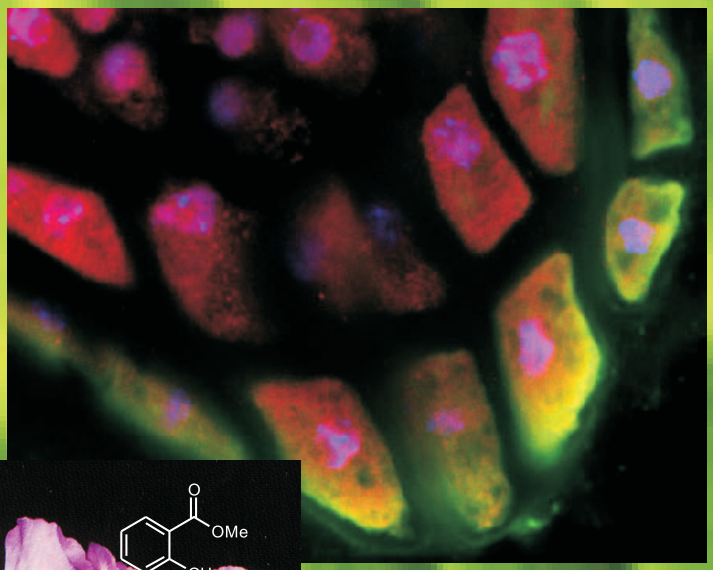
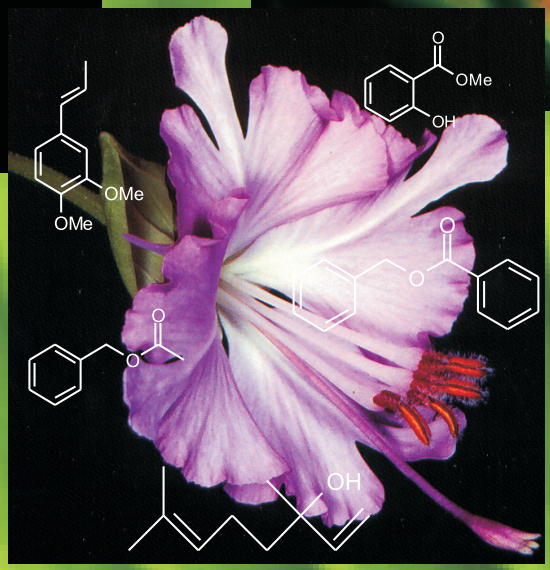
Proceedings of
The Canadian Society of Plant Physiologists
Eastern Regional Meeting

The University of Western Ontario
December 1st, 2007





Délibérations du congrès de la
Société Canadienne de Physiologie Végétale (Congrès régional de l'est)

On the Front Cover:

Left: Flowers of *Lotus japonicus*, a model legume for studying beneficial plant-microbe interactions.
Photo provided by Krzysztof Szczyglowski

Centre: *Clarkia Breweri* flower and some of the volatiles it emits.
Photo provided by Eran Pichersky

Right: Immunofluorescence localization of the flavonoid enzyme, chalcone isomerase (CHI), to both the cytoplasm and nucleus of *Arabidopsis* root tip cells. Fixed whole-mount seedlings were double-labeled with antibodies against CHI (red) and ER-resident protein, BiP (green); nuclei were counterstained with DAPI (Blue).
Photo provided by David E. Saslowsky & Brenda S.J. Winkel

Welcome to the/ Bienvenue au la

2007

Eastern Regional Meeting of the Canadian Society of Plant Physiologists

Société Canadienne de Physiologie Végétale Congrès Régional de l'Est

The University of Western Ontario London, ON, Canada

Saturday December 01, 2007

Local Organizing Committee:
Dr. Norman P.A. Hüner
Dr. Denis P. Maxwell
Dr. Mark A. Bernards

Canadian Society of Plant Physiologists

Société Canadienne de Physiologie Végétale

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SPECIALTY GASES & EQUIPMENT

Research Western

Western Science

biotron
experimental climate change research

Program Overview

Friday November 30, 2007
Pre-Conference Activities:

