural herbivores, *Manduca sexta*, as seen in the plant's signal transduction cascades, secondary metabolite profiles and 'transcriptome', and is, in turn, due to the influence of fatty acid-amino acid conjugates in *Manduca*'s salivary secretions on the plant's wound response. Plant-insect interactions are played out on an arena much larger than the plant itself, and an appreciation of the traits that influence these interactions will be needed to make sense of a plant's molecular organization.

B1-2

STRESS TOLERANCE IN ARABIDOPSIS: MUTANTS, GENES AND SIGNALLING PATHWAYS

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In *Arabidopsis*, the *Salt Overly Sensitive 1* (*SOS1*), *SOS2*, *SOS3* and *SOS4* genes are required for intracellular Na^+ and K^+ homeostasis. Mutations in these genes cause Na^+ and K^+ imbalance and render plants more sensitive toward growth inhibition by salt stress. SOS3 is a myristoylated Ca^{2+}-binding protein that maybe a sensor for cytosolic Ca^{2+} signals elicited by salt stress. *SOS2* encodes a serine/threonine protein kinase. SOS2 physically interacts with and is activated by SOS3. Salt stress up-regulation of *SOS1*, which encodes a plasma membrane Na^+/H^+ antiporter, is partly under control of the SOS3/SOS2 pathway. SOS2 also directly activates the Na^+/H^+ exchanger activity of SOS1. SOS4 encodes a pyridoxal kinase important for the biosynthesis of pyridoxal-5-phosphate, which could regulates SOS1 activity because SOS1 contains a putative pyridoxal-5-phosphate binding motif in its C-terminal cytoplasmic tail. These *SOS* genes define a novel regulatory pathway important for the control of intracellular ion homeostasis and salt tolerance in plants. SOS2-SOS3 interaction is mediated through the regulatory domain of SOS2. We have delimited within the SOS2 regulatory domain, a 21 amino acid motif (designated as the FISL motif) that is both necessary and sufficient for binding to SOS3. On the SOS3 side, no discrete motif could be identified that is sufficient for mediating the interaction with SOS2. It appears that the central EF-hand as well as the N-terminal and C-terminal regions of SOS3 is required for binding to SOS2. Deletion of the FISL motif or a Thr168-to-Asp mutation results in a constitutively active SOS2 that is independent of SOS3 or Ca^{2+}. Expression of the constitutively active SOS2 mutants in yeast or Arabidopsis is partially sufficient for SOS1 activation and salt tolerance. In addition, recent progress on the characterization and cloning of several Arabidopsis mutations that affect osmotic stress and ABA regulated gene transcription will be presented.

B1-3

THE REGULATORY ROLE OF LOW TEMPERATURE IN WINTER CEREALS

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Low temperature stress is a major factor limiting plant productivity and survival. To properly time flowering and cope with low temperature stress, winter cereals regulate their development through adaptive mechanisms that are responsive to light and temperature. These mechanisms are genetically programmed and involve a complex genetic system. To understand the nature of this system and its regulation by low temperature we identified and characterized several genes associated with low temperature and vernalization responses. The use of integrated physiological, biochemical, molecular and genetic approaches have provided valuable information that improved our understanding of their function and their regulation. In addition, these studies have allowed us to propose several hypotheses to elucidate the molecular mechanisms that describe how these genes interact to increase low temperature tolerance, and at the same time ensure the normal transition from vegetative to reproductive stages. We will also discuss different strategies used to manipulate low temperature tolerance in different plant species.

B1-4

SA- AND NO-MEDIATED SIGNAL TRANSDUCTION IN PLANT DISEASE RESISTANCE

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Studies during the past decade have rigorously established that salicylic acid (SA) plays a critical, multifaceted role in plant disease resistance. To help elucidate the mechanisms of SA action, we have identified several tobacco proteins which interact with SA. These include catalase and ascorbate peroxidase. SA inhibits these two major H_2O_2-scavenging enzymes. Another SA binding protein, the chloroplastic SABP3, is carbonic anhydrase. It also has antioxidant activity. SABP2 is a very low abundance protein with high affinity for SA (K_d=90nM). It has been purified >24,000 fold and the sequence of its encoding gene suggests it is a lipase. We have used mutant analyses in *Arabidopsis* to identify several more potential components in the SA-mediated pathway. Among these mutants are those which exhibit constitutive expression of the SA- and pathogen-induced *PR* genes and enhanced resistance to pathogens (e.g. *cep* and cpr22). Another group are suppressor mutants which overcome salicylate insensitivity of our *sai1/npr1-5* mutant (e.g. *ssi1* and *ssi2*). Recently, we showed that *ssi2,* which activates the SA-mediated defense pathway but suppresses the jasmonic acid/ethylene-mediated defense pathway, alters the activity of a fatty acid (stearic acid) desaturase. Moreover, the product of this stearoyl desaturase, oleic acid or a derivative of it, appears to act as a signaling molecule which is required for activation of several jasmonic acid-mediated defenses. Nitric oxide (NO), which plays a key role(s) in innate immune and inflammatory responses in animals, also participates in the tobacco resistance responses to TMV. Following infection, a NO synthase-like activity rises, leading to *PR-1* activation. Several critical players of NO signaling in animals are also operative in plants including guanylate cyclase, aconitase, and the second messengers cGMP, cADP ribose and Ca^{2+}. NO inhibits aconitase enzyme activity and may convert it to an mRNA binding protein that regulates Fe^{2+} homeostasis.

B2 Concurrent: Plant Stress Responses II

B2-1

INFLUENCE OF ABIOTIC STRESS ON THE PLANT GENOME

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All organisms on our planet have to react to constantly changing surroundings. Environmental factors, such as ionizing and UV radiation, heavy metals and various other toxic chemicals represent powerful forces that are continuously shaping the genomes of all species. Studies of the influence of abiotic stress on the plant genome are of special interest, as plants can not avoid harmful environmental conditions. Moreover, germ cells of plants are derived from somatic cells only during late stages of development, thus genetic changes accumulated during somatic growth may be transferred to the gametes and thus inherited by the progeny. We have developed several new methods to study the effects of radiation and toxic chemicals on the plant genome. Using new *Arabidopsis thaliana* bioindicator plants we have shown that ionizing radiation and heavy metals strongly induce somatic homologous recombination as well as point mutations – transitions and transversions, in plants (Kovalchuk et al., *Nature Biotechnology*, 1998; Kovalchuk et al., *Nature Biotechnology*, 2001). We have also developed a new method to study the germline microsatellite mutation in plants on the molecular level and proved that germline microsatellite mutation in radiation-exposed wheat was unusually high (Kovalchuk et al., *Nature*, 2000).

B2-2

INFLUENCE OF PATHOGENS ON PLANT GENOME STABILITY

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Genome instability provides certain, distinct advantages to organisms. Homologous recombination is a well known mechanism for repairing damaged DNA but it also has the potential to generate new alleles through DNA recombination. For example, the major histocompatibility (MHC) locus, because of its high recombination rate, is capable of quickly generating diverse antibodies within the mammalian immune system, thereby protecting the host from diverse and highly mutable pathogens. Similar system could exist in plants. Genome stability in plants is influenced by many environmental abiotic factors (gamma and UV radiation, cold, draught etc). However, besides the exposure to various physical and chemical factors, plants are persistently exposed to diverse biotic stresses, including bacteria, viruses, fungi, nematodes, and insects. Our experiments present strong evidence that Tobacco mosaic virus (TMV) infection causes genome instability. Infected plants exhibited homologous recombination (HR) frequencies that were up to 3 fold higher compared to non-infected control plants. Of particular interest is the fact that grafting experiments demonstrated that the increase of the HR also occurs in non-inoculated tissue and is due to the plant signal generated in infected cells. We suggest that the increase of homologous recombination in non-infected tissue is a specific mechanism of plant defense.

B2-3

HYPOXIA AND SALINITY STRESSES ON JACK PINE (*PINUS BANKSIANA* LAMB.) SEEDLINGS

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The effects of NaCl (0 and 45 mM NaCl) were studied in six-month-old jack pine (*Pinus banksiana*) seedlings growing in solution culture under hypoxic and well-aerated conditions. We tested the hypothesis that hypoxia aggravates plant response to NaCl by its effect on water and salt uptake. Four weeks after treatment imposition, results showed that hypoxia aggravated the effects of NaCl in inhibiting root growth and inducing root mortality. Hypoxia further inhibited stomatal conductance (g_s) and root hydraulic conductance (K_r) of NaCl-treated jack pine. When applied individually or together, neither 45 mM NaCl nor hypoxia affected cell membrane integrity of needles as measured by tissue electrolyte leakage. Shoot sodium and chloride concentrations in NaCl-treated plants were not altered by hypoxia. However, hypoxia reduced the ability of roots to store Na when exposed to NaCl. Root electrolyte leakage of NaCl-treated plants, which was correlated with root Cl^- concentrations, was induced by hypoxia. Our present study suggest that longer-term hypoxic conditions affected root membrane permeability to Cl^- resulting in the increase in Cl^- concentration in the roots and, in turn, in cell membrane injury.

B2-4

REACTIVE OXYGEN SPECIES MEDIATED SIGNALING IN THE ACCLIMATION PROCESS TO STRESS: EFFECTS OF COPPER AND ULTRAVIOLET RADIATION ON THE AQUATIC PLANT *LEMNA GIBBA*

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There is little information on how environmental stressors initiate signaling of plant development. Two common stressors are metals and ultraviolet (UV) radiation. We were interested in studying the combined effects of the heavy metal copper and simulated solar radiation (SSR) containing UV radiation on the aquatic plant *Lemna gibba*. It was found that copper treatment caused reactive oxygen species (ROS) formation. The ROS levels were also higher in SSR than photosynthetically active radiation (PAR) and copper increased these still further in a concentration dependent manner. Higher concentrations of copper caused elevated toxicity as monitored by reduction in growth and pigment contents. This toxicity was more pronounced when copper was combined with SSR. Because the generation of ROS was also higher when copper was combined with SSR, we attribute this enhanced toxicity to elevated levels of ROS. At lower concentrations than those that caused toxicity, copper when combined under either PAR or SSR, induced elevated levels of superoxide dismutase (SOD) and glutathione reductase (GR). Under SSR, GR increased up to a 6 μM copper concentration and then decreased at higher concentrations. Nevertheless, the levels of GR under all copper/SSR conditions were higher than the control level. Strikingly, copper treatment alone in the absence of UV radiation also induced synthesis of the same flavonoids as observed with UV radiation. All of the acclimation processes occurred under copper concentrations that were associated with the onset of the generation of ROS. In conclusion, we found that copper and UV turn on the same acclimation processes, and their induction corresponds to the rise in ROS levels.

B2 Concurrent: Plant Stress Responses II

B2-5

GENOMICS AND PROTEOMICS DECODING OF PLANT DEFENSE PATHWAYS

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The mitogen-activated protein kinase (MAPK) pathway is a key pathway in plant defense. With genomics and proteomics approaches, we studied this pathway in tomato and wheat. After overexpression of *tMEK2*^{MUT}, a constitutively activated tomato MAPK kinase gene, proteins from tomato leaves were analyzed by two-dimensional electrophoresis (2DE) and matrix-assisted laser desorption/ionisation-mass spectrometry (MALDI-TOF). Among the most significantly up-regulated proteins are beta-glucanase and endochitinase. Phospho-proteome analysis revealed components that are regulated by phosphorylation. An array of pathogenesis-related (PR) genes were activated by *tMEK2*^{MUT} at the transcriptional level. Overexpression of *tMEK2*^{MUT} in tomato and wheat enhanced resistance to bacterial and fungal pathogens. Two MAP kinase genes were mined out from a wheat EST database. When wheat was challenged by wheat leaf rust, both were activated but differentially. We will discuss how protein structure determines the function, and the advantages and challenges in this approach.

B2-6

INSECT FOOTSTEPS ON LEAVES INITIATE PLANT DEFENCE RESPONSES WITHIN TWENTY SECONDS

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In response to insect herbivory plants synthesize wound-induced defense proteins within hours. Non-wounding mechanical stimulation of leaves results in the accumulation of gamma-aminobutyric acid (GABA). In animals GABA inhibits neural transmission. GABA accumulation may function as an inducible plant resistance mechanism against herbivory. We demonstrate that insect larvae crawling on leaves cause a 4-fold increase in leaf GABA concentrations within 5 min. Larval footsteps and herbivory result in chlorophyll fluorescence within 20 s, and superoxide production within 10 s. Superoxide functions in wound-induced resistance against herbivory. Thus in contrast to prevailing models, non-wounding larval crawling stimulates the synthesis of resistance compounds within minutes (GABA) or seconds (superoxide).

B2-7

ROLE OF INSECT SALIVARY GLUCOSE OXIDASE IN SUPPRESSION OF PLANT DEFENSE RESPONSES.

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One defensive strategy of plants against insect herbivory is the production of terpenoid and indole volatiles that attract natural enemies of the phytophagous insect. The endogenous signal for volatile biosynthesis is believed to be jasmonic acid (JA) (1,2). However, caterpillar saliva may contain the elicitor glucose oxidase (GOX), which generates hydrogen peroxide (H_2O_2) through the oxidation of D-glucose. H_2O_2 may stimulate salicylic acid (SA) accumulation and through the antagonism between SA and JA signaling pathways, insect herbivory may interfere with plant defense responses (3). In the model legume, *Medicago truncatula*, we examined expression patterns of regulatory genes in the terpenoid biosynthetic pathways in response to herbivory by the beet armyworm, *Spodoptera exigua*. Caterpillars with impaired labial salivary gland secretions were used to examine the role of GOX in modulating gene expression. Findings suggest that insect salivary factors may counteract plant defenses and suppress terpenoid biosynthesis. However, depressed transcript levels of *dxr1*, a pivotal enzyme in the 2*C*-methyl erythritol 5-phosphate (MEP) pathway, appear to be due to direct effects of H_2O_2 and not through interference with signaling molecules.

B2-8

REGULATION OF AN OXYGENASE WITH HOMOLOGY TO ANIMAL CYCLO-OXYGENASES IN SALT AND PATHOGEN-CHALLENGED ROOTS OF TOMATO

Plant AL, Tirajoh A, Aung T, and Diguistini S. Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A 1S6.

A partial cDNA with marked similarity to the C-terminus of a pathogen-induced oxygenase (PIOX) was identified as salt-responsive in tomato roots. In tobacco leaves *piox* gene expression is associated with bacterial infection, wounding, caterpillar feeding, and exposure to the signaling molecules jasmonic acid, salicylic acid and active oxygen species. PIOX catalyzes α-oxidation of fatty acids to hydroperoxy fatty acids and is similar to animal cyclo-oxygenases responsible for directing the synthesis of lipid-derived signals such as prostaglandins. Therefore, it is suggested that PIOX may be involved in signal generation in plants. The expression of *piox* increases in salt-treated tomato roots and enhanced expression follows challenge of roots with *Pythium aphanidermatum*. Aspects of *piox* gene regulation in roots and shoots, in particular the role played by ABA have been investigated. Preliminary data obtained using an ABA-deficient mutant revealed that *piox* gene expression in salt-affected roots may be partially dependent upon ABA; however, salt-dependent gene induction is observed in roots exposed to the carotenoid biosynthetic inhibitor, fluridone. Interestingly, in pathogen-challenged roots, reduction of ABA levels with fluridone leads to enhanced *piox* expression relative to that in roots with wild-type ABA levels. The possibility that this results from an interaction between ABA and ethylene is under investigation.

B3 Concurrents: Plant Biochemistry and Metabolism I

B3-1

PURIFICATION AND CHARACTERIZATION OF TWO PURPLE ACID PHOSPHATASE ISOFORMS FROM MEDIA OF PHOSPHATE STARVED TOMATO SUSPENSION CELLS

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Secreted (culture media) acid phosphatase (AP) activity was increased 800%, 8-d following subculture of P_i-sufficient (+P_i) tomato cells into P_i-free (-P_i) media. Two AP isoforms (LeSAP1 and LeSAP2) were resolved during cation exchange FPLC of media from 8-d-old -P_i cells. LeSAP1 and LeSAP2 were purified 30- and 50-fold to homogeneity and PEP-hydrolyzing specific activities of 241 and 384 U/mg, respectively. Analytical gel filtration, SDS-PAGE, and PAS staining indicated that LeSAP1 and LeSAP2 are glycosylated monomers with M_rs of 84- and 57-kDa, respectively. MALDI-TOF MS analysis of tryptic peptides indicated that LeSAP1 and LeSAP2 are closely related. Spectral studies and insensitivity to tartrate inhibition indicates that both isoforms are purple APs (PAPs). A 9 amino acid internal sequence of LeSAP1 exhibited maximal identity with two putative *Arabidopsis* PAPs. LeSAP1 and LeSAP2 displayed optimal activity at pH 5.3, were respectively activated 135 and 180% by 5 mM Mg^{2+}, exhibited broad substrate selectivity, and were potently inhibited by P_i, molybdate and Zn^{2+}. LeSAP2 specificity constants (V_{max}/K_m) for pNPP, PEP, *O*-phospho-L-tyrosine, phenyl-P, tetrapoly-P, and β-naphthyl-P were significantly greater than those of LeSAP1. Studies are in progress to determine the immunological relatedness of both isofoms. This work should facilitate cloning of cDNAs encoding P_i-starvation inducible tomato PAPs (supported by NSERC).

B3-2

PURIFICATION TO HOMOGENEITY AND CHARACTERIZATION OF NORCOCLAURINE SYNTHASE, THE FIRST COMMITTED ENZYME IN BENZYLISOQUINOLINE ALKALOID BIOSYNTHESIS IN PLANTS

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Norcoclaurine synthase (NCS) catalyzes the condensation of dopamine and 4-hydroxyphenylacetaldehyde to yield norcoclaurine, the common precursor to over 2500 benzylisoquinoline alkaloids (BAs) produced in plants. In opium *poppy (Papaver somniferum* L.) and meadow rue (*Thalictrum flavum ssp. glaucum*), NCS activity was detected in all mature plant organs, but was predominant in the roots and stems. High levels of activity were also found in meadow rue and fungal elicitor-treated opium poppy cell-suspension cultures. NCS basal activity was 5-fold higher in cell cultures of meadow rue than opium poppy. NCS was purified 1590-fold to apparent homogeneity from cell suspension cultures of meadow rue by taking advantage of its high hydrophobicity and low molecular mass. Two-dimensional gel electrophoresis revealed two major and two minor isoforms with pIs between 5.8 and 6.1. Purified NCS had a native molecular weight of approximately 28 kDa, consisting of two 15 kDa subunits, and showed hyperbolic saturation kinetics for 4-hydroxyphenylacetaldehyde, but sigmoidal saturation kinetics for dopamine. These data suggest positive cooperativity between dopamine binding sites on each NCS subunit. Enzymes exhibiting cooperative substrate binding invariably catalyze regulatory, or rate-limiting, steps in metabolism. Product inhibition kinetics performed at saturating levels of the second substrate were consistent with an iso-ordered bi-uni mechanism for NCS with 4-HPAA binding before dopamine.

B3-3

FUNCTIONAL CHARACTERIZATION OF A NOVEL SER/THR PROTEIN KINASE, PHOSPHATE STARVATION-RESPONSIVE GENE GROUP 1 (PSR1), OF *ARABIDOPSIS THALIANA*.

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Plant responses under phosphate limitation and excess are of great interest for agriculture and remediation, respectively. We study genes of regulatory enzymes involved in phosphate related mechanisms. The cDNA sequence for a novel *Arabidopsis* ser/thr protein kinase, PSR1, was cloned into the pGEX-2T *Escherichia coli* expression vector. PSR1 contains a unique acidic C-terminus of unknown function. RNA blots indicate that *psr1* is transcribed in root tissue and that transcription increased under phosphate starvation. The fusion protein, GST-PSR1, expressed as inclusion bodies in *E. coli*, was affinity purified in the presence of sarkosyl. A far-western overlay assay, probed with GST-PSR1, identified myosin heavy chain (MHC) as a substrate. Kinase activity assays, using γ-^{32}P-ATP and MHC, revealed that GST-PSR1 autophosphorylates only in the presence of MHC and also phosphorylates a 25 kDa protein in the sample. Phosphoamino acid analysis, by two-dimensional gel electrophoresis, demonstrated phosphorylation on ser residues. Mutation analysis of PSR1 identified potentially important sites on the protein. Some of these mutations decreased actively, while some significantly increased activity.

B3-4

PURIFICATION AND CHARACTERIZATION OF ENOLASE FROM THE CYANOBACTERIUM *SYNECHOCOCCUS* PCC 6301

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Enolase participates in glycolysis and gluconeogenesis by catalyzing the reversible interconversion of 2-phosphoglycerate (2-PGA) and phosphoenolpyruvate. Cyanobacterial enolase has never been examined. Thus, *Synechococcus* enolase was purified 1,400-fold to homogeneity and a specific activity of 63 units/mg. Analytical gel filtration and SDS-PAGE indicated that this enzyme is a 118-kDa homodimer composed of 56-kDa subunits. Enolase was heat-stable, losing no activity when incubated at 70 ^{o}C for 5 min. It demonstrated optimal activity at pH 8.0, a K_m(2-PGA) of 0.27 mM, an absolute requirement for Mg^{2+} or Mn^{2+}, and non-competitive inhibition by fluoride (K_i = 0.14 mM). A 2- to 4-fold increase in total enolase activity occurred following the first purification step. This was duplicated by warming a clarified extract to 40 ^{o}C for 3 min, but not by desalting or incubating the extract with phosphatases. The effect was reversible since adding increasing volumes of a freshly prepared extract led to a progressive 70% decline in the activity of purified enolase. Results are indicative of a soluble and heat-labile enolase inhibitor protein in *Synechococcus*. Ongoing research includes assessing the physiological relevance of this inhibitor protein, enolase reverse reaction kinetics, and the potential physical interaction between purified enolase and pyruvate kinase from *Synechococcus* PCC 6301. (Supported by NSERC)

B3-5

CHARACTERIZATION OF THE C-TERMINAL EXTENSION OF CARBOXYSOMAL CARBONIC ANHYDRASE FROM *SYNECHOCYSTIS* SP. PCC6803

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Analysis of the carboxysomal carbonic anhydrase (CcaA) from *Synechocystis* PCC6803, *Synechococcus* PCC7942 and *Nostoc* ATCC29133 indicated high sequence identity to the β-class of plant carbonic anhydrases (CA) and conservation of the active site. However, the cyanobacterial enzyme has a C-terminal extension of about 75 aa not found in the plant enzymes. Genes encoding C-terminal truncation products of up to 127 aa were over-expressed in *E. coli* and lysates were analyzed for CA-mediated exchange of ^{18}O between $^{13}C^{18}O_2$ and $H_2{}^{16}O$. Recombinant CcaA proteins with up to 60 aa removed (CcaAΔ60) were catalytically competent, but beyond this there was an abrupt loss of activity. CcaAΔ0, CcaAΔ40 and CcaAΔ60 also catalyzed the hydrolysis of COS, a CO_2 analogue, but CcaAΔ63 and CcaAΔ127 did not, indicating that truncations of 62 aa's resulted in a general loss of catalysis. Yeast two-hybrid analysis revealed that CcaA did not interact with RbcL or RbcS subunits, but there was strong CcaA-CcaA interaction. This protein interaction also ceased with C-terminal truncations in CcaA greater than 60 aa. The correlation between loss of CcaA-CcaA interaction and CcaA catalytic activity suggests that the proximal portion of the C-terminal extension is required for oligomerization and that this oligomerization is essential for catalysis.