Biotron Tours: Friday November 30 @ 16:30
Reception & Registration: Friday Evening at the UWO-Grad Club 19:00-21:00

Saturday December 01, 2007
Main Conference

8:00 - 8:45 am …….Registration and Coffee
8:00 - 8:45 am……..Poster Setup
8:45 - 9:00 am……. Opening Remarks (NCB 113)
Dr. Gerald M. Kidder, Associate Vice-President (Research)
Dr. Richard A. Secco, Assistant Dean (Graduate & International Research)

9:00 - 9:45 am……. 1st Keynote Speaker Dr. Brenda Winkel (NCB 113)
Expecting the unexpected: lessons learned from the flavonoid pathway

9:45 - 10:30 am…..……. 2nd Keynote Speaker Dr. Eran Pichersky (NCB 113)
How Plants Evolve the Ability to Make So Many Scent Compounds

10:30 - 11:00 am.…….…... Refreshment Break (NCB Foyer)

11:00 am - 12:30 pm……...Contributed Papers
Contributed Paper Session A (Abstracts CP-A1 to CP-A6) NCB 113
Contributed Paper Session B (Abstracts CP-B1 to CP-B6) NCB 114

12:00 - 2:00 pm……CSPP Executive Meeting (NCB 301L)
12:30 - 2:00 pm. ……. Lunch and Poster Presentations (NCB Foyer)

2:00 - 2:45 pm……. 3rd Keynote Speaker Dr. Krzysztof Szczyglowski
2:45 - 3:30 pm……..Contributed Papers
Contributed Paper Session C (Abstracts CP-C1 to CP-C3) NCB 113
Contributed Paper Session D (Abstracts CP-D1 to CP-D3) NCB 114

3:30 - 3:45 ……..Refreshment Break (NCB Foyer)

3:45 - 5:00 pm…….……Contributed Papers
Contributed Paper Session E (Abstracts CP-E1 to CP-E5) NCB 113
Contributed Paper Session F (Abstracts CP-F1 to CP-F5) NCB 114

4:30 - 5:00 pm……..Awards Committee Meeting (NCB 301L)
5:00 - 5:30 pm……..Awards Presentations (NCB 113)
5:30 pm……Conference ends

Detailed Scientific Program
Saturday Dec. 01, 2007

8:45 - 9:00 am……. Opening Remarks (NCB 113)
Dr. Gerald M. Kidder, Associate Vice-President (Research)
Dr. Richard A. Secco, Assistant Dean (Graduate & International Research)
Dr. Norm Huner, CSPP-ERM Local Organizing Committee

9:00 - 9:45 am……. 1st Keynote Speaker (NCB 113)
Abstract K1
"Expecting the unexpected: lessons learned from the flavonoid pathway"
Dr. Brenda S.J. Winkel, P. Bowerman, K.C. Crosby, C.D. Dana, M.V. Ramirez, D.E. Saslowsky, and J.I. Watkinson, Virginia Polytechnic Institute and State University, Blacksburg

9:45 - 10:30 am…..……. 2nd Keynote Speaker (NCB 113)
Abstract K2
"How Plants Evolve the Ability to Make So Many Scent Compounds"
Dr. Eran Pichersky, Molecular, Cellular and Developmental Biology Department, University of Michigan

10:30 - 11:00 am.…….…... Refreshment Break (NCB Foyer)

11:00 am - 12:30 pm……...Contributed Papers Concurrent Sessions, A and B

[Presenting author's name underlined; Student presenters further denoted by an asterisk(*)]

Contributed Paper Session A - NCB 113, Chair: Dr. Rima Menassa
(Abstracts CP-A1 to CP-A6)

11:00 CP-A1 "Expression of therapeutic proteins in tobacco BY-2 cell suspension culture"
A. Ahmad* and R. Menassa, Department of Biology, University of Western Ontario and Agriculture and Agri-Food Canada, London

11:15 CP-A2 "Phenotypic analysis of flowering time and segregation distortion in a novel mutant of the MADS-AFFECTING FLOWERING 2 gene of *Arabidopsis thaliana*"
S.M. Rosloski* and V. Grbic, Department of Biology, The University of Western Ontatio

11:30 CP-A3 "A novel expression platform for the production of diabetes-associated autoantigen human glutamic acid decarboxylase (hGAD65)"
X. Wang*, M. Brandsma, D. Maxwell, A.M. Jevnikar, N. Huner, and S. Ma, Department of Biology, University of Western Ontario, (A.M.J., S.M.) Transplantation Immunology Group, Lawson Health Research Institute, London

11:45	CP-A4	"Common bacterial blight of *Phaseolus vulgaris*: Towards the identification of a resistance gene" G. Perry*, Y. Reinprecht, J. Chan and K.P. Pauls, Department of Plant Agriculture, University of Guelph
12:00	CP-A5	"Two *Arabidopsis* plant u-box (atpub) e3 ubiquitin ligases may function redundantly as regulators of plant signalling pathways during gametophytic transmission" D. Yee*, F. Gunawan, and D.R. Goring, Department of Cell and Systems Biology, University of Toronto
12:15	CP-A6	"The role of IAP1 in intercellular salicylic acid accumulation during Age-Related Resistance" J.L. Carviel* and R. K. Cameron, Department of Biology, McMaster University

Contributed Paper Session B - NCB 114, Chair: Dr. Bernie Grodzinski
(Abstracts CP-B1 to CP-B6)

11:00	CP-B1	"Interaction of brassinosteroid with other plant hormones in stress responses of *Arabidopsis* seedlings" U.K. Divi*and P. Krishna, Department of Biology, the University of Western Ontario
11:15	CP-B2	"Increased air temperature during simulated autumn conditions affects electron transfer and energy dissipation in *Pinus banksiana*" F. Busch*, N.P.A. Hüner, I. Ensminger, Department of Biology and The BIOTRON, The University of Western Ontario
11:30	CP-B3	"Growth and development under elevated CO_2 for transgenic lines of *Arabidopsis thaliana* (L.) Heynh. having altered dark respiratory rates" S.M. Weraduwage*, S. Rauf, M.C. Micallef, B. Grodzinski and B.J. Micallef, Department of Plant Agriculture, University of Guelph
11:45	CP-B4	"Rubisco and the response of black spruce to climate warming" D.A. Way* and R.F. Sage, Department of Ecology and Evolutionary Biology, University of Toronto
12:00	CP-B5	"Dynamics of mycorrhizal symbiosis: getting to the roots of metal-stress tolerance" P. Audet* and C. Charest, Dept. of Biology, University of Ottawa
12:15	CP-B6	"Cytokinin profiles during the infection of corn with *Ustilago maydis*" S.A. Bruce*, B.J. Saville and R.J.N. Emery, Watershed Ecosystem Graduate Program, Trent University

12:00 - 2:00 pm……CSPP Executive Meeting (NCB 301L)
12:30 - 2:00 pm. ……. Lunch and Poster Presentations (NCB Foyer)

2:00 - 2:45 pm……. 3rd Keynote Speaker (NCB 113)

Abstract K3

"Cytokinins in symbiosis: new insights into an old hypothesis"

Dr. Krzysztof Szczyglowski, S. Kosuta, B. Karas and M. Held, Department of Biology, the University of Western Ontario and Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London

2:45 - 3:30 pm……..Contributed Papers Concurrent Sessions C and D

Contributed Paper Session C - NCB 113, Chair: Dr. Susanne Kohalmi

(Abstracts CP-C1 to CP-C3)

2:45 pm CP-C1 "Genotype-specific transcriptome changes to osmotic stress and stress recovery within *Zea mays* roots"
T. Wambach* and L. Lukens, Department of Plant Agriculture, University of Guelph

3:00 pm CP-C2 "Expression profile of *Brassica napus* inoculated with the plant growth-promoting rhizobacteria *Pseudomonas putida* UW4"
J.C. Czarny* and B.R. Glick, Department of Biology, University of Waterloo