B3-6

PROPERTIES OF THE N-TERMINAL REGION OF RECOMBINANT DIACYLGLYCEROL ACYLTRANSFERASE-I OF OILSEED RAPE

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Diacylglycerol acyltransferase (DGAT, EC 2.3.1.20) catalyzes the acyl-CoA dependent acylation of *sn*-1, 2-diacylglycerol to generate triacylglycerol and CoASH. We have isolated a cDNA encoding BnDGAT1 from cultures of *Brassica napus* L. cv Jet Neuf. The deduced amino acid sequence of this cDNA exhibited a relatively hydrophilic N-terminal region followed by a number of potential membrane-spanning segments, which was consistent with the membrane-bound nature of the enzyme. As an initial step in gaining insight into the structure/function properties of BnDGAT1, we produced a recombinant histidine-tagged N-terminal fragment of the enzyme ($BnDGAT1_{(1\text{-}116)}His_6$) in *Escherichia coli* BL21 (DE32) (Stratagene) using a pET26b(+) expression vector (Novagen, Inc.). The truncated protein was purified from extracts of ruptured bacteria using immobilized nickel ion affinity chromatography. Lipidex-1000 binding studies demonstrated that $BnDGAT1_{(1\text{-}116)}His_6$ interacted with a number of different ^{14}C-labeled acyl-CoAs, which was consistent with the presence of an acyl-CoA binding signature in the N-terminal segment of the enzyme. $BnDGAT1_{(1\text{-}116)}His_6$ exhibited positive cooperativity via interaction with acyl-CoA and behaved as an aggregate during gel filtration chromatography. Recently, we produced plate-like monoclinic crystals of $BnDGAT1_{(1\text{-}116)}His_6$ as a prelude to investigating the x-ray crystallographic structure of the N-terminal segment of the enzyme.

B3-7

THE p23 SURPRISE!

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The hsp90-based chaperone machinery is implicated in numerous cellular processes, including protein folding, signal transduction, protein trafficking, genomic silencing, and protein degradation. The study of hsp90 complexes, in particular those involving animal steroid receptors, has revealed that in addition to hsp90 several other chaperone components participate in the conformational regulation of hsp90 target proteins. The co-chaperones of hsp90 include p60/Hop/Sti1, hsp70, p23 and the high molecular weight immunophilins. It is proposed that hsp70 first contacts the substrate protein of hsp90 and then facilitates the transfer of the substrate to hsp90. Following loading of the substrate protein with hsp90, p60/Hop and hsp70 dissociate from the heterocomplex, which then progresses to the mature form. The mature complexes are characterized by the presence of p23 and a high molecular weight immunophilin. The role of p23 is to stabilize the complex. The hsp90-based chaperone system is present in plants. To date the components identified include hsp90, hsp70, p60 and the immunophilins. A p23-like activity was not detected in wheat germ lysate. This together with other observations led to the belief that a p23 homolog was not present in plants. Here we report the first demonstration of a plant p23 homolog and discuss its novel characteristics and why it had escaped detection thus far.

B3-8

PURIFICATION AND CHARACTERIZATION OF NOVEL PHOSPHOENOLPYRUVATE CARBOXYLASE ISOFORMS FROM ENDOSPERM OF DEVELOPING CASTOR OIL SEEDS

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Phosphospho*enol*pyruvate carboxylase (PEPC) is believed to play an important role in producing malate as a substrate for fatty acid biosynthesis by leucoplasts of developing castor oilseeds (COS). Two COS PEPC isoforms with differing molecular and kinetic properties were resolved by Superdex 200 gel filtration FPLC and purified to homogeneity. PEPC1 is a typical 415-kDa PEPC homotetramer, whereas PEPC2 is an unusual 700 kDa heteromeric complex consisting of the 114-kDa PEPC1 catalytic subunit and two immunologically unrelated polypeptides of about 83 and 80 kDa. The PEPC2 subunit composition is unprecedented in vascular plants, but is remarkably reminiscent of novel high M_r 'Class 2' PEPC isoforms recently identified in green algae. PEPC1 is less sensitive than PEPC2 to the allosteric inhibitors malate and glutamate. The ratio of PEPC1:PEPC2 increases during COS development, becoming maximal during the most active phase of triglyceride accumulation. We are currently engaged in: (i) a thorough kinetic comparison of the two PEPC isoforms, (ii) assessing the possible role of reversible phosphorylation in their interconversion, and (iii) MALDI-MS analysis of the lower M_r polypeptides that are part of the PEPC2 complex. This study represents the first detailed biochemical characterization of any seed PEPC. (Supported by NSERC)

B4 Concurrent: Plant Physiology I

B4-1

IDENTIFICATION OF *ARABIDOPSIS THALIANA* MUTANTS DEFICIENT IN PLASTIDIC REDOX RESPONSES USING CHLOROPHYLL-FLUORESCENCE IMAGING

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Photoinhibition has been defined as a light-dependent decreased in rates of photosynthesis due to an overreduction of photosystem II (PSII). Cold acclimated plants exhibit a decreased susceptibility to photoinhibition of photosynthesis at low temperature. Furthermore, it has been demonstrated that this decreased susceptibility is as a result of increased excitation pressure on PSII during development. This is reflected as an alteration in the redox state of PSII, which can be monitored *in vivo* using the technique of chlorophyll *a* fluorescence. In this manner, it has been suggest that the redox state of the photosynthetic apparatus can act as an environmental sensor to detect environmentally induced imbalances between photochemistry and metabolism. The localization of the chloroplastic redox sensor/signal has been examined with limited success and remains elusive. In order to identify metabolic components of this redox sensing pathway, we have initiated a molecular genetic approach. A genetic approach to study metabolism implies that introducing mutations in a regulatory metabolic network will result in phenotypic effects, which can then be selected or screened to allow identification. Subsequently, mutated genes can be isolated and characterized, eventually leading to a full description of a regulatory process at the biochemical and molecular level. Currently, we are using a chlorophyll-fluorescence digital-imaging system to screen a population of 40,000 T-DNA-mutagenized *Arabidopsis thaliana* plants for mutants that are altered in their photosynthetic responses to photoinhibition at low temperature in comparison to wild-type *Arabidopsis* plants. The results of our preliminary screens, limitations of the imaging technique and the implications for redox signaling will be discussed.

B4-2

MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF FIBER HEMP (*CANNABIS SATIVA L.)* UPON EXPOSURE TO HEAVY METAL STRESS

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We have been examining the influence of cadmium (Cd) exposure on fiber hemp. Parameters that have been examined in control and treated plants include biomass production, glutathione levels in root tissue, phytochelatin synthesis, and a range of properties associated with the photosynthetic apparatus. Field and controlled lab trials have shown that concentrations of Cd that are toxic to sensitive plant species, such as maize OH-43, appear to have no impact on biomass production in fiber hemp. Only at elevated levels of Cd insult (> 100 μM) do these plants exhibit signs of heavy metal induced stress, including reduced lateral root branching, chlorosis and reduced growth. We postulate that one of the reasons for the relatively high level of tolerance in fiber hemp may be due the naturally high levels of glutathione present in root tissue. Comparative studies of untreated *Cannabis sativa* var. Felina and Fedora with *Zea mays L.* var. OH-43 show glutathione levels of *C. sativa* to be 10 times greater than that of *Z. mays*, suggesting that the higher tolerance of *C. sativa* is correlated to phytochelatin synthesis. These results suggest that fiber hemp may be an ideal species for phytoremediation of soils contaminated by heavy metals.

B4-3

ECOPHYSIOLOGICAL ADAPTATIONS OF WINTER-HARDENED BLACK SPRUCE (*PICEA MARIANA*) AND TAMARACK (*LARIX LARICINA*) SEEDLINGS TO FLOODING

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Black spruce and tamarack are the predominant tree species in boreal peatlands. The effects of flooding on morphological and physiological responses were investigated in greenhouse grown (18 months old) black spruce (*Picea mariana*) and tamarack (*Larix laricina*) seedlings for 34 days. Flooding resulted in reduced root hydraulic conductance, net assimilation rate and stomatal conductance and increased stomatal resistance to water vapor, and needle electrolyte leakage in both species. Flooded tamarack seedlings maintained a higher net assimilation rate and stomatal conductance compared to flooded black spruce. Flooded tamarack seedlings were also able to maintain higher root hydraulic conductance compared to flooded black spruce seedlings at a comparable time period of flooding. Needles of flooded black spruce appeared necrotic and electrolyte leakage increased over time with flooding and remained significantly higher than flooded tamarack seedlings. No visible damage symptoms were observed in flooded tamarack seedlings. Flooded tamarack seedlings developed adventitious roots beginning 14 days after the flooding treatment began. To investigate the possible physiological role of adventitious roots, their hydraulic conductivity was measured and compared with similarly sized flooded roots. Adventitious roots exhibited significantly higher root hydraulic conductivity. Flooded black spruce lacked any such morphological adaptation. These results suggest that tamarack seedlings were better able to adjust both morphologically and physiologically to a prolonged soil flooding than black spruce seedlings.

B4-4

ROLE OF AN *ARABIDOPSIS THALIANA* SULFOTRANSFERASE (*ATST2*) AND ITS SUBSTRATE (12-HYDROXYJASMONATE) IN FLORAL INDUCTION

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Jasmonates (JAs) are derived from linolenic acid and regulate a wide range of developmental processes including seed germination, senescence, and flowering. Recently, we characterized a gene (*AtST2*) from *A. thaliana*, which encodes a sulfotransferase exhibiting strict specificity for 11- and 12-hydroxyjasmonate (12-OHJA). *AtST2* expression was found to be induced by 12-OHJA and to be regulated by photoperiod. Transgenic *A. thaliana* plants expressing *AtST2* in sense and antisense orientations exhibited late and early flowering phenotypes, respectively, suggesting a link between 12-OHJA and flowering time. To further define this link we have analyzed *AtST2* protein levels in the JA biosynthetic mutant, *opr3* and in the JA perception mutants, *coi1* and *cev1*. Our results demonstrate that there is a correlation between the level of expression of *AtST2* and flowering time. The data also suggests 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.

B4 Concurrent: Plant Physiology I

B4-5

PUTATIVELY INACTIVE *CIS*-ISOMERS OF CYTOKININS PREDOMINATE EARLY IN SEED DEVELOPMENT AMONG THREE GENERA OF LEGUMES

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The physiological mechanisms behind high rates of fruit and seed abortion in legumes remains unresolved. Cytokinins (CK) are proposed as signaling molecules that enhance sink strength of developing fruits and seeds by up-regulating cell division. While *trans*-CK are currently believed to be the predominant and active regulators of seed development (i.e. in *Lupinus albus*), recent evidence of *cis*-CK predominating in chickpea (*Cicer arietinum*) questions this belief. Different fruiting patterns of legumes are observed among chickpea, pea *(Pisum sativum)* and lupine (*Lupinus spp.*). Peas are particularly interesting because they have a fruiting pattern similar to chickpea; however, their CK complement has not been rigorously characterized. Developing seeds of chickpea, pea and *L*. *polyphyllus* were harvested early in development while liquid endosperm was accumulating. Seed extracts were purified to isolate and separate 12 forms of CK for analysis by HPLC-electrospray tandem mass spectrometry (LC-MS-MS). Results confirmed the predominance of *cis*-CK in chickpea and revealed that pea seeds had a similar profile. Surprisingly, in contrast to *L. albus*, *L. polyphyllus* also had relatively large amounts of *cis* isomers and no detectable *trans*-CK. This evidence suggests a wider occurrence of *cis* isomers and poses questions about the role of CK form in regulating seed development in legumes.

B4-6

INTERACTIVE ROLE OF ETHYLENE AND POLYAMINES ON SHOOT MORPHOGENESIS *IN VITRO*

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In the genus *Brassica*, some species such as mustard and Chinese cabbage are recalcitrant in shoot regeneration from cultured tissues. To investigate the mechanism controlling shoot regeneration of these recalcitrant species, both chemical inhibitor and molecular approaches were employed. We found that regeneration could be greatly enhanced in the presence of an inhibitor of ethylene synthesis (e.g. aminoethoxyvinylglycine) or action (e.g. $AgNO_3$), or exogenous applications of polyamines (PAs). These results were supported by the responses of transgenic mustard plants expressing sense and/or antisense cDNAs of ACC synthase and ACC oxidase, which are the key enzymes of ethylene biosynthesis. One transgenic line that overexpressed sense ACC synthase RNA was shown to produce more ethylene and tissues were poorly regenerative in culture. On the contrary, transgenic plants expressing antisense ACC synthase or ACC oxidase RNA produced lower amounts of ethylene, and cultured tissues were highly regenerative. The regenerability of antisense explants could be suppressed by MGBG, an inhibitor of SAMDC, but the inhibitory effect of MGBG was abolished by exogenous putrescine. Antisense plants also accumulated PAs, especially spermidine, during shoot morphogenesis in culture. These results suggest that poor regeneration may be attributed to low levels of PAs in cultured tissues resulting from competition of SAM for ethylene synthesis, in view of SAM serves as a common precursor of ethylene and PA biosynthesis.

B4-7

ETHYLENE PRODUCTION, AS CONTROLLED BY THE EXPRESSION OF DIFFERENT ACC SYNTHSE GENES, DETERMINES THE TIME OF YEAR WHEN JAPANESE PEAR RIPENS

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Climacteric fruits are characterized by an increase in ethylene production and respiration at the onset of ripening, whereas non-climacteric fruits do not exhibit these characteristics. Ethylene production by cultivated Japanese pear fruits, which determines their shelf life, varied from 0.1 to 300nl C_2H_4/g. f.w./hr. during ripening. Relatively high ethylene led to reduced storage potential and fruit quality. We have identified RFLP markers tightly linked to the locus controlling ethylene evolution. The study was carried out using two 1-aminocyclopropane-1-carboxylic acid (ACC) synthase genes (*PPACS1, PPACS2*) as probes to cultivars producing different amounts of ethylene during ripening. When total DNA was digested by *Hin*d III and probed with *PPACS1*, we identified a band of 2.8kb specific to cultivars showing very high ethylene production (>10 nl/g f.w./hr) during fruit ripening. Probe *PPACS2* identified a band of 0.8kb specific to cultivars producing moderate amounts of ethylene (0.5 nl/g f.w./hr-10 nl/g f.w./hr) during ripening. These results suggest RFLP analysis with different ACC synthase genes will be useful for determining the maximum potential for ethylene synthesis during fruit ripening in Japanese pear. This RFLP approach using the two ACC synthase genes could be very useful in breeding strategies to improve fruit storage ability of this species.

B4-8

GENE EXPRESSION PROFILES FOR *ARABIDOPSIS* MAPKK/MAPK GENES DURING PLANT GROWTH AND DEVELOPMENT

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In plants, mitogen-activated protein kinase (MAPK) cascades have been implicated in controlling intracellular signaling processes in response to many external stimuli, including hormones, pathogens and abiotic stresses. The hallmark of a MAPK cascades is the participation of three classes of protein kinases (MAPK, MAPKK and MAPKKK) that operate hierarchically to amplify the initial signal. Plant genomes appear to encode an exceptionally rich array of MAPK cascade proteins (at least 20 MAPK and 10 MAPKK homologues have been identified in *Arabidopsis*) but functional analysis of this extensive matrix is just beginning. To gain insight into the specificity/redundancy of MAPKS and MAPKKS, we have used rt-PCR to examine the expression profiles of each of the identified MAPKK and MAPK genes in *Arabidopsis*. Gene expression patterns have been examined in various tissues, at several developmental stages and following a series of stress treatments. The observed patterns reveal that even closely-related MAPK/KK gene family members play discrete roles during plant growth and development, indicating that gene duplication and divergence have created a rich palette of signal transduction specialization during plant evolution.

C1 Plenary: Metabolic Control and Engineering

C1-1

THE PIVOTAL ROLE OF PHOSPHOENOLPYRUVATE IN PLANT METABOLISM

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PEP occurs at a major metabolic junction leading into plant primary and secondary metabolism. Moreover, plant glycolysis is uniquely regulated from the 'bottom up' with maximal flux control exerted at the level of PEP utilization. Thus, the elucidation of the control and integration of plant carbohydrate and energy metabolism necessitates a comprehensive analysis of PEP carboxylase (PEPC; PEP + HCO_3^- → Oxalacetate + Pi) and pyruvate kinase (PK; PEP + ADP → Pyruvate + ATP). PEPC is strictly cytosolic, whereas PK exists as distinct cytosolic and plastidic isozymes (PK_c & PK_p, respectively). To better understand the control of the cytosolic PEP branchpoint we have purified and characterized PEPC and PK_c from a green alga, canola suspension cells, ripening bananas, and germinated castor cotyledons. PK_c and PEPC kinetic/allosteric features are particularly well suited to their role in controlling the provision of: (i) mitochondria with respiratory substrates, &/or (ii) C-skeletons for NH_4^+-assimilation via GS/GOGAT. L-glutamate and L-aspartate appear to be key allosteric effectors that coordinate plant C- and N-metabolism via their potent feedback control of PK_c (inhibited by Glu, activated by Asp) and PEPC (inhibited by both Glu & Asp). Far less is known about the highly labile PK_p. However, feedforward activation of canola cell culture PK_p by 6-*P*-gluconate is suggested to balance NADPH generation (via OPPP) with stromal ATP and pyruvate production (via PK_p) for leucoplast anabolism. Finally, the presumed cyanobacterial origin of plant PK_p is inconsistent with several unexpected kinetic/allosteric properties exhibited by purified *Synechoccocus* PK. (Supported by NSERC)

C1-2

MOLECULAR GENETIC DISSECTION OF WAX BIOSYNTHESIS IN ARABIDOPSIS

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Cuticular waxes coat the aerial surfaces of land plants. Their primary function is to reduce non-stomatal water loss, but they also protect plants against UV radiation, play roles in a variety of plant-insect and plant-pathogen interactions, and are required for pollen fertility. Cuticular waxes are predominantly composed of long-chain aliphatic compounds derived from very long chain fatty acids (C_{26}-C_{32}). The biochemical pathways of fatty acid elongation and wax biosynthesis have been described. However, our knowledge of the enzymes involved in wax biosynthesis, and the genes that encode them is limited. Only five genes required for wax production have been cloned from Arabidopsis, and in most cases sequence analyses provided no assistance in assigning biochemical functions to these genes. The one exception is the *CER6* gene that encodes a key condensing enzyme involved in elongating fatty acid chains during wax production. Wax loads vary depending on developmental and environmental cues, but the mechanisms responsible for these variations have not been determined. A detailed analysis of *CER6* transcription during development and under different environmental conditions allowed us to begin to address this question.

C1-3

ONE-CARBON METABOLISM AND ITS ENGINEERING

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Plant one-carbon (C_1) metabolism is highly active and has some unique pathways, enzymes, and regulatory mechanisms. Three unique features are: (*1*) The enzyme producing methyl groups, methylenetetrahydrofolate reductase, is not feedback inhibited by *S*-adenosylmethionine (AdoMet) as it is in other eukaryotes. This AdoMet feedback loop in other eukaryotes exerts major control over C_1 flux to methionine and AdoMet. *(2)* Plants synthesize *S*-methylmethionine (SMM) and have a futile cycle (the SMM cycle) in which SMM is first synthesized by an AdoMet-dependent methylation of methionine, then reconverted to methionine. This cycle short-circuits the activated methyl cycle and consumes as much as half the AdoMet produced. (*3*) The enzyme 5-formyltetrahydrofolate cycloligase is mitochondrial, not cytosolic as in other eukaryotes. The cycloligase is considered to govern the level of 5-formyltetrahydrofolate, which is a potent inhibitor of various folate-dependent enzymes and may have a regulatory function in C_1 metabolism. All three of the above plant-specific features appear to be evolutionarily ancient. Metabolic engineering, metabolic modeling, and reverse genetics have recently shed light on the functional significance of these features, and shown how features *1* and *2* may be related.

C1-4

FLORAL SCENT - FROM COMPOUNDS TO METABOLIC PATHWAYS AND THEIR REGULATION

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Despite the importance of floral scent compounds in plant biology, our knowledge of their biosynthesis, accumulation and release is still limited. Benzenoid ester, methylbenzoate was used as an example to understand how scent production and emission is regulated in plants. Emission of methylbenzoate in snapdragon flowers is developmentally regulated and occurs in a rhythmic manner, with maximum emission during the day. Methylbenzoate is made primarily in the cytosol of connical cells of upper and lower petal lobes by the action of the biosynthetic enzyme S-adenosyl-L-methionine:benzoic acid carboxyl methyltransferase (BAMT) which catalyzes the transfer of the methyl group of S-adenosyl-L-methionine to the carboxyl group of benzoic acid (BA). Transcriptional regulation of expression of BAMT gene at the site of emission and the level of supplied substrate, BA, for the reaction were found to be the major factors controlling methylbenzoate production, and indirectly methylbenzoate emission during flower development. The level of BA also plays a major role in the regulation of circadian emission of methylbenzoate. in diurnally (snapdragon) and nocturnally (*Petunia cv Mitchell* and *Nicotiana suaveolens*) emitting flowers. Biosynthesis of BA in petal tissue occurs via non-oxidative pathway with benzaldehyde as a key intermediate. This work is supported by NSF grant IBN-9904910.

C2 Concurrent: Plant Molecular Biology

C2-1

SEQUENCE DIFFERENCES BETWEEN THE GENES FOR ENDO-BETA-MANNANASE IN TOMATO FRUIT CULTIVARS PRODUCING ACTIVE AND INACTIVE FORMS OF THE ENZYME

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Endo-beta-mannanase cDNAs were cloned and characterized from ripening tomato fruit of the cultivar Trust which produces an active enzyme and from the cultivar Walter which produces an inactive enzyme. In cv Trust, but not in cv Walter, there is a two-nucleotide insertion located 20 base-pairs upstream from the stop codon of the gene which results in a frame-shift and the addition of four amino acids in the full-length protein. Genomic clones of endo-beta-mannanase from four parental lines of the cv Walter, which produce inactive enzyme, all have the same two-nucleotide deletion. Other unrelated cultivars which either produce active or inactive enzyme show the same presence or absence of the two-nucleotide deletion. In a two-way genetic cross between cv Walter and cv Trust, all progeny from both crosses produced fruit with active enzyme, suggesting this form is dominant and homozygous in cv Trust. Heterologous expression of the two endo-beta-mannanase genes in *E. coli* resulted in active enzyme being produced from cultures containing the cv Trust gene and inactive enzyme being produced from cultures containing the cv Walter gene. Site-directed mutagenesis established key elements in the C-terminus of the endo-beta-mannanase protein which are responsible for full enzyme activity.

C2-2

ISOLATION AND CHARACTERIZATION OF PHYTOCHROME GENES, *PHYB* AND *PHYC* IN *STELLARIA LONGIPES*

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Stellaria longipes grows in a variety of habitats. We selected two ecotypes (dwarf alpine and tall prairie) to understand the role of phytochrome in stem elongation plasticity. We isolated and sequenced two cDNA clones from the prairie ecotype using PCR, RT-PCR, RACE cloning and have deduced the amino acid sequences of two full-length polypeptides. Based on the deduced amino acid sequences, one of the two polypeptides shows 73%, 52% and 50% amino acid identity to the *Arabidopsis phyB, phyA* and *phyC* open reading frame, respectively. We designated this cDNA clone as *Stellaria phyB* phytochrome, which contains 1128 amino acids. The other polypeptide shows 57%, 51% and 49% amino acid identity to the *Arabidopsis phyC, phyA* and *phyB* open reading frame, respectively. We designated this cDNA clone as *Stellaria phyC* phytochrome, which contains 1114 amino acids. These polypeptides (*phyB* and *phyC*) are unique in that they have low sequence identity (47%) with each other. Southern blot analysis indicates that *Stellaria phyB* and *phyC* are single-copy genes.

C2-3

EXPRESSION PATTERN COMPARISON FOR THE TWO GENE COPIES ENCODING *ARABIDOPSIS THALIANA* RIBOSOMAL PROTEIN L23A

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Two gene copies, *RPL23A-1* and *RPL23A-2*, encode *A. thaliana* ribosomal protein (r-protein) L23A, a member of the conserved EL23/L25 r-protein gene family of primary ribosomal RNA (rRNA) binding proteins. RPL23A-1 and RPL23A-2 are 94% identical at the amino acid level, but their genes share only 44% identity within the 5' regulatory regions. In order to investigate gene expression patterns, Arabidopsis plants were transformed with constructs containing the 5' regulatory region of either *RPL23A-1* or *RPL23A-2* upstream of the *uidA* (*GUS*) reporter gene. Slightly different expression patterns from the two regulatory regions were observed in preliminary results, with only the *RPL23A-2* 5' regulatory elements directing expression in sepals. RT-PCR using RNA from wild type and treated Arabidopsis plants was used to generate expression profiles for *RPL23A-1* and *-2.* Preliminary results indicate that both genes are upregulated by auxin, cytokinin, and giberellic acid treatments and are downregulated following abscisic acid treatment, with the responses to these hormones differing quantitatively between the two gene copies. RT-PCR results from heat, cold, and wounding-stressed seedlings will also be discussed. Despite the difference in their primary sequences, the regulatory regions of *RPL23A-1* and *-2* are still able to direct similar expression patterns.

C2-4

DNA RECOMBINATION AND REPAIR PROCESSES IN ARABIDOPSIS: CHARACTERIZATION AND MANIPULATION

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Manipulation of homologous recombination frequency in meiotic and vegetative tissues of plants has important implications for crop improvement through the acceleration of breeding programs and development of practical gene targeting strategies for plants. We are characterising genes associated with DNA recombination and repair processes in Arabidopsis and budding yeast to develop mechanisms to manipulate these processes in crop species. Molecular analysis demonstrates that *AtRAD51* is strongly induced in vegetative tissues when exposed to a DNA damaging agent like gamma radiation, whereas *AtMRE11* is not. Arabidopsis lines homozygous for null alleles of either gene show increased sensitivity to gamma radiation, providing genetic evidence that both genes play a role in DNA repair in plants. In addition, both mutant lines are sterile, implying a role for *AtRAD51* and *AtMRE11* in meiotic DNA recombination in plants. Our work with the yeast model has expedited evaluation of strategies for manipulating recombination frequency in plants. We demonstrate that overexpression in yeast of wild type ScDMC1, ScRAD51 or ScSPO11 increases meiotic recombination frequency whereas overexpression of specific mutant versions of these proteins or their wild-type Arabidopsis homologues confers a dominant-negative effect. Possible biochemical mechanisms for these effects are being investigated by yeast two-hybrid analysis.

C2-5

ISOLATION AND CHARACTERIZATION OF RAPID ALKALINIZATION FACTORS (RALFS) FROM HYBRID POPLAR

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Alkalinization of extracellular pH in cell culture is one of the earliest cell responses to elicitors derived from pathogens and herbivore-wound hormones such as systemin. The biological function of alkalinization of extracellular pH is not understood; investigating this phenomenon will contribute to our understanding of the elicitor-triggered signaling. Using a poplar cell culture, we identified two different medium pH-alkalinizing factors in poplar leaf extracts. One factor induced a pH increase within 5 min, while the second caused alkalinization after approximately 25 min. The factor inducing rapid alkalinization was found to be a peptide, and its N-terminal was determined. Database searches of the peptide sequence showed that it was a tobacco RALF homolog (*R*apid *A*lkalinization *F*actor, Pearce et al., 2001). Two poplar cDNAs (RALF1 and RALF2) encoding RALF peptides were cloned and their expression patterns analyzed. RALF2 was expressed in all tissues except in leaves, and down-regulated by methyl jasmonate (MeJa) in suspension culture. By contrast, RALF1 was expressed in all tissues examined, and MeJa did not affect its expression. Although the specific role(s) of RALF in planta is as yet unknown, the discovery of poplar RALFs is a first step in the discovery of novel signaling pathways in plants.

C2-6

DEVELOPMENT OF AN INVENTORY OF EXPRESSED SEQUENCE TAGS IN SPRUCE AND POPLAR

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In the Canadian forest industry, spruce (*Picea*) is the predominant harvested species for both structural timber and pulp. Poplar (*Populus*) is the most widespread hardwood in Canada and is a "model tree" species. Both spruce and poplar are the subjects of conventional tree breeding programs for growth, wood quality and pest resistance. As a first step towards understanding the gene expression profiles related to wood formation, forest health and tree defense mechanisms, we have initiated the development of large-scale expressed sequence tag (EST) collections (~100,000 ESTs/species). RNA for cDNA library construction has been isolated from a broad range of tissues (e.g. xylem, phloem, flowers, leaves, roots, embryos) and developmental stages, as well as from trees exposed to biotic and abiotic stresses. Normalization strategies have been implemented to minimize the number of redundant sequences. In addition, we are also generating normalized full-length cDNA libraries. Longer EST sequences permit phylogenetic comparisons between the evolutionarily divergent conifers and angiosperms, and are essential to link gene discovery and functional genomics through biochemical approaches. Ultimately, collections of ESTs will be arranged in microarrays to examine global gene expression profiles associated with defense against pests, adaptations to various forms of environmental stress, and specific stages of wood development and embryo maturation.

C2-7

THE ROLE OF ARGININE IN REGULATING LOBLOLLY PINE ARGINASE GENE EXPRESSION *IN VITRO*

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In loblolly pine (*Pinus taeda* L.) seeds, greater than 85% of the storage protein reserve is located in the megagametophyte; the remainder is distributed in the embryo. The largest amount of nitrogen in these proteins is stored as arginine, a very efficient nitrogen storage compound. Following seed germination, storage protein breakdown in the megagametophyte and in the seedling results in a large increase in the seedling's free arginine pool. This arginine is hydrolyzed in the seedling by the enzyme arginase (E.C. 3.5.3.1), which is under strong developmental control. Using an *in vitro* culture system to address the separate impacts of the seedling and megagametophyte tissues on arginase we have shown that arginase expression in the cotyledons is initiated by the seedling itself and not by the megagametophyte as previously proposed. Application of arginine in the absence of the megagametophyte further stimulated arginase gene expression, suggesting the megagametophyte's contribution to arginase regulation is limited to supplying free amino acids to the seedling. Results obtained using this *in vitro* approach have allowed us to propose a model of arginase regulation whereby arginine derived from both the seedling and megagametophyte is responsible for regulating arginase during early seedling growth.

C3 Concurrent: Plant Biochemistry and Metabolism II

C3-1

BIOCHEMISTRY, MOLECULAR GENETICS, CELL BIOLOGY AND GENOMICS OF INDUCED TERPENOID DEFENSES IN *PICEA SPP*.

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Species of spruce (*Picea* spp.) are dominant conifers of Canada's boreal forests. They are hosts to a wide range of insect pests, including bark beetles, weevils and budworms, and insect-associated pathogens. Direct and indirect chemical defenses of conifers depend largely on volatile and non-volatile terpenoids. In spruce, terpenoids accumulate constitutively in cortical stem resin ducts. Stem boring insects, pathogens and treatment with methyl-jasmonate induce *de novo* cell differentiation of resin ducts in developing xylem. This *de novo* differentiation is initiated in cambial meristems, associated with increased gene expression and enzyme activities of terpenoid biosynthesis and accumulation of resin terpenoids. Foliage feeding insects and treatment with methyl-jasmonate also induce diurnal emission of new terpenoid volatiles and methyl-salicylate, associated with increased terpene synthase (TPS) enzyme activities in foliage. Induced volatiles can potentially function in attraction of predators and parasitoids of insect herbivores. Following metabolic profiling of constitutive and induced terpenoid defenses, we isolated and functionally identified a suite of *TPS* genes of direct and indirect defense in spruce. Gene expression profiling, differential protein profiling (2D-SDS PAGE, Q-TOF), and large-scale EST sequencing are being applied to further unlock the molecular genetic and biochemical events of direct and indirect defenses in spruce.

C3-2

SUBSTRATE SPECIFICITY OF MICROSOMAL LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASE FROM CULTURES OF OILSEED RAPE

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Acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT; EC 2.3.1.23) is a membrane-bound enzyme that catalyzes the acyl-CoA-dependent acylation of lysophosphatidylcholine (LPC) to produce phosphatidylcholine (PC) and CoASH. LPCAT activity may affect the incorporation of fatty acyl (FA) moieties at the *sn*-2 position of PC where polyunsaturated fatty acids (PUFAs) are formed. *sn*-1,2-Diacylglycerol (DAG), derived from PC, may be used as substrate in the acyl-CoA- dependent biosynthesis of triacylglycerol (TAG). PC can also serve as a source of *sn*-2 FA moieties in the acyl-CoA-independent biosynthesis of TAG via donation of the FA moiety to *sn*-1,2-DAG. The resulting LPC product could then serve as substrate for LPCAT. Thus, LPCAT activity and the specificity properties of the enzyme may affect the subsequent incorporation of PUFAs into TAG. LPCAT activity from microspore-derived cell suspension cultures of oilseed rape (*Brassica napus* L. cv Jet Neuf) was assayed using [1-^{14}C]acyl-CoA as the FA donor. At acyl-CoA concentrations above 20 µM, LPCAT activity was more specific for oleoyl (18:1)-CoA than stearoyl (18:0)- and palmitoyl (16:0)-CoA. Lauroyl (12:0)-CoA, however, was not an effective acyl donor. LPC species containing 12:0, 16:0, 18:0 or 18:1 as the FA moiety all served as effective acyl acceptors for LPCAT. The failure of LPCAT to catalyze the incorporation of a lauroyl moiety from acyl-CoA into PC is consistent with the exclusion of this FA moiety from the *sn*-2 position of TAG from the seed oil of *B. napus*.

C3-3

TRIACYLGLYCEROL BIOSYNTHESIS IN DEVELOPING FLAX SEED

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Flax (*Linum usitatissamum*) seed triacylglycerols (TAGs) are enriched in omega-3 fatty acids, which have been shown to have number of health benefits. Characterization of TAG biosynthesis in flax will provide valuable information for genetic engineering of flax to further enhance the nutraceutical value of this crop. Under greenhouse conditions, lipid accumulation in developing flax reached a plateau about 20 days after flowering. The proportion of linolenic acid (18:3) in TAG increased markedly during seed development thus accounting for the high level of this fatty acid in the mature seed. Microsomal diacylglycerol acyltransferase (DGAT, EC 2.3.1.20) activity reached a maximum during the active phase of lipid accumulation. DGAT catalyzes the acyl-CoA dependent acylation of *sn*-1,2-diacylglycerol to produce TAG. The activity of DGAT may have a substantial affect on the flow of carbon into seed oil. The incorporation of [1-^{14}C]oleoyl(18:1) moieties into TAG was linear for at least 60 minutes and maximum enzyme activity occurred at an 18:1-CoA concentration of 60 µM. Similar specific activities for microsomal DGAT were determined using 18:1-, 18:3-, eicosapentaenoyl (EPA)- and docosahexaenoyl (DHA)-CoA. The acyl-CoA specificity study suggested that DGAT would not limit the incorporation of EPA and/or DHA into the TAG of flax that was genetically engineered to produce these nutraceutical fatty acids.

C3-4

IMMUNOLOCALIZATION OF ALKALOID-SPECIFIC ENZYMES IDENTIFIES ALKALOID-SYNTHESIZING CELLS IN *PAPAVER SOMNIFERUM*

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Two branch pathways of benzylisoquinoline alkaloid biosynthesis in opium poppy produce the pharmaceuticals morphine and sanguinarine. (*S*)-*N*-methylcoclaurine 3'-hydroxylase (CYP80B1) is a P450-dependant monooxygenase operating in the common pathway leading to both alkaloids. In contrast, berberine bridge enzyme (BBE) represents the first committed step in the sanguinarine branch pathway, whereas the penultimate step in morphine biosynthesis is catalyzed by codeinone reductase (COR). Morphine is a major component of the alkaloid-rich latex in opium poppy. The latex is the cytoplasmic contents of laticifers, which form an internal secretory system associated with the phloem tissues throughout the plant. The alkaloid composition of laticifers varies among different organs, with morphine found primarily in the stem and sanguinarine found mostly in the root, suggesting that alkaloid biosynthesis is controlled by developmental factors. Previous studies have suggested that laticifers are the site not only of product accumulation, but also of alkaloid biosynthesis. We have raised antibodies against CYP80B1, BBE, and COR and used these to localize alkaloid biosynthesis in opium poppy. Our results show that morphine and sanguinarine biosynthesis occurs in the same cell, and that this cell type is distinct from laticifers. Thus, the latex is not the site of alkaloid biosynthesis as previously suggested.

C3-5

REGULATION OF CONDENSED TANNIN IN TREFOIL (*LOTUS*) SPECIES

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Condensed tannins or proanthocyanidins are polymerized units of catechin, arising from a specific and little-understood branch of the flavonoid pathway. Control over condensed tannin levels is desirable, as these are important metabolites in livestock feed and human food. We have sought out candidates for condensed tannin regulation in several plant species. Recently, we isolated a set of basic helix-loop-helix (*myc*-like) genes from the forage trefoils (*Lotus* species). Expression of one of these genes, TAN-1, correlates well with condensed tannin levels in leaves of several forage legume species, ranging from alfalfa and *L. japonicus* (no-tannin species) to sainfoin and *L. uliginosus* (high-tannin species). A homologue in *L. japonicus* is interrupted in a leaf tannin-accumulating mutant, *tan-1*, confirming its role in tannin regulation. We describe this set of genes and discuss the effects of TAN-1 on the flavonoid pathway. This is the first identified regulatory gene to be associated with condensed tannin accumulation.

C3-6

CLONING, CHARACTERIZATION, AND SUBCELLULAR LOCALIZATION OF PLANT 5-FORMYLTETRAHYDROFOLATE CYCLOLIGASE

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5-Formyltetrahydrofolate cycloligase (5-FCL) catalyzes the ATP-dependent conversion of 5-formyltetrahydrofolate (5-CHO-THF) to 5,10-methenyltetrahydrofolate and is the main if not sole means whereby bacteria, yeast, and mammals metabolize 5-formyltetrahydrofolate. 5-CHO-THF is known to occur in plants, and to be particularly abundant in leaf mitochondria. Genomics-based approaches identified an *Arabidopsis* cDNA specifying a protein homologous to 5-FCLs of other organisms but containing a unique N-terminal extension with the features of a mitochondrial targeting peptide. The *Arabidopsis* 5-FCL homolog was shown to have 5-FCL activity by characterizing recombinant protein produced in *E. coli* with or without the mitochondrial targeting peptide. Removal of the N-terminal extension greatly enhanced 5-FCL activity. At5-FCL was active as a monomer, preferred the penta- to the monoglutamyl form of 5-CHO-THF, and otherwise showed properties intermediate between mammalian and bacterial 5-FCLs. Enzyme assays and western blot analyses demonstrated that plant 5-FCL is located in mitochondria, and that the mitochondrial polypeptide is the same size as the processed recombinant protein. Plant mitochondria are rich in 5-CHO-THF, and contain several one-carbon enzymes whose mammalian homologs are inhibited by this folate. 5-FCL could therefore be an important factor in regulation of one-carbon metabolism in plant mitochondria.

C3-7

GENETIC ENGINEERING OF CANOLA WITH DECREASED PALMITIC ACID CONTENT IN THE SEED OIL

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Canola oil is known as healthy oil that typically contains less than 7% saturated fatty acids (SFAs). In recent years, the SFA content of canola oil has exceeded the 7% limit due to an increased reliance by producers on *Brassica napus* varieties. The major products of fatty acid (FA) biosynthesis in plastids of developing seeds of *B. napus* are stearic (18:0) and palmitic (16:0) acid which are formed as a thioester of acyl carrier protein (ACP). A soluble Δ9-18:0-ACP desaturase catalyzes the production of *cis* Δ9-oleoyl (18:1)-ACP. Oleic acid (*cis* Δ 9-18:1) is the predominant FA in canola oil and palmitic acid (16:0) is the major component of SFAs (4% out of 7%). In an attempt to decrease SFA content of canola oil, *B. napus* L. cv Westar was transformed with a cDNA encoding a Δ9-16:0-ACP desaturase from the forest vine, Cat's claw (*Doxanthia uniguis-cati* L.). The goal of the transformation was to convert 16:0-ACP to *cis* Δ9-palmitoleoyl (16:1)-ACP so as to decrease the SFA content of canola oil. The oil of the transgenic plants displayed a significant increase ($P < 0.001$) in 16:1/16:0 ratio compared to control plants but overall SFA content was either unaffected or increased somewhat. Similar results were found in transformations of *Arabidopsis thaliana*. Further studies are being conducted in order to gain insight into the mechanisms accounting for the observed FA composition of the seed oil from transgenic plants.

C3-8

THE BIOCHEMICAL CHARACTERIZATION OF A WOUND-INDUCED PLASMA MEMBRANE NAD(P)H DEPENDENT OXIDASE

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In their native environment, plants are exposed to a variety of environmental challenges including mechanical injuries and pathogen attack. Therefore, a strong defense mechanism is crucial for plant survival and fitness. Plant defenses tend to be site specific such that the affected cells or tissues are healed or isolated from healthy, unaffected ones. In the case of injury, this is usually accomplished by producing high quantities of toxic chemicals (e.g., H_2O_2, secondary metabolites) to kill pathogens followed by the formation of a polymeric barrier next to the infected site (e.g., a suberized layer). Apart from the direct toxic effect of H_2O_2 on pathogens, this reactive oxygen species (ROS) is also required for the oxidative cross-linking of cell wall components. This latter role is important during wound-induced suberization since H_2O_2 is a critical substrate for the peroxidase-mediated polymerization of the suberin poly(phenolic) domain. In the current study, we provide preliminary biochemical characterization data for a plasma membrane NAD(P)H-dependent oxidase responsible for the production of ROS during wound-induced suberization in potato tubers.

C4 Concurrent: Plant Physiology II

C4-1

EFFECTS OF MODERATE LIGHT UNDER LOW TEMPERATURE ON PHOTOCHEMICAL ACTIVITY OF PHOTOSYSTEM II IN INTACT LEAVES

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The functional activity of photosystem II (PSII) in cucumber (*Cucumis sativus*), maize (*Zea mays*), pumpkin (*Cucurbita pepo*), and tomato (*Lycopersicum esculentum L*) leaves exposed to moderate light intensity (300 µmol $m^{-2}.s^{-1}$) at 4°C was analysed using chlorophyll *a* fluorescence rise kinetics. Chlorophyll *a* fluorescence rise in control leaves displayed a typical polyphasic rise comprising of O-J-I-P phases. Moderate light intensity at 4°C drastically altered the fluorescence rise at photochemical phase (J level) and the thermal phase constituting I and P phases as the treatment time increased. In addition, the initial fluorescence intensity (F_o) progressively increased in cucumber, maize and pumpkin leaves under these conditions. The quantum yield of PSII, measured as F_v/F_m ratio, declined by 44% in cucumber, 30-35% in maize and pumpkin leaves. On the other hand, the F_v/F_m ratio decreased by 19% in tomato leaves incubated under similar conditions. This ratio decreased further in leaf segments incubated in diuron solution after moderate white light treatment. For instance, the PS II efficiency declined by 50% in cucumber and pumpkin and by 40% in maize leaves. These changes in F_v/F_m ratios are attributed to the loss of variable fluorescence, F_v in photoinhibited leaves of the four plants used. The data suggests that the PSII of cucumber, pumpkin and maize is highly sensitive to the combined stress of low temperature and moderate light.

C4-2

PHOTOINHIBITION AND RECOVERY IN THE ANTARCTIC GREEN ALGA *CHLAMYDOMONAS SUBCAUDATA*

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Chlamydomonas subcaudata was isolated from a perenially ice-covered Antarctic lake where it grows under the stable environment of low temperatures (4-6º C) and low light (14 micromol/m^2/ s). *C. subcaudata* is not able to undergo state transitions, an important mechanism to balance light absorption. Thus, this green alga is the first example of a natural state transition mutant. Despite adaptation to low irradiances, does this psychrophile possess mechanisms that protect it from low-temperature photoinhibition? The present study examines the sensitivity to and the capacity to recover from photoinhibition in *C. subcaudata* and the mesophile, *C. reinhardtii*. Both species were exposed to 600 µ mol photon/m^2/s at 8ºC for 2h after which they were allowed to recover at 20 µ mol/m^2/s. Fv/Fm and xanthophyll cycle activity were examined. The role of chloroplastic protein synthesis was also examined by monitoring the effects of lincomycin. Fv/Fm decreased 50% during photoinhibition, however, it recovered 72% during the first 20 min at 8º C in *C. subcaudata*. Chloroplastic protein synthesis and the xanthophyll cycle played an insignificant role in both photoinhibition and recovery. This Antarctic alga appears to possess unique mechanism(s) enabling it to tolerate and recover from high light at low temperature compared to *C. reinhardtii*.

C4-3

PHOTOINHIBITORY LIGHT INDUCED ALTERATIONS OF CHLOROPHYLL-PROTEIN COMPLEXES AND ENERGY TRANSFER IN PHOTOSYSTEM I SUBMEMBRANE PARTICLES

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The activity of light-induced oxygen uptake, absorption spectra, low temperature (77 K) chlorophyll (Chl) fluorescence emission and excitation spectra were studied in suspensions of photosystem (PS)I submembrane particles illuminated by strong white light (WL) of 2000 µE m^{-2} s^{-1} at 4°C. A significant stimulation of oxygen uptake was observed during the first 1-4 h of photoinhibitory treatment, while it rapidly dropped down during further light exposure. Chl content gradually declined during the exposure of isolated PSI particles to strong light. A 7-nm blue shift peak was observed at 680 nm in absorption spectra after 6-h illumination. Even more pronounced changes were found in the characteristics of Chl fluorescence. The magnitude of the dominating long-wavelength emission band located at 736 nm in untreated particles was already 5 times reduced after 2-h exposure. The major peak in low-temperature Chl fluorescence emission spectra shifted from 736 to 721 nm after 6-h WL treatment. The absorption spectra of individual Chl-protein complexes differed in response to strong WL. Unlike light-harvesting complexes (LHC), LHCI-680 and LHC-730, which did not exhibit changes in the major peak position, its maximum was shifted from 678 to 671 nm in CPIa complex after PSI submembrane particles were irradiated with strong light for 6 h. The results demonstrated that excitation energy transfer represents the stage of photosynthetic utilisation of absorbed quanta which is most sensitive to light in isolated PSI particles.

C4-4

THE ROLE OF ALTERNATIVE OXIDASE IN REGULATION OF HIGH ENERGY INPUT INTO THE PHOTOSYNTHETIC METABOLISM.

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We have grown *Arabidopsis* plants over expressing alternative oxidase and *Arabidopsis* plants with an anti-sense construct of alternative oxidase. We observe a difference in the capacity to transport electrons in the thylakoid membrane during increasing light (measured as q_P). Furthermore, we also observe differences in the energization of the thylakoid membrane (measured as q_N), between the line over expressing alternative oxidase and the anti-sense line, in the increased light. These lines also show a difference in temperature sensitivity (Dr. McIntosh personal communication). After these changes were observed, we characterised the transformants with respect to ATP/ADP ratios in both normal, growth, light and at higher light intensities (still less than saturating light). We also measured the effect of Oligomycin on q_P, q_N, F'v/F'm and O_2-evolution in these plants.
Further, we have looked at the response of the different lines to low temperature acclimation with respect to PSII photochemistry (q_N and q_P) and low temperature. This is a treatment well defined in producing an extensive high energy input in plants and there is a clear difference between the plant over expressing alternative oxidase and the plant with an anti-sense construct of alternative oxidase with respect to q_P during low temperature acclimation as well as at low temperature stress. We have also looked at the activity of NADP-MDH and Alternative Oxidase expression in these plants, both at normal growth temperature and throughout the low temperature acclimation process.