3:15 pm CP-C3 "The cDNA library screen and functional characterization of host proteins associated with Plum pox virus (PPV) infection"
T.S. Huang *and A. Wang, Department of Biology, the University of Western Ontario and Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London

Contributed Paper Session D - NCB 114, Chair: Dr. Sheila Macfie

(Abstracts CP-D1 to CP-D3)

2:45 pm CP-D1 "Co-immunopurification of phosphorylated bacterial- and plant-type phosphoenolpyruvate carboxylases with the plastidial pyruvate dehydrogenase complex from developing castor oil seeds"
R.G. Uhrig* and W.C. Plaxton, Department of Biology, Queen's University

3:00 pm CP-D2 "The 'surfaceome' of *Catharanthus roseus* is enriched for monoterpenoid indole alkaloid biosynthesis: Cloning and characterization of loganic acid methyltransferase from epidermis enriched cDNA library of *Catharanthus roseus*"
J. Roepke*, J. Murata and V. De Luca, Centre for Biotechnology, Brock University

3:15 pm CP-D3 "Hydroponics induces apoplastic flow to the xylem in *Arabidopsis*"
Kyle Bender* and Ewa Cholewa, (K.B.) Department of Biology, Queen's University, (E.C.) Department of Biology, Nipissing University

3:30 - 3:45 ……..Refreshment Break (NCB Foyer)

3:45 - 5:00 pm…….……Contributed Papers Concurrent Sessions E and F

Contributed Paper Session E - NCB 113, Chair: Dr. Mark Gijzen
(Abstracts CP-E1 to CP-E5)

3:45 pm CP-E1 "Dual targeting of the tRNA nucleotidyltransferase in plants – not just the signal"
S.S. von Braun, A. Sabetti, P.J. Hanic-Joyce, J. Gu, E. Schleiff and P.B.M. Joyce, (E.S.) LMU München, VW-Research group, Department of; (A.S., P.B.M.J.) Department of Biology and Centre for Structural and Functional Genomics, Concordia University, and (P.J.H., J.G., P.B.M.J.) Department of Chemistry and Biochemistry, Concordia University

4:00 pm CP-E2 "A functional genomics approach to dissect the pathogenicity of *Sclerotinia sclerotiorum*"
D. Liberti, D. Qutob, M. Gijzen and K.F. Dobinson, Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada

4:15 pm CP-E3 "Identification of a *Phytophthora sojae* avirulence effector that elicits cell death in soybean plants carrying Rps3a"
J. Tedman-Jones, S. Dong, B. Tyler, and M. Gijzen, Southern Crop Protection and Food Research Centre, Agriculture and Agri-food Canada, (B.T) Virginia Bioinformatics Institute

4:30 pm CP-E4 "TF989, a novel myb-like transcription factor, acts as activator for CHS8 gene expression in soybean"
J. Yi and S. Dhaubhadel, Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada

4:45 pm CP-E5 "Plant recombinant antibodies for the prevention of foot-and-mouth disease virus"
J.J. Joensuu, K. Brown, A. Clavijo, R. Menassa and J. Brandle, Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, (A.C.) Canadian Food Inspection Agency

Contributed Paper Session F - NCB 114, Chair: Dr. Susan Lolle
(Abstracts CP-F1 to CP-F5)

3:45 pm CP-F1 "A predicted interactome for Arabidopsis: expanding the Bio-Array Resource's collection of large-scale data sets available for hypothesis generation"
R. Ammar, J. Geisler-Lee, N. O'Toole, A.H. Millar, M. Geisler and N.J. Provart, Department of Cell & Systems Biology, University of Toronto, (J.G-L, M.G.) Department of Plant Biology, Southern Illinois University Carbondale, (N.O.) ARC Centre of Excellence in Plant Energy Biology, University of Western Australia

4:00 pm CP-F2 "Impact of mycorrhiza and growth rate on vascular maturity in roots"
J. H. Taylor, Department of Biology, Slippery Rock University

4:15 pm CP-F3 "Genome instability in hothead mutants: Outcrossing is not the only answer"
M.T. Hopkins, R. Yochim, and S. J. Lolle, Dept. of Biology, University of Waterloo

4:30 pm	CP-F4	"Redox modulation of starch biosynthesis" M.M. Burrell, I.J. Tetlow, M.J. Emes, Department of Molecular and Cellular Biology, University of Guelph
4:45 pm	CP-F5	"Methodologies for the quantification of ferric and cupric reductase activities by iron-limited algal cells" H.G. Weger, M.B. Fink and C.N. Walker, Department of Biology, University of Regina

4:30 - 5:00 pm	**……..**	**Awards Committee Meeting (NCB 301L)**
5:00 - 5:30 pm	**……..**	**Awards Presentations (NCB 113), Chair: Dr. Malcolm Campbell**
5:30 pm	**……..**	**Conference ends**

Posters for the 2007 CSPP-ERM
Presenting author's name underlined; Student presenters further denoted by an asterisk(*)

No.	Authors	Affiliation	Title
P1	E.T. Hamanishi, O. Wilkins, S. Raj, and M.M. Campbell	Faculty of Forestry (E.T.H., M.M.C.), and Centre for the Analysis of Genome Evolution & Function, Department of Cell & Systems Biology (S.R., O.W., M.M.C), University of Toronto,	Populus drought transcriptome: intra-specific variation in transcriptome activity
P2	J. Romano*, C. Dubos, and M.M. Campbell	Centre for the Analysis of Genome Evolution & Function, Department of Cell & Systems Biology, University of Toronto,	A central regulator of the plant transpiration stream
P3	C. Dubos, C. Vriet and M.M. Campbell	Centre for the Analysis of Genome Evolution & Function, Department of Cell & Systems Biology, University of Toronto,	Transcriptional regulation of AtMYB61 expression by nutritional cues
P4	M. Stokes, M. Waller and M.M. Campbell	Centre for the Analysis of Genome Evolution & Function, Department of Cell & Systems Biology, University of Toronto	Dissection of the AtMYB61 regulatory circuit by chemical genetics
P5	O. Wilkins and M.M. Campbell	Centre for the Analysis of Genome Evolution & Function, Department of Cell & Systems Biology, University of Toronto	Drought and the model tree: a genomics approach
P6	S. Raj, O. Wilkins, E.T. Hamanishi, and M.M. Campbell	Centre for the Analysis of Genome Evolution & Function, Department of Cell & Systems Biology (S.R., O.W., M.M.C), and Faculty of Forestry (E.T.H., M.M.C.), University of Toronto	Populus drought transcriptome: spatial and temporal effects on transcriptome activity
P7	H.L. Chen, L. Constan, P. Seguin, R. Bodo, and S.H. Jabaji	Department of Plant Science, McGill University	Gene expression and isoflavone concentrations in soybean sprouts treated with chitosan