C4-5

EFFECTS OF WATER DEFICIT STRESS ON ROOT WATER FLOW PROPERTIES IN TREMBLING ASPEN

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Effects of water deficit stress on root water flow properties were studied in trembling aspen (Populus tremuloides Michx.) seedlings grown in sand and in solution culture. An exodermis was present in sand-grown roots systems, but absent from seedlings growing in solution culture. Root hydraulic conductivity (Lpr) declined with increasing water deficit stress in aspen grown in both media and increased in sand-grown plants following rewatering. Root respiration increased with increasing water deficit stress. Mercuric chloride resulted in reduction of Lpr due to inhibition of water flow through water channels (aquaporins, AQP). A relatively lower mercuric inhibition of Lpr in stressed seedlings suggests that AQP-mediated transport contributed less to root water flow in stressed plants. Mercuric inhibition was greater in solution culture-grown aspen, which was likely due to lack of an exodermis. The results on this study indicate that AQP activity may decrease with increasing water deficit stress as a means of regulating root water flow into and out of roots during water deficit stress. The exodermis may also play a role in conferring stress resistance by increasing resistance to root water loss.

C4-6

REGULATION OF WATER FLOW PROPERTIES IN ASPEN (*POPULUS TREMULOIDES*) SEEDLINGS BY PHOSPHOPRYLATION AND DEPHOSPHORYLATION OF AQUAPORINS

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Cell-to-cell water transport is an important pathway for water flow in roots. Water transport across the cell membranes is facilitated by water channel proteins (aquaporins). Their phosphorylation status may be one of the gating mechanisms for water permeation through the membranes. In the present study, we examined the modulation of aquaporin activity by phosphorylation using different phosphorylation and dephosphorylation inhibitors that were applied to the whole root systems. Aspen seedlings were grown in solution culture under controlled environmental conditions. When the seedlings were eight-weeks-old, their shoots were removed and the steady state root flow rates were measured using the hydrostatic method. Sodium orthovanadate (1mM, 5mM) decreased the root flow rates, while root respiration was not affected. Potassium fluoride (1mM, 5mM) and H-7 (1-(5-isoquinolinylsulfonyl)-2-methylpiperazine) (50 μM) also inhibited the water-flow rates. Okadaic acid, a phosphatase inhibitor, stimulated the steady-state root water flow. Phosphorylation and dephosphorylation of aquaporins are likely the principal processes regulating aquaporin activity. While for some inhibitors we observed a quick response in the steady-state root flow without an effect on root oxygen uptake, other phosphorylation and dephosphorylation inhibitors slowly affected aquaporin activity, likely through their effects on other metabolic processes.

C4-7

MEDICAGO INC, MANUFACTURING ALFALFA-MADE PHARMACEUTICALS

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Medicago Inc is a world leader in plant-made pharmaceuticals, specializing in the transformation of alfalfa into a living factory to produce high-value active molecules. Medicago inc is committed to provide a cheap, biosafe and renewable source of recombinant molecules for use in the biopharmaceutical, cosmaceutical and processing industries. The current forecast is that bioactive molecules will occupy 15-20% of the whole pharmaceutical market in 5 years from now, representing a market value of 50M$ with 15M$. Alfalfa (*Medicago sativa*) has been chosen for its perennial characteristics, its high protein content, and numerous environmental benefits. Since its incorporation in 1997, Medicago inc is conducting R&D activities to establish its technology platform. Medicago's efforts have focused on the development of proprietary expression cassettes, alfalfa transformation technologies, biomass production capacity, recovery and quality control processes. These efforts have generated a strong IP portfolio, several scientific publications and the establishment of important strategic alliances and partnerships. In 2001, Medicago entered its manufacturing phase following the signature of its first commercial prototype development contracts. Medicago plans to increase its workforce with major goals of increasing productivity, reducing the time from gene to plant, reaching optimal product conformity, building capacity for larger scale, satisfying regulatory requirements and further strengthening FTO and IP position.

C4-8

EVIDENCE FOR SINGLE CARBON METABOLISM IN NON-GREEN PINE TISSUES

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Methanol (CH_3OH) and formaldehyde (HCHO) are generally considered to be toxic but are among various volatile compounds that have been found in plants. There have been no previous investigations of CH_3OH and HCHO in relation to secondary growth. To understand heterotrophic carbon assimilation better, quantitative analyses were done of those endogenous compounds in cambium and developing xylem of eastern white pine (*Pinus strobus* L.). Trees (12 years old) were harvested at various times during the annual cycle of growth and dormancy, microscopy done to determine the stage of development in the cambial region, and corresponding tissues isolated into liquid nitrogen following bark peeling. CH_3OH was quantified by combined GC/MS in selected-ion monitoring mode, using a stable heavy isotope internal standard. An improved method for HCHO quantification was developed. Using $H^{14}CHO$ as internal standard, extracted HCHO was coupled to dimedone and, following C18 Sep-Pak prepurification, quantified as formaldemethone by reversed-phase gradient-elution HPLC (UV detection), assessing recovery by liquid scintillation spectroscopy. Levels of endogenous CH_3OH differed between cambium and developing xylem, and changes in both CH_3OH and HCHO levels in relation to the phenology of cambial growth were noted. CH_3OH levels were high during dormancy and dropped with springtime cambial reactivation, then increased with xylogenesis.

POSTER SESSION

8:00 PM- 11:00 PM

SUNDAY, JUNE 9, 2002

Poster Abstracts

P1

DIRECT MEASUREMENT OF 4-HYDROXYBUTYRATE (GHB) IN CRUDE PLANT EXTRACTS BY LIQUID CHROMATOGRAPHY/API-ES MASS SPECTROMETRY

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GHB (4-hydroxybutyrate) is a small hydroxycarboxylic molecule that is present in most organisms such as bacteria and mammals. However, it has not previously been detected in plants. , GHB is usually detected and quantified by gas-chromatography-mass spectrometry. In this paper, a simple, direct method was developed to detect and quantify 4-hydroxybutyrate (GHB) in crude plant extracts by liquid chromatography/atmospheric pressure ionization-electrospray mass spectrometry (LC/API-ES MS). The chromatographic separation was achieved on a aqueous reverse-phase column isocratically with 10 mM ammonium formate (pH 3.0) and 1% methanol. GHB and its lactone, gammabutyrolactone (GBL) were monitored by API-ES mass spectrometry using selective ion monitoring (SIM) in negative mode. Even though GHB and GBL co-eluted at 3.4 minutes, they could clearly be distinguished by their unique mass-to-charges ratios. The compounds were quantified using deuterated 4-hydroxybutyrate (GHB-d6) as a suitable internal standard. The method was linear up to 20 nmol, with a detection limit of 0.1 nmol, regardless of whether GHB was made up in 10 mM ammonium formate (pH 3.0) or added to tobacco leaf extracts. GHB concentrations in tobacco leaves ranged from 1.9-31.4 nmol g^{-1} FW and in *Arabidopsis* leaves from 1.9- 49.6 nmol g^{-1} FW.

P2

RESPONSE OF BARLEY, FIELD PEA AND CANOLA TO ETHYLENE EXPOSURE

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In the field, emissions of ethylene from point sources such as those from industrial facilities might have negative effects on plants. To gain information on the potential effects of ethylene exposure, experiments conducted in a growth chamber gas exposure system were designed to: 1) study the response of crops to short- and long-term exposures to ethylene; 2) determine the critical duration of exposure at 50 ppb that would cause significant effects on yield; 3) test whether the sensitivity of barley to ethylene varied with time of day; and 4) develop a dose-response relationship that describes the response of various crops to ethylene. No significant effects of short-term (<12h) exposure to ethylene up to 1200 ppb were observed in barley, field pea and canola. Symptoms of ethylene exposure were observed in plants exposed for more than 3 days. In the cultivars studied, barley was found to be more sensitive to ethylene than field pea and canola. The response of plants to long-term ethylene exposure depended on concentration and length of exposure. The sensitivity of barley varied with time of day of ethylene exposure. A mathematical expression of dose was developed to describe the yield loss of crops exposed to ethylene.

P3

A POSSIBLE ROLE FOR CLP PROTEASE IN THE DEGRADATION OF CYANOBACTERIAL LIGHT-HARVESTING COMPLEXES

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Phycobilisomes are large pigment-protein complexes that harvest light energy for photosynthesis in cyanobacteria. When the cyanobacterium *Synechococcus elongatus* is starved for an essential nutrient such as sulfur, the phycobilisomes are degraded in a rapid and orderly manner. To date the identity of the protease(s) involved in phycobilisome degradation has remained elusive. One possible candidate is the Clp protease, which is composed of separate proteolytic and regulatory ATPase subunits. Three isomers of the proteolytic subunit exist in *Synechococcus*, ClpP1-3. Two other Clp proteins, ClpC and ClpX are likely partners in Clp proteolytic complexes as the ATPase subunits. Here we report that *Synechococcus* cells in which the *clpP1* gene has been inactivated do not degrade their phycobilisomes to the same extent as wild-type cells when starved of sulfur. The extent of degradation can be estimated by determining phycocyanin pigment content in cell cultures since virtually all phycocyanin is assembled into phycobilisomes. In wild-type cultures the level of phycocyanin decreased by 80% after 96 hours whereas in Δ*clpP1* cultures phycocyanin content dropped by only 20%. Coomassie staining of phycobilisomes isolated after 24 hours of growth in sulfur-deficient media confirmed a loss of the outermost polypeptide components in wild-type but not in Δ*clpP1* cultures. Levels of all three Clp isomers in the wild-type increased during sulfur deprivation, as did ClpX, whereas the level of ClpC remained constant.

P4

GIBBERELLIN TRANSPORT DURING EARLY SEEDLING GROWTH

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The regulation of gibberellin (GA) levels in young pea seedlings (*Pisum sativum* L. cv. Carneval) was examined by transport studies of [^{14}C]GA_{20}. GA_{20} is considered a potential transport form of GA that is subsequently metabolized to biologically active GA_1. [^{14}C]GA_{20} was surface applied to the shoot of 3-day-old seedlings, and plants were harvested at 6, 12, 24 and 48 hours after treatment. Seedlings were separated into shoots, roots and cotyledons, and frozen. GA transport from shoots into the other tissues was determined by oxidizing tissues, and collecting and counting radioactivity. Twenty five percent of the total ^{14}C applied to the shoot was transported to the cotyledon within the 6-hour treatment, while only 4% to the root. ^{14}C transport continued to increase during 12 hours of treatment for both cotyledons and roots but only slightly thereafter. Over the 48-hour experimental period cotyledons received four fold more ^{14}C than did the roots. Future experiments will examine cotyledon and root transport and metabolism of GAs to understand the dynamics of GA transport during early seedling growth.
Supported in part by AARI grant # 2001 J230.

P5

DORMANCY, CHLOROPHYLL FLUORESCENCE AND FLOWERING IN RHODODENDRON

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Two azalea cultivars, "Vuyk's Scarlet" and "Noordthiana", and a rhododendron cultivar, "Molly Ann", were grown outside in Victoria,B.C., and each month from October,2000 to March,2001 were brought into a 10C night greenhouse to be forced into flower. After 5 hours in the greenhouse, Fv/Fm, Fo, Fm, Fv and chlorophyll were determined. Dates and times to anthesis were also determined. As plants remained outside at lower temperatures longer, the time of forcing into flower in the greenhouse was progressively
shorter, indicating a breaking of dormancy outside in all three cultivars. This was especially pronounced between October, 2000 and December, 2000. At the same time, between October, 2000 and November, 2000, there were decreases in Fv/Fm, Fm and Fv in all three cultivars, and between October, 2000 and December, 2000 in "Molly Ann" rhododendrons. Chlorophyll levels remained relatively constant fron October, 2000 to March, 2001. Decreases in chlorophyll fluorescence may indicate the completion of part of the dormancy breaking process.

P6

THE EFFECTS OF REDUCED AND OXIDIZED GLUTATHIONE ON WHITE SPRUCE SOMATIC EMBRYOGENESIS

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Redox reagents are powerful tools in the study of plant growth and development. The glutathione - glutathione disulfide redox pair has been well studied in plant and animal systems as an antioxidant, where it is has been shown to play a central role in the ability of cells to manage oxidative stress. The balance between the reduced and oxidized forms appears to influence diverse developmental pathways. The white spruce somatic embryo system is a well-established experimental system that is also an important tool used for the micropropagation of plants. However, the system continues to present many challenges despite recent advances in micropropagation techniques. Overall embryo quality and quantity have yet to reach their optimum for this coniferous species. The manipulation of the tissue culture environment to a more oxidized state, via glutathione disulfide, promotes the development of better quality embryos. This quality is observed as a function of the shoot apical meristem, where glutathione disulfide promotes improved cotyledon formation and apical cell morphology. Furthermore, this improved quality is reflected by an increased conversion frequency. The manipulation of redox compounds presents an opportunity to improve white spruce somatic embryogenesis with the intent to obtain greater numbers of quality plants.

P7

INORGANIC CARBON ACQUISITION IN MARINE HAPTOPHYTE ALGAE.

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Haptophyte algae are primarily unicellular biflagellates occurring mainly in seawater. Inorganic carbon acquisition has been investigated in the marine haptophytes *Dicrateria inornata, Isochrysis galbana* and *Ochrosphaera neapolitiana*. The presence of external carbonic anhydrase (CA) was detected by potentiometric and mass spectrometric assays. External CA was present when cultures of *Dicrateria* and *Isochrysis* were grown on air (0.034% CO_2) and completely repressed when the algae were grown on 3-5% CO_2. External CA was not detected in *Ochrosphaera* regardless of the CO_2 concentration in the growth medium. The occurrence of an active CO_2 transport was investigated by mass spectrometry. Illumination of air-grown cells of *Dicrateria* incubated with 100 □M $H^{13}CO_3^-$, and treated with the CA inhibitor acetazolamide, caused a rapid drop in CO_2 concentration too fast to be accounted for by the uncatalzyed dehydration of HCO_3^-, indicating that cells were actively taking up CO_2 from the medium. Similar results were found for *Isochrysis* and *Ochrosphaera*. Active CO_2 transport in all species was not repressed by growth under high levels of CO_2. The photosynthetic electron transport inhibitor DCMU inhibited CO_2 uptake in air-grown cells of all species, demonstrating that the energy to support an active CO_2 transport was derived from photosynthetic electron transport.

P8

CHARACTERIZATION OF THE INDUCTION OF ALUMINUM RESISTANCE IN *PHASEOLUS VULGARIS* L.

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In long-term dose response experiments snapbean (*Phaseolus vulgaris* L.) cultivar Dade is 1.8 fold more resistant to aluminum than snapbean cultivar Romano. However, short-term experiments show that differences in sensitivity to aluminum injury are only apparent after two or more days of exposure to aluminum, suggesting that aluminum resistance may be induced in Dade. Differential citrate exudation is also measured in response to aluminum exposure. Citrate exudation is apparent at least 24 hours earlier than the recovery from aluminum injury in Dade. Exclusion from the cytoplasm by aluminum-chelating compounds is a potential mechanism of aluminum resistance. Visualization of aluminum injury over time is used to further characterize the induction of aluminum resistance in Dade.

P9

A NOVEL COLD REGULATED TRANSMEMBRANE PROTEIN FAMILY IN CEREALS

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Cold acclimation allows hardy plants to develop efficient tolerance mechanisms needed for winter survival. To get further insight on the genetic nature of these mechanisms, we have isolated cold regulated genes encoding novel integral membrane proteins (COR413). Sequence database survey has revealed that the genome of freezing tolerant cereals such as wheat and barley contain three members of the COR413 family (α, β and γ) while low temperature sensitive cereal such as rice and maize contain only two (α and γ). Northern blot analyses showed that *Tacor413*α and γ transcripts are up regulated by low temperature treatments while *Tacor413*β is down regulated. Sequence analyses using several prediction softwares has led us to propose that *Ta*COR413α and β are integral plasma membrane proteins while *Ta*COR413γ is an integral chloroplast membrane protein. The leaf specific expression of *Tacor413*γ transcript confirms its chloroplast localization. All the three proteins are found to have five transmembrane domains. Additional sequence analysis of COR413 homologous proteins from different species revealed the presence of a conserved phosphorylation site and a glycosylphosphatidylinositol anchor site at the C-terminal end. Structure prediction and overall structural comparison allow us to suggest that the COR413 family encodes putative G-protein coupled receptors.

P10

GENOMIC IDENTIFICATION OF CYCLIC NUCLEOTIDE BINDING PROTEINS IN *ARABIDOPSIS THALIANA*

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Cyclic nucleotides regulate a wide variety of processes in mammalian tissues, as well as glucose sensing in bacteria. However, the role of these ubiquitous second messengers has been controversial in plants. By searching the completed genome of A. thaliana for the cyclic nuclotide binding motif, we have identified 30 putative proteins which may be
regulated by either cGMP and cAMP. Of these proteins, 29 appear to be cyclic nucleotide ion channels (either K or Ca channels) while one protein shows strong homology to the regulatory subunits of mammalian cyclic nucleotide dependent kinases. This work forms the basis of a discussion of the possible roles of cyclic nucleotides within plants and provides a framework for their study.

P11

ENHANCER-TRAP LINES FOR THE INVESTIGATION OF VASCULAR DEVELOPMENT.

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As part of a conditional activation tagging project we have generated a collection of 6,000 enhancer trap lines in Arabidopsis, utilizing the pBIN mGAL-mGFP5 HDEL #15 construct (produced and tested by Dr J. Haseloff, Cambridge, UK). Insertion of this construct leads to the expression of the yeast transcription factor GAL4 in a variety of patterns, which can be revealed through a GFP reporter gene under control of the GAL4 target sequence, UAS. When crossed into the appropriate enhancer trap line, any gene of interest under UAS control can be expressed in the respective tissue specific pattern.
To date approximately 3000 lines have been screened and reporter gene expression characterised at the seedling stage. Lines identified as exhibiting a vascular pattern of GFP expression in foliar tissues are being characterised throughout development and in all organs using confocal microscopy.
The project is expected to: (a) provide Arabidopsis lines with specific GAL4 transactivation patterns for use in the larger scientific community and to this end a database for online searches is in preparation. (b) identify new genes with roles in the development or physiology of vascular tissues and (c) new tools for the targeted manipulation of vascular tissue patterns with potential applications in plant biotechnology and agriculture.

P12

TRANSACTIVATIONAL PROPERTIES OF THE ARABIDOPSIS TGA FAMILY OF bZIP TRANSCRIPTION FACTORS

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The TGA factors are a group of basic leucine zipper (bZIP) transcription factors that have been implicated in the control of gene expression in response to environmental stress. There are at least seven members to the TGA family in Arabidopsis, which can be divided into three subclasses based on primary structure. We have recently shown in yeast two-hybrid screens and in vitro that members of subclass II and III interact with the NPR1 protein, an important positive regulator of systemic inducible plant defense responses, while subclass I factors do not. Importantly, NPR1 enhances the ability of subclass II and III factors to bind cognate promoter elements in vitro, but does not influence the DNA binding of subclass I factors. In this study, we analyzed the ability of Arabidopsis TGA factors to transactivate gene expression in a yeast model system. Results demonstrate that subclass I factors (TGA1 and TGA4) are capable of transactivating a reporter gene under the control of the CaMV35S promoter. This transactivation was abolished by mutation to the as-1 element, a classical TGA factor binding site present in the CaMV35S promoter. Deletion analysis of TGA1 identified the N-terminal as an important domain for transactivation.

P13

INORGANIC CARBON ACQUISITION BY THE ALGA *EUSTIGMATOS VISCHERII*

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Inorganic carbon uptake by the eustigmatophyte soil alga *Eustigmatos vischerii* has been investigated by mass spectrometry. No external carbonic anhydrase (CA) was detected in cells of *E.vischerii.* On illumination of the cells, there was rapid decrease in the concentration of CO_2 in the suspending medium to a level below the equilibrium CO_2 concentration. Addition of bovine CA at this point raised the CO_2 concentration to its equilibrium concentration, indicating that the alga actively and selectively takes up CO_2. The continued evolution of O_2 at the CO_2 compensation concentration indicated that the cells also had the capacity to take up bicarbonate as a source of substrate for photosynthesis. An inhibitor of photosynthetic electron transport, DCM, had no immediate effect on inorganic carbon uptake, but the active uptake of CO_2 and bicarbonate was inhibited by 500 mM cyanide or azide, suggesting that mitochondrial respiration provided the energy to support inorganic carbon uptake.

P14

BIOCHEMICAL CHARACTERIZATION OF POLYPHENOL OXIDASE (PP0) FROM HYBRID POPLAR

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Polyphenol oxidases are wide-spread, thylakoid-localized enzymes, which oxidize *o*-diphenolic compounds to *o*-quinones, reactive compounds with diverse biological effects. PPO has been demonstrated to be part of the inducible defense against insect herbivores in a number of plants, including in hybrid poplar which is a model system for forest tree molecular biology. Molecular and biochemical studies indicated that there are at least two major forms of PPO in poplar. In addition to the defense-related PPO (Plant Physiol 124: 285-295), a second PPO protein (PP0-2) is constitutively expressed in conducting tissues of hybrid poplar. Characterization of semi-purified PPO preparations indicates that these two PPOs have distinct substrate preferences and other biochemical properties. cDNA cloning of the constitutive PPO is under way. Based on both biochemical analyses and expression data, we propose that the two PPO protein forms have distinct functions.

P15

REGULATION OF ANTHOCYANIN PRODUCTION IN TEINTURIER GRAPES

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Highly coloured teinturier grapes are used by the wine and food industries to improve the colour of wines and other foods. Unfortunately teinturier varieties have undesirable flavour characteristics which limit the percentage that can be used in wine production. While anthocyanin production is limited to the skins of the berries of traditional wine grape varieties, anthocyanins are found in both berry skin and flesh of teinturier varieties. As a route to understanding how anthocyanin production is controlled in grapes, we are identifying markers for loci responsible for the teinturier phenotype. This work is intended to facilitate the development of traditional *Vitis vinifera* varieties with enhanced anthocyanin content.

P16

HIGH CO_2 SUPPRESSES GROWTH IN TWO MARINE DINOFLAGELLATES

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Inorganic carbon uptake was investigated in two marine dinoflagellates, *Amphidinium carterae* and *Heterocapsa oceanica*. Mass spectrometry and potentiometric assays indicated that both species lacked external carbonic anhydrase (CA). The presence of internal CA was demonstrated by a potentiometric assay. The capacity for bicarbonate transport was investigated by comparing the calculated rate of spontaneous CO_2 formation with the rate of photosynthesis after the addition of 100 mM $NaHCO_3$. Neither species appeared to have the capacity for direct bicarbonate uptake. Monitoring of CO_2 and O_2 fluxes in *Heterocapsa* by mass spectrometry demonstrated a rapid uptake of CO_2 on illumination, to concentrations below the CO_2 equilibrium concentration, indicating an effective, selective uptake of CO_2. When both species were grown on high CO_2 there was a suppression of growth and a rapid decrease in pH from 8 to 6.9. This suppression of growth was not due to pH, since both species are able to grow at pH 7. Growth under high CO_2 had no effect on the internal CA activity of *Heterocapsa*.

P17

DYNAMICS OF NECTAR PRODUCTION BY THE TWICE-ACTIVE FLORAL NECTARIES OF CARAWAY (CARUM CARVI L., APIACEAE)

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Despite their tiny size, the perfect florets of many members of the Apiaceae present a significant opportunity to investigate separated phases of nectar production by the same nectaries. Florets of caraway were protandrous and dichogamous. Nectar secretion began during a floret's male phase (stamen elongation and anther dehiscence) but then ceased in an intermediate, neutral phase of stamen loss and style elongation before a second bout of secretion resumed during the female phase (stigma receptivity). Female-phase florets produced a total of 2.2 times more nectar sugar than male-phase florets, and 1.5 times more on a daily basis. Total reabsorption of male-phase nectar did not result in greater nectar-sugar production by the same florets once in the female phase. Also, nectar-sugar composition differed between the two separated sexual phases, being hexose-rich initially but hexose-dominant during the female phase. Therefore, despite involvement of the same nectaries, it appears that nectar secretion in each sexual phase of caraway florets is at least a partially-independent process. Currently we are utilizing stereology of transmission-electron micrographs to quantitatively investigate ultrastructural features of the nectaries throughout floret phenology, hoping to elucidate the basis for some of these observed differences between sexual phases.

P18

APHID INFESTATION OF ALPINE AND PRAIRIE ECOTYPES OF *STELLARIA LONGIPES* GROWN AT AMBIENT AND ELEVATED CONCENTRATIONS OF ATMOSPHERIC CARBON DIOXIDE.

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Global increases in atmospheric carbon dioxide (CO_2) may alter plant-herbivore interactions. Intra-specific differences in herbivory across habitat gradients may result from changes in the physiological or morphological responses of plants to elevated CO_2. We examined patterns of aphid infestation in alpine and prairie ecotypes of *Stellaria longipes*, a herbaceous perennial, in response to CO_2 enrichment. Green peach aphids, *Myzus persicae*, were allowed to infest alpine and prairie ecotypes of *S. longipes* under long-day-warm (LDW) greenhouse conditions during the flowering period. Plants were then transferred to a minimum 90-day simulated winter and subsequently placed in CO_2 controlled environment chambers under LDW maintained at ambient CO_2 (365 ppm) or elevated CO_2 (1000 ppm) concentrations for 21 days. After 21 days, the number of aphids per ramet on the prairie ecotype was 3 fold lower at elevated CO_2 compared to ambient CO_2. The opposite response was observed in the alpine ecotype, which had a 16 fold increase in aphids per ramet at elevated CO_2 compared to ambient CO_2. The effect of ambient and elevated CO_2 on aphid infestation, plant morphology and leaf nitrogen content of both ecotypes will be discussed.

P19

ETHYLENE PRODUCTION IN PRAIRIE AND ALPINE ECOTYPES OF *STELLARIA LONGIPES* GROWN AT AMBIENT AND ELEVATED CONCENTRATIONS OF ATMOSPHERIC CARBON DIOXIDE.

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Ethylene is an important phytohormone that regulates many aspects of growth and development in plants. Global increases in atmospheric carbon dioxide (CO_2) may alter endogenous ethylene production in plant tissue. We exposed alpine and prairie ecotypes of *Stellaria longipes* to ambient CO_2 (365 ppm) and elevated CO_2 (1000 ppm) in controlled environment chambers for a period of 21-days. Ethylene was measured at 0, 4, 7, 9, 14, 18 and 21 days. Growth parameters were measured on day 0, 7, 9 and 14. Ethylene production was generally higher at ambient CO_2 than elevated CO_2 for both ecotypes. At both CO_2 concentrations, ethylene production peaked on day 4 and was generally greater in the alpine ecotype than in the prairie ecotype. Fresh weight and stem length per ramet in both ecotypes was greater at elevated CO_2 than ambient CO_2 however, the effect was more pronounced in the prairie ecotype. In conclusion, ethylene production did not increase at elevated CO_2 in contrast to observations recorded previously in tomato, sunflower and corn. However, increases in growth were observed at elevated CO_2 for both ecotypes.

P20

CHARACTERIZATION OF A PSII EXCITATION PRESSURE-REGULATED GENE ENCODING A NOVEL *O*-METHYLTRANSFERASE

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A cDNA clone encoding a novel *O*-methyltransferase was isolated from a cDNA library prepared from rye leaves. The open reading frame predicts a protein of 355 amino acids with a calculated Mr of 38 kDa. Database searches revealed that the cDNA clone shares the highest identity (57%) at the amino acid level with the maize safener-binding protein and the lowest (26%) with the *Arabidopsis* flavonol 3'-*O*-methyltranferase. The recombinant enzyme was expressed in a tagged form in *XL1-Blue* cells where it displayed an exclusive methylation of 7,8-dihydroxycoumarin (daphnetin). It did not accept the 6,7-dihydroxy isomer, esculetin nor any of the common phenylpropanoids or flavonoids that were tested as potential substrates. The identity of the methylated product was putatively established as 7-hydroxy-8-methoxycoumarin by autoradiography, chromatography and MS analyses. Northern blot analysis and enzyme activity assays revealed that the cDNA transcript and corresponding enzyme activity are up-regulated by both low temperature and photosystem II excitation pressure. Using various phenylpropanoids and flavonoids substrates, we demonstrate that cold acclimation of rye leaves increases *O*-methyltransferase activity not only for daphnetin but also with the lignin precursors caffeic acid and 5-OH-ferulic acid. The significance of this inducible enzyme is discussed in relation to its putative role in modulating cold acclimation and PSII excitation pressure.

P21

FRET PROBES FOR THE DETECTION OF A SNP LINKED TO A DISEASE RESISTANCE ALLELE IN LACTUCA SATIVA (LETTUCE)

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We describe the development of a non-electrophoresis-based PCR assay for the allelic discrimination of a tightly linked polymorphic locus flanking the recessive cor resistance allele in lettuce (Lactuca sativa). The assay is based on the release of fluorescence upon the adjacent annealing of two hybridization probe by FRET and analysis of melting curve fluorescence profiles to detect SNPs. Probes and primers for the assay were designed after the characterization of a SNP in $SCO07_{600}$, a polymorphic SCAR (Sequence Characterized Amplified Region) associated with Rhizomonas suberifaciens resistance. The FRET hybridization probe procedure allowed a fast and reliable genotyping of breeding material directly in the PCR vials. The lack of fragment separation makes this technique suitable for applications that require automation and high-throughput analyses. This assay could advantageously be used for the improvement of plant varieties in a large-scale marker-assisted selection program.

P22

REGULATION OF STARCH SYNTHESIS IN NON-PHOTOSYNTHETIC TISSUES

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The aim of the work described was to investigate the importance of potential regulators of starch synthesis in non-photosynthetic tissue. Phosphate concentrations were manipulated in potato tuber discs by incubation with mannose which, is phosphorylated by hexokinase to mannose-6-phosphate and then takes no further part in metabolism. Labelling with ^{14}C glucose was used to measure flux into starch, sucrose, cell wall and glycolysis. There was no increase in the rate of starch synthesis. However, hexose phosphate concentrations and the ATP/ADP ratio were reduced in mannose incubated *discs,* suggesting that substrate limitation of starch synthesis may be important.
In order to investigate the role of 3-phosphoglyceric acid (3PGA) in the regulation of starch synthesis, transgenic potato plants over expressing the Arabidopsis cytosolic phosphoglycerate kinase (PGK) gene were produced. The expression of the transgene was confirmed and the activity of PGK was approximately 10 fold that of WT plants. The effect on starch synthesis will be discussed.
In order to study the factors regulating starch synthesis in more detail amyloplasts were isolated from developing pea embryos. The rate of incorporation of U-^{14}C glucose-6-phosphate (G6P) into starch has been measured in the presence of varying concentrations of ATP, G6P, and 3PGA.

P23

PLASMA MEMBRANE LOCALIZATION OF A TEMPERATURE REGULATED RNA HELICASE

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The cyanobacterial temperature regulated RNA helicase CrhC, was found to be inner membrane associated utilizing immunogold labelling and membrane fractionation. Immunogold particles were localized on both the plasma membrane and the septa separating adjacent cells. Immunoblots of membrane fractionation by SDS-PAGE showed that CrhC is only detected in the plasma membrane fraction of cold shocked cells. The nature of CrhC association with the membrane was investigated by treating membranes with detergents and 0.1 M Na_2CO_3 pH 11. CrhC was not removed from the membranes by 0.1 M Na_2CO_3 pH 11, suggesting it is not a peripheral protein. Among the detergents used (1% Triton X-100, 1% Octyl D-galactoside, 1% Sarcosyl) only 1 % SDS completely removed CrhC from the membrane suggesting the strong association of CrhC with the plasma membrane as an integral membrane protein. Computer analysis did not reveal any potential transmembrane domains in CrhC. However, CrhC was released from membranes treated with phospholipase C. These results suggest that CrhC is an integral membrane protein which is associated with the inner plasma membrane through a carbohydrate-lipid anchor.

P24

OVER-EXPRESSION OF TRYPTOPHAN DECARBOXYLASE IN TRANSGENIC POPLAR AND TOBACCO GENERATES HIGHER LEVELS OF TRYPTAMINE AND REDUCES INSECT PREDATION

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The enzyme tryptophan decarboxylase (TDC) converts tryptophan to tryptamine, a neuroactive substance that may affect insect behavior, development and physiology. To examine the feasibility of improving pest tolerance by modifying tryptamine levels, explants from the hybrid poplar clone Populus tremula x P. alba INRA 717 and tobacco Nicotiana tabacum were transformed with a TDC1 cDNA driven by the CaMV35S promoter. Putative *transgenic lines* were confirmed by PCR detection of the presence of the TDC1 gene sequence, and by expression analysis of the transgene mRNA and encoded protein. Chemical and radiotracer analysis suggested that the major accumulated product of tryptophan decarboxylation was tryptamine. No visible phenotypic changes were associated with ectopic TDC expression in either species. Plants from the high, medium and low expression groups were selected for insect bioassays using Malacosoma disstria (forest tent caterpillar) and Manduca sexta (tobacco horn-worm) on poplar and tobacco, respectively. In both interactions, larvae consumed significantly more leaf tissue and gained more weight on the foliage of plants with low TDC1 gene expression in comparison to the foliage of plants with high TDC1 gene expression and higher levels of tryptamine. This suggests that ectopic expression of TDC can allow sufficient tryptamine to accumulate in poplar and tobacco leaf tissue to deter predation by specific herbivorous insect pests.

P25

ANALYSIS OF A SYNECHOCYSTIS sp. PCC6803 MUTANT DEFECTIVE IN PHOTOSYNTHESIS

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An insertional inactivation construct was generated containing the complete sequence of the putative bicarbonate transporter slr1515, disrupted at a unique restriction site by a spectinomycin resistance (spc^R) cassette. This construct was transformed into wild-type cells of the cyanobacterium Synechocystis PCC6803. The spc^R transformants were screened via Southern hybridization to identify a completely segregated slr1515 mutant. The results of the Southern analysis suggest that the mutants obtained are merodiploids, possessing both a wild-type and an insertionally inactivated copy of the slr1515 sequence. One of the spc^R transformants obtained was unable to grow photoautotrophically. This mutant was able to grow in the presence of glucose under both high and low inorganic carbon conditions; however, it failed to grow in the absence of glucose under either Ci condition. Mass spectrometric analysis demonstrated that it possessed CO_2 uptake capabilities and was able to generate an intracellular inorganic carbon pool. However, the cells were unable to evolve O_2 in the light. The mutant also exhibited increased levels of CO_2 leakage to the media, but, this observation alone is insufficient to explain the observed phenotype. These results suggest that the mutant possessed a lesion in some component of the photosynthetic apparatus.

P26

EFFECTS OF CARBON NUTRITION ON THE PHYSIOLOGICAL EXPRESSION OF HCO_3^- TRANSPORT AND THE CO_2-CONCENTRATING MECHANISM IN THE CYANOBACTERIUM CHLOROGLOEOPSIS sp. ATCC 27193.

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Chlorogloeopsis sp. ATCC 27193 cells grown under photoautotrophic conditions in the presence of limiting or replete levels of inorganic carbon (Ci), or grown under mixotrophic (light) or chemoheterotrophic (dark) conditions in the presence of sucrose retained both active CO_2 and Na^+-independent HCO_3^- transport activity. Two distinct effects on the kinetic properties of HCO_3^- transport were observed, however, which segregated on the basis of phototrophic and chemoheterotrophic growth. In the former, the $K_{0.5}$ HCO_3^- transport varied (12-fold) in response to the growth Ci or mixotrophy while V_{max} HCO_3^- transport was constant. In the latter case, the $K_{0.5}$ value was unchanged from the starting value (35 µM) of Ci-limited photoautotrophic cells, but transport capacity declined 3-fold. Modulation of the $K_{0.5}$ value required light. Mixotrophic and chemoheterotrophic growth resulted in a diminished ability to concentrate Ci internally. The relationship between photosynthetic carbon fixation and the internal Ci pool varied by 2-fold with high Ci-grown cells being the most efficient and mixotrophically-grown cells the least, indicating that there was limited capacity to modulate this relationship in response to changes in carbon nutrition. Within broad limits this relationship appeared to be a fixed trait of the strain and a significant factor in determining growth rate.

P27

CHEMISTRY, BIOCHEMISTRY AND MOLECULAR GENETICS OF DIRECT AND INDIRECT DEFENSES IN NORWAY SPRUCE (*PICEA ABIES*)

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Picea abies (L.) Karst. (Norway spruce) forms a suite of resin terpenoids and volatile terpenoids as constitutive and induced defenses against insects and pathogens. An array of terpenoids is induced in stems of Norway spruce after treatment of trees with methyl jasmonate (MeJA) (Martin *et al.*, 2002). Induced accumulation of terpenoids is associated with increased terpenoid synthase (tps) transcript accumulation, terpenoid synthase enzyme activities, and *de novo* differentiation of xylem resin ducts. In this study, two full-length monoterpenoid synthase cDNAs, *PaJF67* and *PaJF104*, were isolated and the recombinant enzymes were expressed in *E. coli* and functionally characterized *in vitro*. *PaJF67* enzyme forms stereospecifically (+)-3-carene (78 %) together with minor acyclic and cyclic monoterpenes, including the mechanistically closely related terpinolene (11 %). The enzyme encoded by *PaJF104* forms mainly ()-□-pinene (57 %) and ()-□-pinene (27 %), similar to the grand fir ()-pinene synthase (Bohlmann *et al.* 1997), but also ()-□-phellandrene (11 %). Additional *TPS* genes isolated from spruce encode monoterpene synthases, sesquiterpene synthases and diterpene synthases. The suite of new spruce *TPS* genes is involved in MeJA induced traumatic resinosis and induced volatile emissions of spruce.

Martin et al 2002. Plant Phys. Accepted.
Bohlmann et al 1997. J. Biol. Chem. 272: 21784-21792.

P28

ION UPTAKE AND MEMBRANE PERMEABILITY IN PINUS BANKSIANA TREATED WITH SODIUM CHLORIDE AND SODIUM SULFATE

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Soil salinization due to agricultural and industrial land use is of increasing importance worldwide. Although NaCl is most commonly used in salinity studies, plant ion uptake and physiological effects may differ greatly between plants treated NaCl and with Na_2SO_4, a common salt in terrestrial soils. This study tested the hypothesis that a greater Na uptake and translocation in the presence of Cl is related to increased membrane permeability. One-year-old jack pine (*Pinus banksiana*) seedlings were grown in sand culture and treated for 5 weeks with NaCl or Na_2SO_4 solution. In a second experiment, transpiration of treated plants was measured as the loss of water from a sealed system. Sodium uptake and root-to-shoot transport rates were greater in treatments containing Cl. A delay in root-to-shoot transport of both Na and Cl indicate retention of these ions in the roots. Uptake of Ca and Mg were more reduced by Na_2SO_4 treatment than by NaCl treatment. Electrolyte leakage of needles was more closely related to treatment chloride concentrations than treatment sodium concentrations. The flux of sodium and chloride ions to the shoot was related to differences in permeability of the root sytem to these ions, and was not related to transpiration rate.

P29

MOLECULAR AND STRUCTURAL ANALYSES OF A NOVEL TEMPERATURE STRESS INDUCED LIPOCALIN FROM WHEAT AND *ARABIDOPSIS*

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Two cDNAs corresponding to a novel lipocalin were identified from wheat and *Arabidopsis*. The two cDNAs designated *Tatil* for *Triticum aestivum* L. temperature induced lipocalin and *Attil* for *Arabidopsis thaliana* temperature induced lipocalin, encode polypeptides of 190 and 186 amino acids respectively. Structure analyses indicated the presence of the three structurally conserved regions that characterize lipocalins. Sequence analyses revealed that this novel class of plant lipocalin share homology with three evolutionarily related lipocalins: the mammalian apolipoprotein D, the bacterial lipocalin and the insect Lazarillo. The comparison of the putative tertiary-structures of both the human apolipoprotein D and the wheat *Ta*TIL suggest that the two proteins differ in membrane attachment and ligand interaction. The 570 bp open reading frame of *Tatil* was expressed in *E. coli* as a fusion protein using pTrcHis vector. The recombinant protein of 25kDa with a theoretical isoelectric point of 5.7 was affinity purified and used to raise a polyclonal antibody. Northern analyses demonstrated that *Tatil* and *Attil* transcripts are upregulated during cold acclimation and heat-shock treatment. The putative functions of this novel class of plant lipocalins during temperature stresses are discussed.

P30

STRUCTURAL STUDIES OF NOVEL PETALS IN *BRASSICA* PLANTS EXPRESSING AN *ARABIDOPSIS* CYCLIN-DEPENDENT KINASE INHIBITOR

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The cell cycle in plants is governed by the cyclin-dependent kinases (CDKs). In order to address the question about how cell cycle regulation affects plant organ development, we investigated the effects of overexpressing a CDK inhibitor in *Brassica napus* L. Previously, the *Arabidopsis* CDK inhibitor gene *ICK1* was identified (1) and constitutive overexpression of *ICK1* resulted in inhibition of cell divisions and plant growth as well as modification of plant morphology (2). Tissue-specific expression of *ICK1* driven by the *Arabidopsis AP3* promoter resulted in transgenic *Brassica* plants with modified petals (3). In these *AP3-ICK1* plants, petal modifications ranged from simply smaller petals to a total absence of petals, but also included two types of novel petals - tubular or trumpet shaped and filamentous. The expression of a cell cycle regulator thus had altered the fundamental shape of a plant organ from a sheet-like structure to a trumpet- or filament-like structure. These novel petals are characterised structurally. Results from scanning electron microscopy and light microscopy of these modified petals will be presented and discussed in relation to the cell cycle and petal development.

1. Wang H, Fowke LC, Crosby WL (1997) *Nature* 386: 451
2. Wang H, Zhou Y, Gilmer S, Whitwill S, Fowke LC (2000) *Plant J* 24: 613
3. Zhou Y, Wang H, Gilmer S, Whitwill S, Keller W, Fowke LC (2002) *Planta* (in press)

P31

BIOCHEMICAL AND GENETIC CHARACTERIZATION OF LEUCOCYANIDIN REDUCTASE IN RIBES, LOTUS, ROBINIA AND BARLEY

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Leucocyanidin reductase (LCR) one out of a sub-class of NADP(H)-dependent oxido-reductases called leucoanthocyanidin reductases (LAR) which function to direct unstable flavan-3,4-cis-diols into the proanthocyanidin rather than the anthocyanin pathway. This activity has been characterized previously as a function of plant development. Otherwise, little is known about these enzymes and their relationship to other components of proanthocyanidin biosynthesis. In this study, we purified and characterized LCR activity from three plant species which accumulate large quantities of proanthocyanidin and compared it with activity in barley seed. Distinct forms of LCR activity emerge. *Robinia pseudoacacia* and *Lotus uliginosis* leaf activities have broad optima at pH 5.5, while *Ribes sanguineum* floral activity and *Hordeum vulgare* seed activities are optimal at 6.5. Activity was strongly influenced by specific metal ions and by several of the mutations recovered in barley seed coat *ant* lines.

P32

GEOGRAPHIC VARIATION IN ECOPHYSIOLOGICAL TRAITS OF BLACK COTTONWOOD (*POPULUS TRICHOCARPA*) FROM WESTERN NORTH AMERICA

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Geographic variation in photosynthesis and water-use were examined within and between populations of black cottonwood in a common garden experiment in Surrey, BC. Mid-day photosynthetic rate (*A*) and instantaneous water-use efficiency (WUE_i) were measured on 2-year-old stecklings under full sun conditions in July. Leaf disks were analysed for stable carbon isotope composition ($\Delta^{13}C$) to provide a more long-term measure of water-use efficiency. Photosynthetic rate per unit leaf nitrogen was used as a measure of nitrogen-use efficiency (NUE). There was significant ($p<0.01$) variation between populations in *A*, which increased with latitude ($r^2=0.732$) on either a mass or area basis. There were also significant differences ($p<0.01$) in WUE_i, which increased with distance from the Pacific coast ($r^2=0.523$). NUE increased with elevation ($r^2=0.211$). Variation within populations was observed in $\Delta^{13}C$ value ($p<0.01$) and NUE ($p=0.018$). Differences in *A* were attributed to stomatal factors (conductance, density, adaxial:abaxial ratio) as well as nitrogen content. Higher WUE_i appeared to result from lower conductance rather than greater photosynthetic capacity. WUE_i may increase for interior provenances due to lower precipitation, whereas the increase in NUE with elevation of origin may reflect lower available soil nitrogen. Northern provenances may have inherently higher *A* to compensate for shorter growing seasons.

P33

MANIPULATION OF STEROL BIOSYNTHESIS BY UNCOUPLING HMGR FROM PHOSPHORYLATIONAL CONTROL BY SNRK1

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HMG-CoA reductase (HMGR) is a key enzyme at the gateway of carbon flux into sterol and sesquiterpene biosynthesis in plants. Its activity is controlled at many levels, one of which being post translational modification of the enzyme by reversible phosphorylation. HMGR is phosphorylated by AMP dependent kinase (AMPK) in animals and by SnRK1 in plants. Both enzymes are homologues of the yeast protein kinase SNF1. In yeast SNF1 is considered to be a global regulator of carbon metabolism. It is activated by low glucose levels and is involved in the derepression of genes required for the utilization of alternative carbon sources and the inactivation of biosynthetic enzymes.
In animals, the homologous AMPK's are known to control pathways involved in anabolic processes which consume ATP. In plants, SnRK1 phosphorylates and deactivates key metabolic enzymes, including HMGR, sucrose phosphate synthase and nitrate reductase. SnRK1 also activates genes for enzymes involved in reserve synthesis and mobilisation, implicating SnRK1 as playing a role at the heart of carbon partitioning in plants. We have attempted to increase carbon flux into sterol biosynthesis, by removing HMGR from control by SnRK1, using site directed mutagenesis of the SnRK1 phosphorylation site in HMGR.

P34

LEARNING OBJECTS: A NEW JARGON FOR AN OLD PROBLEM

Educational Committee, CSPP. John Hoddinott (Chairperson), Biological Sciences, University of Alberta, Edmonton, AB, T6G 2E9.

The Learning Technology Standards Committee of the IEEE defines a learning object as "any entity, digital or non-digital, which can be used, re-used or referenced during technology supported learning." A Canadian project, the Campus Alberta Repository of Educational Objects (CAREO), extends the definition to include "simulations, tutorials, drill and practice modules, content databases and multi-media exercises." It even includes "administrative objects such as calendars and quiz programs," research-related "items such as discussion papers and research results", and also "content creation tools such as databases, graphics and animation tools". Two major problems arise after objects are created: where to keep them and how to find them? MERLOT is a distributed public repository while Bio-DiTRL is a more rigorously peer reviewed subscription based archive. To make objects discoverable, accessible or searchable in such repositories, metadata is used to describe and categorize them. Metadata is "structured data about data" and CanCore is a Canadian metadata initiative. In a series of posters the CSPP Educational Committee will review learning objects, repositories and metadata and present problem based learning objects developed by Peter Jolliffe, Plant Science, UBC. Discussants are invited to explore how the CSPP community may collaborate in the development and sharing of learning objects.

P35

PROGRESS IN THE POSITIONAL CLONING OF THE Cer7 WAXLESS MUTANT OF ARABIDOPSIS THALIANA

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Plant epicuticular waxes are exuded over the surfaces of higher plant cuticles. By forming the primary interface between the plant and the environment, they play important roles in plant responses to abiotic and biotic factors. A number of waxless (Cer) mutants have been isolated from *Arabidopsis thaliana*. The *cer* mutations identify gene functions required for wax biosynthesis and allow cloning of these genes. Thus, they are an invaluable resource for advancing our understanding of wax production. The Cer7 mutant was chosen for this study, because it has almost undetectable levels of *CER6* transcript in bolting stems. Because *CER6* encodes a key wax biosynthetic enzyme essential for wax production in the stem, the absence of the *CER6* mRNA in the Cer7 mutant suggests that *CER7* may encode a transcriptional regulator which controls *CER6* expression in Arabidopsis stems. To investigate this hypothesis, we are isolating the *CER7* gene using a positional cloning approach. As a first step, we have fine-mapped the chromosomal location of the gene using simple sequence length polymorphism (SSLP) marker analysis of an F2 mapping population. Progress towards the cloning of *CER7*, as well as genetic and phenotypic analysis of the Cer7 mutant, will be presented.

P36

IDENTIFICATION OF *Arabidopsis thaliana* MUTANTS DEFICIENT IN PLASTIDIC REDOX RESPONSES USING CHLOROPHYLL-FLUORESCENCE IMAGING

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Photoinhibition has been defined as a light-dependent decreased in rates of photosynthesis due to an overreduction of photosystem II (PSII). Cold acclimated plants exhibit a decreased susceptibility to photoinhibition of photosynthesis at low temperature. Furthermore, it has been demonstrated that this decreased susceptibility is as a result of increased excitation pressure on PSII during development. This is reflected as an alteration in the redox state of PSII, which can be monitored *in vivo* using the technique of chlorophyll *a* fluorescence. In this manner, it has been suggest that the redox state of the photosynthetic apparatus can act as an environmental sensor to detect environmentally induced imbalances between photochemistry and metabolism. The localization of the chloroplastic redox sensor/signal has been examined with limited success and remains elusive. In order to identify metabolic components of this redox sensing pathway, we have initiated a molecular genetic approach. A genetic approach to study metabolism implies that introducing mutations in a regulatory metabolic network will result in phenotypic effects, which can then be selected or screened to allow identification. Subsequently, mutated genes can be isolated and characterized, eventually leading to a full description of a regulatory process at the biochemical and molecular level. Currently, we are using a chlorophyll-fluorescence digital-imaging system to screen a population of 40,000 T-DNA-mutagenized *Arabidopsis thaliana* plants for mutants that are altered in their photosynthetic responses to photoinhibition at low temperature in comparison to wild-type *Arabidopsis* plants. The results of our preliminary screens, limitations of the imaging technique and the implications for redox signaling will be discussed.

P37

A RAPID TRANSIENT ASSAY SYSTEM FOR DETERMINING CONSTRUCT FUNCTIONALITY IN *ARABIDOPSIS THALIANA*

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Stable transformation of Arabidopsis is a lengthy process involving up to 3 months of plant growth and seed selection. While using a number of constructs, with *uidA* (*GUS*) expression driven by various promoter/regulatory elements, we found it useful to develop a rapid transient assay system to test the constructs for functionality in plants before undertaking stable transformation. Arabidopsis seedlings grown on plates for 2 weeks were vacuum infiltrated with *Agrobacterium* cultures carrying promoter::*GUS* constructs and resuspended in a simple sucrose medium. After 48, 72, and 96 hours the seedlings were fixed and stained for GUS activity. Promoters that were tested in this system include the CaMV *35S* promoter, the upstream regulatory region of both a heat-inducible gene and a ribosomal protein gene. A binary vector construct lacking *GUS* was included as a negative control. The resulting staining percentages of transformed seedlings varied depending upon the construct used, with the CaMV 35S promoter producing the highest percentages. The data suggest that, unlike the viral promoter, the endogenous plant promoters were regulated in a tissue-specific/developmental manner.

P38

CHARACTERIZATION OF BETA-D-XYLOSIDASE AND ALPHA-L-ARABINOFURANOSIDASE DURING AND FOLLOWING SEED GERMINATION IN TOMATO

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The modification of cell walls is an important aspect of plant cell growth. Changes in the cell wall are mediated by cell wall hydrolases, including beta-D-xylosidase and alpha-L-arabinofuranosidase that participate in the breakdown of xylan or arabinoxylan. However, very little is known about these enzymes during and following seed germination. The activity of both enzymes increased during and after germination. Two beta-D-xylosidase cDNAs (*LeXYL1* and *LeXYL2*) and one alpha-L-arabinofuranosidase cDNA (*LeARF1*), isolated from ripening tomato fruit, were used for Northern blot analysis during and following seed germination; both *LeXYL2* and *LeARF1* transcripts increased strongly. Expression of *LeXYL1* was not observed during or following germination. These cDNAs were also used to investigate gene expression in the various seed parts (embryo, micropylar endosperm and lateral endosperm). *LeXYL2* mRNA transcripts were highest in the embryo and quite low in the micropylar and lateral endosperm, whilst *LeARF1* mRNA transcripts were higher in the lateral and micropylar endosperm, but were not observed in the embryo. Thus, beta-D-xylosidase and alpha-L-arabinofuranosidase genes show different spatial expression patterns. These data indicate that beta-D-xylosidase and alpha-L-arabinofuranosidase have different roles during and following seed germination.

P39

ETHYLENE IN EARLY PEA FRUIT DEVELOPMENT

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Two naturally occurring auxins, indole-3-acetic acid (IAA) and 4-chloroindole-3-acetic acid (4-Cl-IAA) are present in *Pisum sativum* (I_3-Alaska type) fruit, however, they have different effects on pericarp growth. Exogenous IAA at 50 □M is inhibitory to pollinated deseeded pea pericarp elongation both when applied alone (Reinecke et al. 1995) and in conjunction with gibberellic acid (GA_3) (Reinecke and Ozga 1995). 4-Cl-IAA applied to the tissue at the same concentration will promote elongation. In combination with GA_3, 4-Cl-IAA shows a synergistic growth effect on the pea pericarp (Ozga and Reinecke 1999). When pericarps are treated with GA_3 and IAA, growth is less than when pericarps are treated with GA_3 alone. However, pretreatment of pericarps with silver thiosulfate (STS, an ethylene action inhibitor) will reverse the inhibitory effect of IAA on GA_3-promoted growth (Reinecke and Ozga 1995). Our current results show that pea pericarps naturally produce ethylene. 4-Cl-IAA and IAA treatment stimulate pericarp ethylene evolution to maximum levels 12 h after treatment, and ethephon treatment can mimic IAA's inhibitory effect on pericarp growth. These results support that ethylene is a regulator of pea fruit development. Supported in part by NSERC grant #138166-97.

P40

ETHYLENE AND NAPHTHENIC ACIDS AFFECT WATER TRANSPORT IN ASPEN (*POPULUS TREMULOIDES*) SEEDLINGS

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Effects of ethylene and naphthenic acids on water transport in solution culture grown aspen (*Populus tremuloides*) seedlings were examined in two separate experiments. Short-term exposures of roots to ethylene significantly increased root hydraulic conductivity (L_p), stomatal conductance (g_s), and root oxygen uptake in hypoxic seedlings. An ethylene action inhibitor, silver thiosulphate, significantly reversed the enhancement of L_p by ethylene. A short-term exposure of excised roots to ethylene significantly enhanced the root water flow (Q_v), measured by pressurizing the roots at 0.3 MPa. The Q_v values in ethylene-treated roots declined significantly when 50 □M $HgCl_2$ was added to the root medium and this decline was reversed by the addition of 20 mM 2-mercaptoethanol. A longer, 3-5-week, exposure of roots to sodium salt of naphthenic acids significantly decreased L_p and g_s. Root-absorbed naphthenic acids also decreased leaf chlorophyll contents, net photosynthesis and leaf growth. A short-term exposure of excised roots to naphthenic acids significantly decreased Q_v with a concomitant decline in root respiration. The response of root water flow to ethylene involved mercury-sensitive water channels and root-absorbed ethylene enhanced water permeation through roots resulting in an increase in root water transport and stomatal opening in hypoxic seedlings. Naphthenic acids metabolically inhibited L_p, likely by affecting water channel activity and that this inhibition could be responsible for the observed reduction in gas exchange and leaf growth.

P41

TaVERF: A TRANSCRIPTION FACTOR ASSOCIATED WITH VERNALIZATION RESPONSE IN CEREALS.

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The molecular basis of vernalization, the promotion of flowering by cold treatment, remains unknown. To understand this mechanism, we cloned and characterized a regulatory gene that we named *Taverf*. Molecular analysis indicates that this gene encodes a transcription factor that belongs to the MADS-box family that characterizes several flowering control genes in *Arabidopsis*. However, there was no homology with any of the *Arabidopsis* genes outside the MADS-box domain. Using several wheat genotypes and near-isogenic lines that differ in vernalization requirement, we demonstrated that this gene was associated with the vernalization response. In spring habit wheat that does not have vernalization requirement, *Taverf* was constitutively expressed. On the other hand, winter habit wheat requires extended cold acclimation to induce the expression of *Taverf*. The transcript accumulation was associated with the vernalization response reaching maximum level at the vernalization saturation point, the stage where the transition from vegetative to reproductive phase occurs. Using different deletion lines for homoeologous group 5 chromosomes, we localized this gene to the *VRN 1* locus on the long arm of chromosome 5A, a region associated with vernalization and cold tolerance in wheat. The functions of *Taverf* in regulating the vernalization response in wheat will be discussed.

P42

PHOTOPROTECTION IN EVOLUTIONARY DIVERGENT SPECIES.

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Plants must balance energy absorbed through photochemistry versus energy either utilized or dissipated as heat. An imbalance in energy budget may result in photoinhibition and photodamage to the photosynthetic apparatus. The potential for photoprotection was assessed by xanthophyll cycle activity and Lhcb phosphorylation, while D1 repair was monitored by the capacity for D1 phosphorylation. How much variation is exhibited in the mechanisms of photoprotection in evolutionary divergent species? All plant species examined were photoinhibited at 1200 micromol m^{-2} s^{-1} at 20□C and 5□C. Xanthophyll cycle activity was detected in all species. However, mosses exhibit the lowest xanthophyll cycle activity. Similarly, phosphorylation of D1 was not detected in either mosses or ferns. In contrast , all plant species exhibited the capacity to phosphorylate Lhcb. High light inhibited the phosphorylation of Lhcb to the greatest extent in gymnosperms and angiosperms. Chloroplast ultrastructure in the plant species studied indicated that lower plants possessed loosely appressed thylakoid membranes while higher plants, such as gymnosperms and angiosperms, contained large, tightly appressed grana stacks. Although all species examined exhibited xanthophyll cycle activity and the phosphorylation of Lhcb, granal stack membrane appression was correlated with the capacity to phosphorylate the PSII D1 protein only in seed plants.

P43

ROLE OF ETHYLENE AND LIGHT QUALITY IN GROWTH AND DEVELOPMENT OF *STELLARIA LONGIPES*.

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Though most members of the *S. longipes* complex display considerable phenotypic plasticity in response to changing light environment, some genotypes are highly plastic (tall prairie), others less so (dwarf alpine). Based on previous studies and recent observation of alpine and prairie genotypes growing under low (0.7), normal (1.9) and high (2.7) Red/Far Red (R/FR) ratios, we suggest that R/FR ratio is the major factor influencing phenotypic plasticity responses in differing light environments. We have found distinct and significant differences in stem elongation rate, leaf shape, color and expansion, and timing of inflorescence appearance in both genotypes under different R/FR ratios. The prairie ecotype shows a decrease in growth as R/FR ratio increases, while the alpine ecotype show an increase in growth. For the prairie ecotype the inflorescence appears earlier under lower R/FR ratios, while for alpine ecotype it appears earlier under higher R/FR ratios. We have also found that ethylene production is greater for the alpine genotype under enhanced R, relative to the prairie ecotype. We thus postulate that there is a phytochrome-mediated response in ethylene biosynthesis for the alpine ecotype, at least, of *S. longipes*. The possible role of blue light (as a separate effector) in phenotypic plasticity of *S. longipes* will also be discussed.

P44

PARTICIPATION OF DIFFERENT SYSTEMS OF SECOND MESSENGERS IN PLANT HARDENING

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Adaptation of plant to low temperature is very complicated process that joins structural and functional reconstructions of cell metabolism and pass with assistance of different regulatory systems. Although considerable successes has been obtained in investigation of the signal-transduction processes in plant cell and there is a special interest to participation of second messengers in response reactions of plant on external stimulus, their involvement to the development of termoadaptive reactions to the low temperature has not been elucidated enough. We investigated the participation of different system of second messenger in formation frost resistance seedlings of winter wheat by use inhibitors these systems. Full stopping of growth seedlings of winter wheat by their placing in climatic chamber (2^{O}C) accompanied increasing endogenous content of calcium and cyclic AMP. It evidenced about switching metabolism of cell on adaptation and preceded forming the frost resistance. Addition of forskolin (activator of adenylate cyclase) and inhibitor of phosphodiesterase - 3-isobutyl-1-methylxantine (IBMX) increased the concentration the c-AMP in peak, which has been obtained from cold acclimation. Moreover the exogenous addition of c-AMP increased frost tolerance as well. The antagonists of calmodulin (chlorpromazine, fluphenazine, W7), Ca^{2+}-channel blockators (La^{3+}, diltiazem) inhibited the rise frost tolerance. The same time forskolin and IBMX considerably increased low temperature resistance.

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P45

STUCTURE AND EVOLUTION OF FOUR PHYTOCHROME A GENES IN *STELLARIA LONGIPES*

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The phytochrome photoreceptors have been demonstrated to play a critical role in plant perception of red and far-red light in higher plants. Phy family consists of three to five genes in monocot and dicot. A single or two *phyA* seem to be present in most species. We isolated and sequenced four cDNA clones coding for type A phytochrome from *Stellaria longipes*. They share 71% to 75% amino acid identities with the *Arabidopsis phyA* and 50% to 52% with the *Arabidopsis phyB* open reading frame. We designated these cDNA clones as *phyA1, phyA2, phyA3* and *phyA4*. Based on the deduced amino acid sequence, *phyA1* shares 96% amino acid identity with *phyA2*. Portions of these two genes, encoding amino acids from 1 to 975 are much more conservative than the rest (148 amino acids) which share 99% identity with each other. *phyA3* shares 98% amino acid identity with *phyA4,* except that *phyA4* shows a deletion of 187 amino acids between 684 and 871. We classified these four *phyA* genes into 2 subfamilies (*phyA1/phyA2*, *phyA3/phyA4*).

P46

MOLECULAR REGULATION OF ESSENTIAL OIL COMPOSITION IN PEPPERMINT: METABOLIC FATE OF (+)-PULEGONE.

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(+)-Pulegone is a key intermediate in the biosynthesis of the monoterpene constituents of peppermint (*Mentha X piperita L*) essential oil. This branch point metabolite may be reduced to (-)-menthone by pulegone reductase (PR), or oxidized to the hepatotoxin (+)-menthofuran by menthofuran synthase (MFS). To elucidate regulation at this branch point, we altered the expressions of *mfs* and *pr* in independently transformed peppermint plants. Overexpression of *pr* failed to improve overall PR enzyme activity levels in transgenic plants, suggesting that the expression of this gene is post-transcriptionally regulated. Overexpression of *mfs* led to increased menthofuran production, and downregulation of expression of this gene resulted in reduced menthofuran biosynthesis. Surprisingly, the reduction of pulegone to menthone was inhibited in *mfs*-overexpressors, and was enhanced in *mfs*-knockouts. This outcome was a consequence of transcriptional downregulation of *pr* by menthofuran. The *mfs*-knockouts also expressed a monoterpene epoxidase (EPO) that catalyzed the formation of (-)-*trans*-piperitone oxide in these plants. These findings demonstrate that menthofuran acts as a negative transcriptional regulator of at least two monoterpene biosynthetic genes, including *pr* and *epo*, in peppermint.

P47

TEMPERATURE-SENSITIVITY OF DIHYDROFLAVONOL REDUCTASE GENE EXPRESSION AND PIGMENT DEPOSITION IN SEEDLING LEAVES AND SEED COAT OF *BRASSICA CARINATA*

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Metabolic components in a yellow-seeded line (Y line) of *Brassica carinata* were compared to those of a near-isogenic brown-seeded line (B line) and the differences used to identify a genetic block in pigment biosynthesis. Results from this targeted metabolic profile lead to a comparison of dihydroflavonol reductase (*DFR*) gene expression in developing seeds and seedling leaves of the two lines. Temperature affected *DFR* gene expression only in the Y line. At 20 to 25 ^{o}C, seedling leaves exhibited reduced expression of *DFR*, contained less anthocyanin and the seed coat was more transparent compared to the respective tissues from plants grown at cooler temperatures. When Y line seedlings were grown at 15 to 18^{o}C or were chilled for several hours, seedling leaves produced anthocyanins and were similar in colour to the leaves of B line seedlings. When flowering plants remained under cooler temperatures (15 to 18^{o}C), mature seed from the Y line was a brownish-yellow whereas seed that matured under consistently warm temperatures (20 to 25^{o}C) was a brighter yellow. Comparable B line tissues were unaffected by these temperature changes. These results are suggestive of a temperature-sensitive regulator of *DFR* in the yellow-seeded *Brassica carinata*.

P48

DEGRADATION OF PHENANTHRENE AT THE SOIL/ROOT INTERFACE IN CONTAMINATED SOILS

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Terrestrial soils contaminated with petroleum hydrocarbons (PHs) present a major challenge to Alberta's oil and gas industry. Recently, rhizosphere-assisted bioremediation has received widespread attention as a mechanism of PH attenuation. A model plant/soil/contaminant system was set up to elucidate the pathway of a PH when exposed to a plants' influence. A polycyclic aromatic hydrocarbon, phenanthrene, was selected as a model contaminant. Screening tests to determine a model plant were conducted using various levels of soil phenanthrene in artificially contaminated soils. Experiments with wheat, alfalfa and canola demonstrated wheat to be the ideal model plant due to its extensive fibrous root system, low variability, ability to sustain high root microbial growth, and acceptable tolerance to soil phenanthrene. Plate counts from artificially contaminated soils demonstrated an increase in heterotrophic and phenanthrene-degrading microorganisms in the rhizosphere with increasing soil phenanthrene concentration. Contaminated field soils from Guy Lake, Alberta, show a similar increase in heterotrophic microorganisms compared to control soils, but the number of phenanthrene-degrading microorganisms does not increase uniformly. Degraders isolated from this soil will be used in future studies to trace the transport of PHs through a model soil/plant/microbe system.

P49

PURIFICATION AND PROPERTIES OF ARABIDOPSIS THALIANA TYPE ONE PROTEIN PHOSPHATASE (PP1)

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The Arabidopsis thaliana type one protein phosphatase catalytic subunit was released from its endogenous regulatory subunits by ethanol precipitation and purified by anion exchange and microcystin affinity chromatography. The enzyme was identified by MALDI-TOF mass spectrometry from a tryptic digest of the purified protein as a mixture of PP1 isoforms (TOPP1-6) indicating that at least 4-6 of the 9 known PP1 proteins are expressed in sufficient quantities for purification from Arabidopsis thaliana suspension cells. The enzyme had a final specific activity of 8950 mU/mg using glycogen phosphorylase *a* as substrate, had a subunit molecular mass of 35 kD as determined by SDS-PAGE and behaved as a monomeric protein of approximately 39 kD on Superose 12 gel filtration chromatography. Similar to the mammalian type one protein phosphatases, the Arabidopsis thaliana enzyme was potently inhibited by Inhibitor-2 (IC_{50} =0.65 nM), tautomycin (IC_{50} =0.06 nM), microcystin-LR (IC_{50} =0.01 nM), nodularin (IC_{50} =0.035 nM), calyculin A (IC_{50} =0.09 nM), okadaic acid (IC_{50} =20 nM) and cantharidin (IC_{50} =60 nM). The enzyme was also inhibited by fostriecin (IC_{50} =22 µM), NaF (IC_{50} =2.1 mM), Pi (IC_{50} =9.5 mM), and PPi (IC_{50} =0.07 mM).

P50

LIFE IN THE FAST LANE: ACTIN-BASED MOTILITY OF PLANT PEROXISOMES

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Peroxisomal shape, distribution, motility, and interactions with cytoskeletal elements were examined during interphase in living leek (*Allium porrum* L.) epidermal cells transiently transformed with a construct encoding the green fluorescent protein bearing a carboxy-terminal type 1 peroxisomal targeting signal. Confocal laser scanning microscopy and time-course analysis revealed that labeled peroxisomes were either spherical or rod-shaped and possessed several types of motility including random oscillations, slow and fast directional and bidirectional movements, and stop-and-go movements. Co-localization studies indicated that most peroxisomes were in close association with actin microfilaments, while treatment of cells with the actin-disrupting drug cytochalasin D blocked all types of peroxisomal movements. In contrast, the overall spatial organization of peroxisomes and the microtubule cytoskeleton were different and the microtubule-destabilizing agent oryzalin had no obvious effect on peroxisomal motility. These data indicate that the peroxisome in plant cells is a highly dynamic compartment that is dependent upon the actin cytoskeleton, not microtubules, for its subcellular distribution and movements.

P51

MULTIPLE TARGETING SIGNALS ARE REQUIRED FOR THE SORTING OF A 22KDA *ARABIDOPSIS* INTEGRAL MEMBRANE PROTEIN TO PEROXISOMES

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The 22 kilodalton *Arabidopsis thaliana* integral peroxisomal membrane protein (PMP22) has four predicted transmembrane domains (TMDs) and has been proposed to function as a pore-forming channel. We have investigated the topological orientation and membrane peroxisomal targeting signals (mPTSs) of PMP22 both in vivo and in vitro. Immunofluorescence microscopic analyses revealed that epitope-tagged PMP22 sorted exclusively to peroxisomes in transiently-transformed tobacco BY-2 cells. Results from differential permeabilization experiments indicated that PMP22 inserted in the peroxisomal boundary membrane with an $N_{cytosol}$-$C_{cytosol}$ topology. In vitro, epitope-tagged and non-tagged PMP22 inserted into purified sunflower peroxisomal membranes with similar efficiencies. Modified versions of PMP22 containing specific amino acid changes allowed for the identification of at least three distinct regions within the protein as putative mPTSs, as mutations within each region caused a decreased efficiency of targeting to or insertion into peroxisomal membranes in vivo or in vitro. Surprisingly, minimal portions of PMP22 that included one or more putative mPTS were not sufficient to redirect a reporter protein to the peroxisomal boundary membrane. Efficient sorting of PMP22 was observed only when at least three of the four TMDs within PMP22 were included. The role of the PMP22 TMDs in subcellular sorting will be discussed.

P52

EFFECTS OF SODIUM CHLORIDE ON C*ORNUS STOLONIFERA* SEEDLINGS: A FOCUS ON WATER RELATIONS AND CELL WALL ELASTICITY

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Red-osier dogwood (*Cornus stolonifera* Michx.) is relatively well adapted to saline conditions compared with other species in the boreal forest. To survive in moderately saline areas plants must undergo physiological changes to maintain water uptake required for growth and survival. Plant water relations and cell wall properties play an important role in regulating water uptake. The objective of this study was to determine the effect of NaCl on cell wall elasticity and water relation parameters (water potential, relative water content, solute potential and pressure potential) of red-osier dogwood seedlings. Water potential, relative water content at turgor loss, and solute potential at full turgor did not differ between control and salt treated seedlings. Treated seedlings had more rigid cell walls, lower solute potential at turgor loss, and higher pressure potential at full turgor than control seedlings. Seedlings treated with NaCl showed lower growth, transpiration and photosynthetic rates. The findings of this study suggest that differences observed in cell wall elasticity, solute potential and pressure potential may be a result of decreased growth rather than the direct effect of NaCl.

P53

BROAD-SPECTRUM RESISTANCE OF POTATO PLANTS EXPRESSING ANTIMICROBIAL PEPTIDE MsrA2, OR HOW COULD THE FROGS HELP POTATOES FIGHT THE LATE BLIGHT

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Dermaseptin B1 is very potent antimicrobial peptide found in the skin secretions of the frog Phyllomedusa bicolor. Its broad-spectrum antimicrobial activity makes it an extremely promising tool in plant antimicrobial warfare. We expressed this cationic peptide (MsrA2) under the control of the duplicated-enhancer CaMV 35S promoter in the potato (Solanum tuberosum L.) cultivar Desiree. The stable incorporation of msrA2 gene into plant genome was confirmed by PCR and expression by RT-PCR. The expression of MsrA2 peptide gives the transgenic potatoes potent resistance to a variety of bacterial and fungal phytopathogens, including the most devastating potato pathogen - Phytophthora infestans.

P54

FUNCTIONAL ANALYSIS OF THE DOUGLAS-FIR BIP PROMOTER IN *ARABIDOPSIS*

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The luminal binding protein BiP is a ER resident HSP70 molecular chaperone localized in the endoplasmic reticulum and assists in the folding and processing of newly synthesized proteins targeted to the secretory pathway. The expression of Douglas-fir BiP is subject to both developmental and environmental regulatory cues. To elucidate regulatory elements responsible for the transcriptional control of Douglas-fir BiP, a promoter sequence was isolated and functionally characterized. Transient expression analysis showed this promoter to be active in germinating Douglas-fir embryos and revealed that minimal promoter elements reside within a -261 to +16 bp region. Deletion analysis of promoter activity in stably transformed *Arabidopsis* showed that elements upstream of the -261 to +16 region were necessary for higher levels of expression and suggest that transcriptional control may be linked to pathways controlling the expression of cell wall proteins. Histochemical staining revealed strong staining associated with actively dividing/expanding cells, secretory tissues of developing seedlings and in cells surrounding a wound site. Correlation of the observed expression pattern with the known function of BiP suggests that pathways controlling expression are highly conserved between angiosperms and gymnosperms.

P55

CHARACTERIZATION OF A HIGHLY CONSERVED PLANT MAGO NASHI HOMOLOG

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The subcellular localization of mRNA is a widely employed mechanism important in targeting proteins to regions of the cell where they are required. Localized mRNAs are typically trafficked in the form of large ribonucleoprotein particles that may contain many copies of the RNA as well as motor proteins, RNA-binding proteins, and translation factors. Mago Nashi (Mago) is a protein that was first identified as having an important role in mRNA localization in *Drosophila* oocytes. Mago was recently shown to interact with an RNA-binding protein and is involved in shuttling spliced mRNAs from the nucleus into the cytoplasm. A homolog of Mago was identified in rice, and DNA sequencing showed that it was 77% identical to the *Drosophila* protein, indicating a conservation of function of this protein across kingdoms. The rice Mago protein is expressed in developing root, leaf and seed tissues, and two copies are present in the rice genome. As observed in animal systems, Mago protein in plants is primarily localized to the nucleus. We are currently identifying the proteins and mRNAs that are associated with the Mago complex in plants and we are determining the effect of over- and under-expression of Mago on plant development.

P56

NEW GIBBERELLINS FROM *POPULUS* CAPSULES

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Developing capsules of *Populus deltoides* and growing shoots of *P. deltoides* and *P. trichocarpa* were analyzed for gibberellins (GAs) by gas chromatography-mass spectrometry. GAs of the early 13-hydroxylation pathway, and their catabolites, predominated in all sources. A number of GAs not previously reported from *Populus* were identified by comparison of their Kovats retention indices and mass spectra with those of standards. In capsules these included 16□,17-dihydro-17-hydroxy GA_{20} and three new GAs - 12□-hydroxy GA_{53} (GA_{127}), 16□,17-dihydro-17-hydroxy GA_{53}, and 16□,17-dihydro-16,17-dihydroxy GA_9, with structures confirmed by partial synthesis. Evidence was also found of 16,17-dihydro-16,17-dihydroxy GA_{12}, 12-hydroxy GA_{14}, GA_{34}-catabolite and several hydroxy- and dihydroxy-GA_{12}-like compounds. The catabolites of GA_1 and GA_4 (the active GAs) or of significant precursors, hydroxylated at C-2 in stems and either C-2, C-12, C-17, or C-16,17 in capsules, were the major proportion of the GAs. These results suggest that in developing capsules hydroxylation at locations other than C-2 (in particular C-17 and C-16,17) may be important in regulating the pools of substrates for two of the key enzymes in GA biosynthesis - GA20-oxidase (GA_{12} and GA_{53}) and 3□-hydroxylase (GA_9 and GA_{20}).

P57

THE ROLE OF ATP8 IN CYTOPLASMIC MALE STERILITY OF CARROT

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Petaloid-type cytoplasmic male sterility (cms) in carrot is characterized by the homeotic-like conversion of stamens to petals. The physical map of the mitochondrial genome of petaloid carrot has three recombinationally-active repeated sequences, one of which contains part of the *atp*8 (*orf*B) gene. Sequencing revealed that two different versions of *atp*8 may be encoded as a result of recombination within the repeat. Both ORFs have long carboxy-terminal extensions relative to other plant *atp*8 genes and both types of extensions are transcribed without expected stop codons created by RNA editing. It has been suggested by other researchers that the presence of one of these *atp*8 ORFs (*atp*8-1) correlates with petaloid cms. However, we can identify *atp*8-1 in both sterile and fertile lines. The other *atp*8 we have identified in cms carrot (*atp*8-2) was detectable only in sterile lines. Another allele (*atp*8-3) has been detected only in fertile lines. The expression of fertility-restoring nuclear alleles (Rf or ms) did not affect the transcription or RNA editing of *atp*8-1 or *atp*8-2, indicating that if either of these alleles is involved in mediating petaloid cms in carrot, it is not at a DNA or RNA level.

P58

EFFECTS OF VANADIUM ON GROWTH AND NITRATE REDUCTASE ACTIVITY IN SOYBEAN LEAVES IN RELATION TO MOISTURE OF SOIL I

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The purpose of this work was to examine the effect of seed treatments with vanadium on nitrate reductase (NR) activity and the dry matter accumulation of soybean plants in relation to soil moisture. The experiments were performed under greenhouse conditions using the pots culture. The soybean (Glycine max., L. Merr) plants in the flowering stage were maintained temporary (10 days) at drought conditions (35% of field water capacity) and the control plants at 70% - value is consider as optimal moisture for soybean plants. The untreated plants by V served as the control .The application of micronutrient (0.030-0.045%) showed an increase in the dry matter accumulation of the leaves. The final yield of the plants treated with V evaluated as dry weight of entire plant, was higher than the control. Vanadium limitation caused reduction in plant growth. The seed treatments with low concentration of element (0.015%) did not modify the NR activity and the content of nitrates in leaves! at the optimal moisture of soil. Increasing V supply (to 0.045%) resulted in the enhancement of the nitrogen and nitrates of the tissues compared to control plants.. The water deficit at the flowering stage caused a significant ($P<0.05$) decrease in NR activity of leaves. However increasing NR activity (by 14%) due to nutrition of V was significantly observed in leaves of plants grown at water deficit.

P59

A ROOT-SPECIFIC CONDENSING ENZYME FROM *LESQUERELLA FENDLERI* THAT ELONGATES VERY-LONG-CHAIN SATURATED FATTY ACIDS

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Very-long-chain fatty acids (VLCFAs; chain lengths ≥20) are synthesized by sequential additions of C2 moieties from malonyl-CoA to C18 fatty acyl precursors. Each cycle of elongation involves four enzymatic reactions, the first of which is a condensation reaction. It is the substrate specificity of the condensing enzyme that determines the spectrum of VLCFA products made in a given cell type. A gene, *LfKCS45*, was isolated by probing a *Lesquerella fendleri* genomic library with the *Arabidopsis FAE1* gene. *FAE1* encodes a seed-specific condensing enzyme. At the amino acid level LfKCS45 was found to share 70% sequence identity with *Arabidopsis* FAE1. Fusion of the *LfKCS45* promoter to the *uidA* reporter gene resulted in expression of □-glucuronidase activity exclusively in the root-tip region of transgenic *Arabidopsis* plants. Expression of the *LfKCS45* in *Saccharomyces cerevisiae* lead to an increase in the accumulation of saturated VLCFAs (>C26). *Arabidopsis* plants have been transformed with *LfKCS45* driven by a seed-specific promoter. The fatty acid profile of seeds from the transgenic lines is currently being analyzed. The substrate specificity of *LfKCS45* is also being investigated using *in vitro* assays.

P60

BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF THIOL AND CHLORIDE METHYLATION IN PLANTS, AND ITS IMPLICATIONS FOR PEST AND STRESS RESISTANCE

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Certain plants, algae and fungi can methylate organic thiols and/or Cl^- ions. Our group and others have purified, characterized and cloned several methyltransferases that catalyze these reactions using *S*-adenosyl-methionine as the methyl donor. Despite catalytic similarities between these novel groups of enzymes (thiol- and chloride-methylators), their putative metabolic roles and physiological functions are quite distinct. Thiol methyltransferases (TMTs) are present in Brassicaceae and other families that contain glucosinolates. Upon pest attack, glucosinolates are hydrolyzed by a separately compartmentalized myrosinase enzyme to release thiocyanate, bisulfide and thiolates. TMTs methylate these substrates and thus potentially reduce their toxicity to the plant. In addition, several products of these reactions are volatile and have been implicated in plant-insect and plant-pathogen interactions. On the other hand, methylation of Cl^- ions by chloride methyltransferases in halophytes and marine algae has been viewed as a mechanism for Cl^- detoxification via its volatilization to chloromethane. However, the exact nature and role of these enzymes remains controversial. Our recent progress in characterizing these metabolic sectors and the genes involved, and the implications of this research for biotechnological interventions to improve plant defences against stress and pests will be presented.

Comment: Typo? This is from the original.

P61

BOTH OVER-EXPRESSION AND SUPPRESSION OF A REDOX-ACTIVATED MAPK RENDER TRANSGENIC TOBACCO PLANTS OZONE-SENSITIVE

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In plants, the role of MAPKs in ROS (reactive oxygen species)-based signal transduction processes is elusive. Despite the fact that ROS can induce rapid and specific MAPK activation, as well as formation of necrotic lesions, no direct genetic evidence has linked ROS-induced MAPK activation functionally with cell death. In tobacco, the major ROS-induced MAPK is Salicylate-Induced Protein Kinase (SIPK). We report here through gain-of-function and loss-of-function approaches that both over-expression and RNAi-based suppression of SIPK render the plant sensitive to ROS stress. Transgenic lines over-expressing a non-phosphorylatable version of SIPK were not ROS-sensitive. Analysis of the MAP kinase activation profiles in ROS-stressed transgenic and wild type plants revealed a striking interplay between SIPK and another MAPK (Wound-Induced Protein Kinase; WIPK) in the different kinotypes. During continuous ozone exposure, abnormally prolonged activation of SIPK is seen in the SIPK-overexpression genotype, without WIPK activation, while strong and stable activation of WIPK was observed in the SIPK-suppressed lines. One role of activated SIPK in tobacco cells upon ROS-stimulation thus appears to be control of the inactivation of WIPK.

P62

BIOCHEMICAL ANALYSIS OF A DIFFERENTIAL GERMINATION RESPONSE TO OSMOTIC STRESSES UNDER BLUE LIGHT IN A MALE-STERILE TOMATO MUTANT

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A photoperiod-sensitive male-sterile mutant(*7B-1*) in tomato shows light-dependent resistance to osmotic stresses, as compared to the wild type (WT), and this resistance is accentuated in blue light. In the dark, there was no difference in seed germination between the mutant and the WT in the presence of mannitol (100 mM). However, in light 80% reduction in germination was recorded in the WT compared to no inhibition in the mutant. Seed germination was always better in the mutant exposed to various types of osmotic and other abiotic i.e., salt, stresses and grown under light. To investigate the possible cause(s) of this differential behavior, a biochemical approach is being used to analyze the proteins, by two-dimensional gel electrophoresis, in the embryos of *7B-1* and WT. In the dark, and in the presence of mannitol, during the initial period of germination (at 24h) only minor differences were observed in the protein spots between the mutant and WT. However, in blue light, variation in protein spots was much more pronounced between the embryos of the two lines exposed to mannitol. The characterization of proteins, particularly the cryptochromes (blue light receptors) in the mutant and WT under various stress and non-stress conditions is currently underway.

P63

CYTOKININS AND ETHYLENE IN RELATION TO DARK-INDUCED LEAF SENESCENCE IN P_{SAG12}-*IPT* TRANSGENIC AND WILD TYPE TOBACCO

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Transgenic tobacco plants (*Nicotiana tabacum* cv. Wisconsin) expressing *ISOPENTENTL TRANSFERASE* (*IPT*) (gene encoding isopentenyl transferase) with the SAG12 promoter (P_{SAG12} - a highly specific senescence associated gene) inhibited leaf senescence. Tobacco plants containing P_{SAG12}-*IPT* retarded senescence in intact leaves as well as in leaf disks in the dark. Application of cytokinins, dihydrozeatin (DZ) or benzylamino purine) BAP at 10^{-4}M retarded senescence of leaf disks of wild-type tobacco but had little effect on leaf senescence of P_{SAG12}-*IPT* transgenic plants. On the other hand, 2-chloroethyl phosphonic acid (CEPA - an ethylene releasing compound) (10^{-4}M) accelerated senescence of leaf disks of wild-type tobacco but had little effect on leaf senescence of P_{SAG12}-*IPT* transgenic plants. Cytokinin levels in wild-type and P_{SAG12}-*IPT* transgenic plants were analyzed by enzyme-linked immunosorbent assay. The relationship between endogenous cytokinins and leaf senescence in P_{SAG12}-*IPT* transgenic plants and wild-type plants will be discussed.

P64

^{15}N-NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPIC STUDIES OF NITROGEN REYCLING DURING PHENYLALANINE METABOLISM IN *LENTINUS LEPIDEUS*

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Lentinus lepideus, a wood-decaying basidiomycete, displays high phenylalanine ammonia-lyase (PAL) activity and produces large amounts of methylated phenolic acids, especially *p*-methoxymethylcinnamate. The metabolic fate of the PAL-generated ammonium ion in *L. lepideus* was investigated. ^{15}N-L-Phenylalanine was administered to four-day old mycelium of *L. lepideus* in the dark. Analyses of the ^{15}N-labeled metabolites by ^{15}N-nuclear magnetic resonance spectroscopy indicate that this nitrogen is first incorporated into the amide moiety of L-glutamine and then into L-glutamate by the glutamine synthetase (GS)/glutamate synthase (GOGAT) pathway. ^{15}N is also incorporated into γ-aminobutyric acid (GABA) and alanine by a direct transamination at the level of phenylalanine. A new nitrogen recycling mechanism during phenylalanine
metabolism in fungi is proposed.

P65

EFFECTS OF ELEVATED CO_2 ON GROWTH OF ALFALFA (*MEDICAGO SATIVA* L.) INOCULATED WITH FUNGAL PATHOGENS

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Two cultivars of alfalfa differing in disease resistance were grown at CO_2 concentrations of 360 ppm (ambient) or 720 ppm (double ambient) in a gas exposure system. Six-week old plants were inoculated with a strain mixture of fungal isolates. Three days after inoculation, half of the plants were treated with fungicide to control the development of leaf disease and to test whether elevated CO_2 affected fungicide efficacy. Plants were grown for an additional six weeks and harvested. Over the course of the experiments, chlorophyll *a* fluorescence and chlorophyll content of the leaves were measured. Observations of disease incidence were also made. At the end of experiments above- and below-ground biomass, total soil nitrogen and disease severity were assessed. Elevated CO_2 caused an increase in above- and below-ground biomass of non-inoculated plants in both cultivars. In the disease-resistant cultivar inoculated with fungal pathogen decreases in biomass accompanied by loss of chlorophyll content and decreased chlorophyll *a* fluorescence were observed. Fungicide only partially alleviated the effects of the fungal pathogens. Inoculation did not affect photosynthetic characteristics and growth in the disease-sensitive cultivar. Visual observations of disease incidence and severity confirmed differential responses of the two cultivars of alfalfa grown at elevated CO_2.

P66

REGULATORY ASPECTS OF PII PROTEIN IN *ARABIDOPSIS THALIANA*

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The PII protein is one of the most widespread and highly conserved regulatory proteins with homologues found in archaebacteria, bacteria, and eukaryotes. PII is known as the central signal transmitter in bacterial nitrogen metabolism. The role of PII in higher plants is also known to be involved in nitrogen control, although details of its function remain to be understood. Our experiments extended some regulatory aspects of bacterial PII proteins to the higher plant PII. We have demonstrated that *Arabidopsis* PII binds to small effector molecules ATP and 2KG, and with lesser affinity to OAA. The oligomeric structure of *Arabidopsis* PII was determined as a homotrimer, again similar to bacterial PII proteins, and by sequence similarity we expect the plant PII to bind one ATP and one 2KG molecule per subunit. Based on our *in vitro* binding studies, we predict that in the cell plant PII is continually bound by ATP, and its ligand-bound state varies only with respect to the degree of 2KG binding. The function of plant PII as a 2KG sensor is suggested.

P67

INFECTIVITY OF *HETERODERA SCHACHTII* ON *BRASSICA NAPUS*

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Infection of canola (*Brassica napus*) by the sugarbeet cyst nematode, *Heterodera schachtii*, has been demonstrated and compared to infection levels observed in cabbage (*Brassica oleracea*), a known host of this endoparasitic nematode. In examining cyst production on infected plants, no significant difference was found between the two *Brassica* species, 1 - 31 cysts/plant were found in canola compared to 4 - 76 cysts/plant in cabbage. Comparison of growth rates between infected and uninfected plants within each species demonstrated that infection caused a significant decline in above ground growth in both. This finding was supported by examining physical changes occurring in plants with increasing time of infection. At 9 days infection, little difference was seen between infected and uninfected plants. At 24 days infection, both species showed signs of wilting, stunting of growth and leaf discoloration. By 38 days infection, all infected plants were severely stunted and leaves showed extensive discoloration. At this stage uninfected canola was beginning to bolt. Infected canola showed no signs of bolting and lacked eventual seed formation. Similarly, root development in both species was severely affected by nematode infection. Changes included significantly smaller root systems, decreased production of secondary roots, and severe pigmentation of all root tissue. Given the increasing identification of *H. schachtii* in Canadian agricultural lands, the pervasive nature of the dormant cysts, and the potential for population amplification, understanding of the plant/pathogen interaction is critical.

P68

ISOLATION AND CHARACTERIZATION OF GENES DIFFERENTIALLY EXPRESSED IN AL-SENSITIVE AND -TOLERANT WHEAT CULTIVARS WITH AN IMPROVED SSH PROCEDURE.

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Aluminum (Al) is considered to be the principal factor limiting plant productivity in acid soils. Since 78,4% of arable soils are acid (pH<5,5) with 40,9% in North America, especially in Canada, thus it is important to understand and overcome the phytotoxicity of aluminum. In wheat, the phenotype of tolerance is dominant, hereditary, supported by the nuclear genome. The root apex is the first target of Al toxicity. To identify new genes that may be involved in Al toxicity and tolerance, the Suppression Subtractive Hybridization (SSH) technique was applied between root apex mRNA of tolerant and sensitive varieties of wheat exposed to different Al concentrations. To find rare genes and reduce the number of false positives, several modifications of the SSH procedure were made. The major changes consist in avoiding the *Rsa* I digestion of the driver cDNA and performing PCR in the presence of excess driver RNA as inhibitor of amplification for genes expressed in both cultivars. We have isolated two very rare genes: a hypersensitive response gene and a hypothetic transporter of oligopeptides. The expression of these two genes in various cultivars is currently analyzed and will be discussed.

P69

MEETING THE METHYLATION REQUIREMENTS OF PLANT DEVELOPMENT

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We are investigating activities of two enzymes, *S*-adenosylhomocysteine hydrolase (SAHH) and adenosine kinase (ADK), associated with the activated methyl cycle and the changes in the methylation needs of plants. Essentially all stages of plant growth require methylation, principal examples being the synthesis of pectin, lignin, phosphatidylcholine as well as modification of nucleic acids. SAHH and ADK transcript and protein abundance have been monitored in developing siliques and stems to examine the correspondence between their expression levels and the methylation requirements of these organs. Transcripts and protein have been localized by in situ hybridization and immunofluorescence detection, respectively. Conditions that incur the synthesis of methylated products, such as pathogen attack or growth in short days have been used to evaluate the responsiveness of these enzymes to biotic and abiotic stresses. Based on these and earlier results, we propose that SAHH and ADK activities rise in response to increased methyl demand and that metabolites of the methyl cycle act as initial signals or mediators. To test this hypothesis we are introducing promoter::reporter gene constructs for each ADK and SAHH gene-promoter into wild-type and mutant Arabidopsis plants. As well, metabolite analysis of ADK and SAHH mutants is being carried out to evaluate the mechanism of SAHH and ADK regulation.

P70

THE IDENTIFICATION AND ISOLATION OF PLANT PHOSPHATASE-1 (PP1) REGULATORY SUBUNITS FROM THE HIGHER PLANT *ARABIDOPSIS THALIANA*

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Protein phosphatase-1 (PP1) is a member of the PPP family of protein phosphatases, which dephosphorylate phosphoserine and phosphothreonine residues. Type 1-protein phosphatases are highly conserved and well studied in both fungi and mammals, but much less is known about the role and regulation of PP1 in higher plants. PP1 and its associated regulatory subunits were purified from crude extracts of *Arabidopsis thaliana* using anion exchange and microcystin affinity chromatography. Putative PP1 regulatory subunits were identified by MALDI-TOF mass spectrometry from a tryptic digest of the purified proteins. Far western experiments using digoxigenin-conjugated recombinant PP1 as a probe were performed to positively identify *A. thaliana* PP1 regulatory subunits. Several putative PP1 regulatory subunits were identified. These findings provide valuable insights into the localization of PP1 in the cell and its cellular targets.

P71

ICE RECRYSTALLISATION INHIBITION PROTEIN FROM WINTER WHEAT (*TRITICUM AESTIVUM L.*)

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Low temperature (LT) and Freezing tolerance (FT) in cereals is a complex multigenic trait. To elucidate the molecular and genetic basis of this trait, we searched for new genes using the virtual screening of a cold acclimated winter wheat cDNA library. Using this functional genomic approach, we identified a gene with high homology with Ice Recrystallisation Inhibition protein. Northern analysis demonstrated that the transcript level is exclusively upregulated during cold acclimatation. The transcripts accumulated in leaf and to a lesser extent in crown and root. The transcript was found to accumulate in FT species such as wheat and rye compared to the less tolerant species barley and oat. There was no accumulation in sensitive rice and corn. The cDNA encode a protein of 279 amines acid with a theorical isoelectric point of 8.23. The bioinformatic analysis indicate that the protein has a N-terminal signal, transmembrane domain, 6 repeats that characterize the ice recrystallisation inhibition domain and a region homologue to kinase receptor. The analysis also suggest that IRI domain is in the apoplasts while the kinase receptor in the intracellular part. The putative function of this protein during freezing is discussed.

P72

MECHANICAL GATING OF AQUAPORINS IN CORN ROOT CELL: EFFECT OF ABA

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A reversible gating function of water transport was found in root cortical cells of maize in response to pulses in cell turgor, when the cell-water-permeability (L_p) was measured by the cell pressure probe. Magnitude of the applied pressure pulses affected half-time of water exchange of individual cell ($T_{1/2}$) which is inversely proportional L_p. After several large pressure pulses $T_{1/2}$ changed from 0.59 to 5.0 seconds. Usually, the changes were irreversible. Medium size peaks induced longer $T_{1/2}$ values that could be reversed with small pressure relaxations. Small pressure difference did not change short $T_{1/2}$ even measuring individual cell for six hours. ABA (abscisic acid) affected gating in that irreversible changes of $T_{1/2}$ tended to be wholly or partially reversible by adding ABA. Reversed cells repeatedly switched to longer $T_{1/2}$ again by applying large pressure pulses. Mercurial reagent inhibited L_p when the cells were in the state of high-water-permeability. The L_p changed from 3.4 □ 10^{-6} to 0.53 □ 10^{-6} m s^{-1} MPa^{-1}. However, it did not affect L_p when the cells had been induced by big pressure pulses into the state of low-water-permeability. Those evidences demonstrated that there exists a gating-like system in maize cell membrane to mediate reversibly water transport.

P73

SUPPRESSION OF DEOXYHYPUSINE SYNTHASE EXPRESSION IN *ARABIDOPSIS THALIANA* DELAYS LEAF SENESCENCE

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A full-length cDNA clone encoding deoxyhypusine synthase (DHS) was isolated from a cDNA expression library prepared from senescing leaves of *Arabidopsis thaliana*. DHS mediates the first of two enzymatic reactions that convert inactive eucaryotic translation initiation Factor-5A (eIF-5A) to an activated form able to facilitate translation. Southern blot analysis indicated that DHS is encoded by a single-copy gene in *Arabidopsis*. During leaf development, the abundance of DHS mRNA peaked at weeks 4 and 7 after planting, corresponding to the initiation of bolting and the later stages of leaf senescence, respectively. Levels of DHS transcript also increased in detached leaves coincident with the onset of post-harvest senescence. DHS was suppressed in transgenic plants by expressing antisense full-length *Arabidopsis* DHS cDNA under regulation of the constitutive cauliflower mosaic virus (CMV-35S) promoter. Plants expressing the antisense transgene had reduced levels of leaf DHS protein and exhibited delayed natural leaf senescence as well as enhanced seed yield in comparison with wild-type plants grown under identical conditions. Suppression of DHS also delayed the onset of premature leaf senescence induced by drought stress and resulted in enhanced survival in comparison with wild-type plants. In addition, post-harvest senescence of detached leaves from transgenic plants was delayed.

P74

PLASMA MEMBRANE FERRIC CHELATE REDUCTASE ACTIVITY AND THE IRON-LIMITED GROWTH RATE IN UNICELLULAR GREEN ALGAE

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Four species of green algae (*Chlorella kessleri*, *Chlorococcum macrostigmatum*, *Haematococcus lacustris*, *Stichococcus bacillaris*) were grown in iron-limited chemostats over a range of iron-limited growth rates. Plasma membrane ferric chelate reductase activity was enhanced by iron limitation in all species, and suppressed by phosphate limitation and iron sufficiency. While imposition of iron limitation led to enhanced activities of ferric chelate reductase in all species, the relationship between per cell ferric chelate reductase activity and degree of iron limitation varied. In contrast to the other species, there were large effects of iron-limited growth rate on *H. lacustris* cell volume; ferric chelate reductase activity declined with decreasing degree of iron limitation when calculated on a cell volume basis. Calculation of ferric reductase activity on a per chlorophyll basis allowed for a clear differentiation between iron-limited and iron-sufficient cells. Based on the per chlorophyll data, it may be feasible to use an in situ ferric chelate reductase assay to investigate the absence or presence of iron limitation in natural waters. As well, the results may also provide for insights into the regulation of ferric reducing capacity in plant roots.

P75

OXYGEN CONSUMPTION ASSOCIATED WITH IRON ACQUISITION BY IRON-LIMITED ALGAL CELLS

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Plasma membrane ferric chelate reductase activity was enhanced five-fold under iron limitation in the unicellular green alga *Chlorella kessleri*. Furthermore, ferric chelate reductase activity in iron-limited cells was approximately 50% higher in the light compared to dark. In contrast, iron uptake rates of iron-limited cells were unaffected by light versus dark treatments. Rates of iron uptake were much lower than rates of ferric reduction, averaging approximately two per cent of the dark ferric reduction rate. Ferric reduction was associated with an increased rate of O_2 consumption in both light and dark. The stimulation of O_2 consumption was non-respiratory, and was almost completely abolished by the addition of BPDS, a strong chelator of Fe^{2+}. Anaerobic conditions or the presence of exogenous superoxide dismutase affected neither ferric reduction nor iron uptake. We suggest that the O_2 consumption associated with ferric reductase activity resulted from superoxide formation from the aerobic oxidation of Fe^{2+}, which is the product of ferric reductase activity. At saturating concentrations of Fe^{3+}-chelates, ferric reductase activity is much greater than the iron uptake rate, leading to rapid oxidation of Fe^{2+} and superoxide generation. Therefore, O_2 consumption is not an integral part of the iron assimilation process.

P76

ANALYSIS OF INTERCELLULAR CALCIUM CONCENTRATIONS IN EMBRYOGENIC MICROSPORES OF *BRASSICA NAPUS*

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Cultured *Brassica napus* microspores undergo a shift in development from sporophytic to embryogenic when incubated at 30°C. Since intercellular calcium increases coincide with fertilization in other species, we hypothesize that intercellular calcium contributes to embryogenic induction in *B. napus* microspores. To test this hypothesis, cultured microspores were treated with inhibitors that affect components of the calcium-signalling pathway. The treatments' impacts were evaluated using flow cytometric measurments of cell viability and embryo counts after a three-week incubation period. Inhibition of DAGK and PP1/2A enhanced embryogenic potential in 30°C microspore cultures while inhibition of $InsP_3$ and PIP_2 abolished the embryogenic response. Also, the degree of up-regulation of a putative calcium stimulated RopGAP was determined by screening cDNA from embryogenic microspores. Degenerate PCR primers were designed from an *Arabidopsis thaliana* sequence similar to *Homo sapiens* CAPRI. Three PCR products corresponding to partial ORFs of RopGAPs were characterized. Preliminary data suggests that these potential isoforms of RopGAP*Bn* are transciptionally up-regulated in embryogenic microspores. Ongoing experiments shall determine the effects of other calcium-pathway inhibitors/activators on embryogenesis and to clone other isoforms of RopGAP. Understanding intercellular calcium's role during embryogenesis may allow the development of novel approaches to increase the regenerative potential of cultured microspores.

P77

AN EXAMINATION OF CYCLOBUTANE PYRIMIDINE DIMER (CPD) DNA PHOTOLYASE OF THE ALPINE AND PRAIRIE ECOTYPES OF *STELLARIA LONGIPES* (CARYOPHYLLACEAE) TO ULTRAVIOLET RADIATION.

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With a reduction of the stratospheric ozone layer, ultraviolet radiation (UVR) has increased proportionally. Plants are at high risk of UVR damage since they are immobile. UVR is known to cause damage to membranes, proteins, and nucleic acids. Direct damage to genomic DNA causes lesions that impede replication and transcription, leading to cell death. Currently, knowledge of UVR effects in plants is limited compared to bacterial and animal systems. There are various strategies plants use against UVR. One mechanism includes avoidance, either by use of cuticular modifications to block UVR penetration, or anthocyanins to absorb UVR. Another mechanism involves DNA repair mechanisms (ie. DNA photolyases) to directly repair UVR-induced DNA damage. We exposed alpine and prairie ecotypes of *Stellaria longipes* to UV-B. After exposure, the alpine ecotype recovered while the prairie ecotype did not. We examined cyclobutane pyrimidine dimer DNA photolyase in these two ecotypes. Based upon dot blots, we show the presence of CPD photolyase gene in both ecotypes. Initial studies indicate possible CPD DNA photolyase expression differences between the ecotypes.

P78

A GENOMIC AND BIOCHEMICAL SEARCH FOR FUNCTIONAL APOPTOSIS GENES IN WHEAT AND THE STUDY OF THEIR RESPONSE TO WHEAT LEAF RUST

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Some defense signal pathways are common between plant and mammalian cells. However, there are no clear homologues of many mammalian defense and cell-death control genes in the complete *Arabidopsis* genome, and many pathways, pathway components, or functions of those components are unique to plants (1). To evaluate the possible involvement of apoptosis in response to pathogen attack in wheat, we mined a wheat EST database for caspases and poly(ADP-ribose) polymerases (PARPs). Both EST database and protein database searches did not reveal wheat homologues to known caspases or PARPs. Antibodies against caspase-7 or cleaved caspase-7 did not show cross-reactions to wheat proteins. However, PARP-like proteins were detected in immuno-analysis. PARP antibody detected polypeptide bands at 55 kDa and 45 kDa and both polypeptides decreased in both BBB- and TJB-challenged Lr1 wheat leaves. Using antibodies against cleaved-PARP, a signal at 12 kDa was significantly increased in samples challenged by race TJB but not by race BBB. Our preliminary study has suggested that a functional PARP-like protein may exist in wheat which is activated in response to wheat leaf rust invasion.

1. Xing T. and Jordan M. 2000, Genetic engineering of signal transduction mechanisms. *Plant Mol. Biol. Rep.* 18: 309-318.

P79

DISSECTION OF PROGRAMMED CELL DEATH PROCESS DURING WHEAT-LEAF RUST INTERACTIONS

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Programmed cell death (PCD) is an active process of cellular suicide triggered by a variety of physiological and stress stimuli (1) . We used genomics, biochemical and histochemical approaches in the study of incompatible and compatible interactions of wheat and leaf rust. We identified several PCD components in a wheat EST database. Although we found no caspases or poly(ADP-ribose) polymerases (PARPs), there are accumulative evidence that support the existence of caspase-like proteins (CLPs) and PARP-like proteins in plants. DNA laddering and terminal transferase-mediated dUTP nick-end labeling (TUNEL) analysis have revealed detailed progress of PCD during the infection process. Differences exist between incompatible and compatible wheat-leaf rust interactions. Our study has suggested that PCD pathways may play a role in plant defense in this pathosystem.

1. Fan T. et al. 1998, *J. Exp. Med.* 187: 487-496.

P80

IDENTIFICATION OF THE TRANSCRIPTIONAL FACTOR MEDIATING THE ACTIVATION OF TOMATO PR1B1 GENE DOWNSTREAM OF A MAP KINASE PATHWAY

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Constitutive activation of *tMEK2*, a mitogen-activated protein kinase kinase (MAPKK), in tomato activated pathogenesis-related (PR) 1b1 gene (1). We mined *Arabidopsis* EST database for potential transcriptional factors (TFs) downstream of MAP kinase (MAPK) using WU-Blust2 against known mammalian TFs that mediate MAPK pathways. To search for binding sites in PR1b1 promoter for TFs downstream of MAPK, these *Arabidopsis* TFs were analyzed in Transfac database, a transcriptional factor database, with MatInspector program. The analysis indicated that the promoter contains binding sites for Elf1 and AP1. A protoplast co-expression system was used in deletion analysis. Deletion of Elf1 binding site blocked activation of PR1b1 gene by tMEK2. Deletion of AP1 binding site did not affect this activation. GCC box and W box, two regulatory elements found in some defense-related genes, also exist in the PR1b1 promoter region. Deletion of each of them did not affect the activation of PR1b1. Other evidence also indicated that Elf1 may mediate MAPK pathways in plants.

1. Xing T. et al. 2001, Activation of tomato PR and wound-related genes by a mutagenized tomato MAP kinase kinase through divergent pathways. *Plant Mol. Biol.* 46: 109-120.

P81

METABOLIC PROFILING OF *ARABIDOPSIS* VOLATILES THAT MODIFY FLEA BEETLE ELECTROPHYSIOLOGICAL BEHAVIOR

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Plant volatiles play an important role as cues for herbivorous insects when they are locating host plants. Crucifer crops are hosts for several different insect species, including flea beetles (*Phyllotreta cruciferae*) (Coleoptera: Chrysomelidae). These insects are the most serious pest of canola in Canada. In this investigation, *Arabidopsis thaliani* was used as model crucifer plant to identify the volatiles which are recognized by flea beetles. Rapid mini-scale methods were developed to profile volatiles by GC and GC/MS using tissues which are most susceptible to flea beetle damage in the canola crop. Results revealed that *Arabidopsis* cotyledon volatiles are mainly composed of green odor compounds, isothiocyanates and nitriles in a profile that is very distinct compared to selected crucifer breeding germplasm.. Electrophysiological responses of flea beetle antennae to *Arabidopsis* volatiles were tested using a GC-electroantennographic detector coupling technique. Initial results showed that lower b.p. compounds (green odour chemicals and isothiocyanates) elicited higher electrophysiological responses than higher b.p. compounds (nitriles). The technique was also used to profile volatiles from several *Arabidopsis* mutants with altered flea beetle feeding.

P82

EVIDENCE FOR THE EXISTENCE OF ALTERNATIVE OXIDASE RELATED TO NON-PHOTOCHEMICAL PQ-POOL REDUCTION

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The relationship between reduction of plastoquinone by added NADPH during dark adaptation and reoxidation by molecular oxygen of intact thylakoïds membranes was studied using the OJP rise of chlorophyll*a* fluorescence induction kinetics. A drastic increase of F_J was observed under anaerobic conditions but the initial OJP rise was restored by aeroby. Propyl gallate (PG) an inhibitor of quinol oxidases displayed a significant increase of F_J comparable to that observed in thylakoïds incubated under anaerobiosis. Interestingly, when PG was added to heat-treated thylakoïds in a medium containing NADPH, the shape of the Chl*a* fluorescence transient changed. F_P was less quenched compared to the same treatment in the absence of PG and started to increase at around 100 ms. F_O and F_J fluorescence levels were increased and the maximum level of F_J was achieved within 0.8 to 0.9 ms (compare to 2 ms for control). Otherwise, F_O and F_J were strongly affected by PG and anaerobiosis. F_P-level was more affected by heat treatment. Our results supply additional evidence for the existence of a chlororespiratory pathway.

P83

CELLULAR ASCORBIC ACID REGULATES THE ACTIVITIES OF MAJOR PEROXIDASES IN GERMINATING WHITE SPRUCE SOMATIC EMBRYOS

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In previous studies we have demonstrated that ascorbic acid (AA) is required for white spruce somatic embryo conversion. In order to determine the effects of AA on cellular peroxidases, the activities of guaiacol-, ferulic acid-, and ascorbic acid-dependent peroxidases were measured after altering the endogenous AA content of the germinating embryos. This was achieved by using exogenously supplied AA, L-galactono-lactone (GL), the last precursor of the de novo synthesis of AA, and lycorine (L), which inhibits the conversion of GL to AA. Our studies demonstrated a negative correlation between cellular AA content and activities of both guaiacol and ferulic acid peroxidases. In addition to decreasing the post-embryonic performance of the embryos, a decrease in the endogenous AA level resulted in an increased rate of both guaiacol and ferulic acid oxidation. High levels of cellular AA did not have any effect on embryo conversion, but inhibited the activities of both peroxidases. Isoenzyme profile and in vitro studies also demonstrated that AA plays an important role in inhibiting total peroxidase activity in germinating embryos. Inhibition of cellular peroxidases, including those responsible for the cross-linking of wall components, may be required for promoting wall relaxation in the meristematic cells of germinating embryos.

P84

TRANSGENIC TOBACCO PLANTS WITH WOUND-REGULATED PATHOGENE RESISTANCE

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To engineer broad-spectrum disease resistance in plants, use of a wound/infection inducible promoter has distinct advantages. To evaluate the use of a wound inducible promoter (*win3.12*) from poplar (Hollick &Gordon) in tobacco, we amplified 823 bp downstream fragment using high fidelity PCR and specific primers based on the nucleotide sequence from GenBank Database. Two vectors with transcriptional fusion between the *win3.12*-promoter and either the ß-glucuronidase (GUS) reporter gene or a modified cecropin-melittin chimeric gene (which encodes a peptide with antimicrobial activity) were constructed and introduced into tobacco plants via *Agrobacterium*-mediated transformation. Stable gene integration into plants was confirmed with PCR and Southern blot analysis. Quantitative RT-PCR and Northern analyses showed significantly increased levels of transgene expression in response to mechanical wounding (simulating pathogene damage): up to 10-fold and more comparing to the unwounded background levels. Strong positive correlation between transgene copy number and *win3.12*-driven transcription was observed. Preliminary bioassay of transgenic plants using co-cultivation of leaf explants in the presence of *Fusarium ssp*. showed spectrum of plant resistance to the pathogenic fungus that corresponds to the data obtained from Northern analysis. These plants will be tested with other phytopathogens. Research supported by an NCE- CBDN grant to S.Misra.

P85

HORMONAL REGULATION OF GA3ox GENE EXPRESSION IN EARLY PEA FRUIT DEVELOPMENT

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Normal pea fruit growth requires the presence of developing seeds. The effect of seeds on fruit development is assumed to involve plant hormones. The GA3ox gene product, GA 3_-hydroxylase is responsible for 3β-hydroxylation of GA_{20} to the biologically active GA_1. To understand further how seeds, auxins (4-Cl-IAA, IAA) and gibberellins (GAs) regulate GA biosynthesis in pea (*Pisum sativum* L.) pericarp at the molecular level, real time PCR was used to quantitate GA3ox transcript levels in this tissue. The split-pericarp method was used to apply hormone treatments to 2 DAA deseeded pericarps, and study their effect on GA3ox expression over a 24-h period. Pericarp GA3ox mRNA levels with seeds (SP) were low and relatively stable; however when the seeds were removed (SPNS) the pericarp transcript declined to almost undetected levels throughout the treatment period. When the auxin 4-Cl-IAA (stimulates pericarp growth) was applied, GA3ox mRNA levels increased dramatically at 4h, then decreased to low levels by 12h after hormone treatment. GA_3 and the auxin IAA (does not stimulate pericarp growth) decreased pericarp GA3ox mRNA to almost undetected levels within 2h after application.
These data show that seeds, auxin (4-Cl-IAA) and gibberellins are involved in the regulation of pea fruit development. Supported by NSERC grant#138166-97

P86

ISOLATION OF THREE GLUTATHIONE S-TRANSFERASES FROM ELICITED TOBACCO CELL SUSPENSION CULTURES AND A POSSIBLE ROLE IN PHENOLIC METABOLISM

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Glutathione *S*-transferases (GSTs) are a ubiquitous group of dimeric soluble enzymes that conjugate various cytotoxic, xenobiotic compounds to the tripeptide glutathione. However, GSTs are as multifunctional as they are abundant. The ability of GSTs to detoxify xenobiotic compounds, such as herbicides has been well documented, but the possibility of GSTs acting as ligandins involved in the translocation of various endogenous compounds has not been extensively studied. There is compelling evidence that this may be the case. GSTs are required in the last step of the anthocyanin biosynthetic pathway in both maize (*Bz2*) and petunia (*An9*), and function in the sequestering of cytotoxic anthocyanins to the vacuole. In addition, the conjugating activity of a type III GST isolated from opium poppy was inhibited in the presence of various phenolic compounds. This suggests GSTs may be involved in the transport of certain phenolics, localizing them to the cell wall. Three different GSTs have been isolated from an elicited tobacco cell culture cDNA library. We are investigating the possibility that one or more of these enzymes are involved in the transport of phenolics and represents a novel function for GSTs in tobacco.

P87

DIFFERENTIATION OF GOLGI DURING SECONDARY XYLEM LIGNIFICATION IN *PINUS*

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Differentiation of secondary xylem is a dramatic developmental transformation, starting with thin-walled cambial cells undergoing mitosis and finishing with lignification and programmed cell death of mature tracheids. Because of the buildup of hydrolytic enzymes in the vacuole, prior to autolysis, it is difficult to study cell structure in developing tracheids. Cryofixation using high pressure freezing was used to preserve developing xylem from *Pinus contorta*, followed by freeze substitution and transmission electron microscopy. Developing tracheids contained prominent Golgi with unusual branching trans-Golgi networks and large, darkly staining vesicles. To test if these vesicles were involved in secretion of lignin precursors, developing xylem was treated with piperonylic acid, an inhibitor of cinnamate-4-hydroxylase, an important enzyme of phenylpropanoid metabolism. Piperonylic acid treatment resulted in altered Golgi morphology and disappearance of vesicles in developing xylem tracheids only, while adjacent parenchymous rays and the cambium did not show any cell structure changes. A model for conifer lignification is proposed where Golgi-mediated deposition of monolignols produces an early, organizing, lignin in the middle lamella. Following release of cell contents, monolignol glucosides stored in the vacuole are released into the cell wall where the action of glucosidases and peroxidases lead to lignification throughout the secondary wall.

P88

CHARACTERIZATION OF *BANYULS*, A POSITIVE REGULATOR OF FLAVONOID BIOSYNTHESIS IN THE *ARABIDOPSIS* SEED COAT, BUT NOT A LEUCOCYANIDIN REDUCTASE GENE

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Proanthocyanidians (PAs) are plant flavonoid polymers formed by the condensation of flavan-3-ol units with flavan-3,4-diols. Leucocyanidin reductase (LCR) is a key enzyme at the branchpoint in the flavonoid pathway leading to the production of PAs. LCR uses a flavan-3,4-diol to synthesize catechin (a flavan-3-ol). This enzyme has been characterized as a function of development in several plant species, but has not been cloned yet. Recently, the *Arabidopsis* seed coat gene, *BANYULS*, was suggested to be LCR (Devic et al., 1999). We expressed *BAN* in *E. coli* to determine whether it is functional, but the expressed protein did not show any LCR activity. When transformed into *Arabidopsis* under the control of a CaMV35S promoter, *BAN* transcripts were newly detected in leaves of transgenic plants, while seeds were a darker colour. However, no LCR enzyme activity was detected in leaves. Addition of the recombinant BAN protein to leaf extracts of *Lotus uligonosis* (which normally have very high LCR activity) did not increase the formation of PA dimers or trimers. This suggests that *BAN* is not a structural gene involved in flavan-3-ol production or condensation. Analysis of a range of knockout lines of *Arabidopsis* may shed more light on the true function of *BAN* in PA biosynthesis.

P89

BIOCHEMICAL AND GENETIC CHARACTERIZATION OF LEUCOCYANIDIN REDUCTASE IN RIBES, LOTUS, ROBINIA AND BARLEY

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Leucocyanidin reductase (LCR) one out of a sub-class of NADP(H)-dependent oxido-reductases called leucoanthocyanidin reductases (LAR) which function to direct unstable flavan-3,4-cis-diols into the proanthocyanidin rather than the anthocyanin pathway. This activity has been characterized previously as a function of plant development. Otherwise, little is known about these enzymes and their relationship to other components of proanthocyanidin biosynthesis. In this study, we purified and characterized LCR activity from three plant species which accumulate large quantities of proanthocyanidin and compared it with activity in barley seed. Distinct forms of LCR activity emerge. *Robinia pseudoacacia* and *Lotus uliginosis* leaf activities have broad optima at pH 5.5, while *Ribes sanguineum* floral activity and *Hordeum vulgare* seed activities are optimal at 6.5. Activity was strongly influenced by specific metal ions and by several of the mutations recovered in barley seed coat *ant* lines.

P90

METABOLIC PROFILING OF *ARABIDOPSIS* VOLATILES THAT MODIFY FLEA BEETLE ELECTROPHYSIOLOGICAL BEHAVIOR

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Plant volatiles play an important role as cues for herbivorous insects when they are locating host plants. Crucifer crops are hosts for several different insect species, including flea beetles (*Phyllotreta cruciferae*) (Coleoptera: Chrysomelidae). These insects are the most serious pest of canola in Canada. In this investigation, *Arabidopsis thaliani* was used as model crucifer plant to identify the volatiles which are recognized by flea beetles. Rapid mini-scale methods were developed to profile volatiles by GC and GC/MS using tissues which are most susceptible to flea beetle damage in the canola crop. Results revealed that *Arabidopsis* cotyledon volatiles are mainly composed of green odor compounds, isothiocyanates and nitriles in a profile that is very distinct compared to selected crucifer breeding germplasm.. Electrophysiological responses of flea beetle antennae to *Arabidopsis* volatiles were tested using a GC-electroantennographic detector coupling technique. Initial results showed that lower b.p. compounds (green odour chemicals and isothiocyanates) elicited higher electrophysiological responses than higher b.p. compounds (nitriles). The technique was also used to profile volatiles from several *Arabidopsis* mutants with altered flea beetle feeding.