P8	M.C. Micallef, L. Tian*, L. I. D'Silva, J. Robertson, and B. J. Micallef	Department of Plant Agriculture, University of Guelph	Photoperiodic injury in tomato is linked to diel N metabolism and the circadian clock
P9	A. L. Marcellus and E. Cholewa	Department of Biology, Nipissing University	Vitality stains reveal senescence pattern of Eriophorum vaginatum corm
P10	N.R. Roscoe and E. Cholewa	Nipissing University Biology Department	Characterization of Eriophorum vaginatum seed tissues and seedling growth
P11	E. Cholewa, B. Duquette, B. Dew, and P. Babady-Bila	Department of Biology, Nipissing University	The extracts from sweet fern (*Comptonia peregrina*) have high antioxidant and antibacterial properties
P12	A. Al-Shammari, F.Tran, W.Liang, X.Wen and O.Rowland	Department of Biology and Institute of Biochemistry, Carleton University	Development of a Genome Information Resource for the Identification of Genes Involved in Plant Cuticular Wax Biosynthesis
P13	A.Facciuolo*, D.Falcone, and B.A. Moffatt	Department of Biology, University of Waterloo, (D.F.) Department of Biological Sciences, University of Massachusetts, Lowell	The identification of transcript and protein products arising from the apt1 locus in Arabidopsis thaliana
P14	C.W. Beninger, R.R. Cloutier, B. Grodzinski	Department of Plant Agriculture, University of Guelph	The iridoid glucoside, antirrhinoside, from Antirrhinum majus L. has differential effects on two generalist insect herbivores
P15	B.A. Adeniji*, S.M. Macfie and M.A. Bernards	Department of Biology, the University of Western Ontario	Differential accumulation of cadmium in durum wheat: the roles of low molecular weight organic acids.
P16	S.M. Clark*, R. Dileo, R.T. Mullen, and B.J. Shelp	Departments of Plant Agriculture and (R.T.M) Molecular and Cellular Biology, University of Guelph	Overlapping, but non-redundant roles for multiple tomato GABA transaminases
P17	Z.B. Armstrong, E. Cheng, G. Thillainadesan, and S.E. Kohalmi	Department of Biology, The University of Western Ontario	Subcellular localization of Arabidopsis thaliana ADT1-GFP fusion proteins
P18	T.V. Humphrey, K.E. Haasen, S. Mehta and D.R. Goring.	Department of Cell and Systems Biology, University of Toronto	The Proline-rich, Extensin-like Family of Receptor Kinases Play a Role in Cell Elongation

P19	H.A. Saeed, L.O. Vodkin, F. Fauteux and M.V. Stromvik	Dept of Plant Science, McGill University, (L.O.V.) Dept of Crop Sciences, University of Illinois at Urbana-Champaign	In Silico and In Planta Analyses of Promoters from Soybean Seed Lectin Homologues
P20	C. Poo*, M. Grbic and V. Grbic.	Department of Biology, University of Western Ontario	Genetic Analysis of Arabidopsis-Spidermite Interaction.
P21	D. Yee, F. Gunawan, and D.R. Goring	Department of Cell and Systems Biology, University of Toronto	Two arabidopsis plant u-box (atpub) e3 ubiquitin ligases may function as regulators during transmission
P22	Wesley A. Farquharson, Arthur G. Szabo and Matthew D. Smith	Departments of Biology (WAF and MDS) and Chemistry (AGS), Wilfrid Laurier University	Functional analysis of the chloroplast protein import GTPase receptors using fluorescence spectroscopy
P23	M. Feeney*, Y. Cui and R. Menassa	Department of Biology, University of Western Ontario; (Y.C, R.M.) Agriculture and Agri-Food Canada, London	Protein trafficking and localization in leaves
P24	X. Wang*, A. Wang, S. Kohalmi, H. Sanfacon and L. Tian	Department of Biology, the University of Western Ontario, (X.W., A.W., L.T.)) Agriculture and Agri-Food Canada,London, ON, (H.S.) Agriculture and Agri-Food Canada, Summerland	Study of plant eIF4E genes for Plum pox virus (PPV) resistance
P25	K. Chin, J. Baxter, W. Urquhart, W. Moeder, K. Yoshioka	Department of Cell and Systems Biology, Center for the Analysis of Genome Evolution and Function, University of Toronto	The Arabidopsis Cyclic Nucleotide-Gated Ion Channels, ATCNGC11 and ATCNGC12 influences cation stress responses
P26	S. Rauf*, S.M. Weraduwage, M.C. Micallef, B. Grodzinski and B.J. Micallef	Department of Plant Agriculture, University of Guelph	Arabidopsis thaliana (L.) Heynh. having altered expression of mitochondrial pyruvate dehydrogenase kinase show enhanced growth and development under elevated CO_2
P27	S.M.H. Slater *and B.J. Micallef	Department of Plant Agriculture, University of Guelph	Identification of a null mutant for cytosolic fructose-1,6-bisphosphatase in Flaveria linearis

P28	M.M. De Decker and M.A. Fieldes	University of Waterloo, (M.M.D.D. and M.A.F.) Wilfrid Laurier University	Increased LEAFY transcript levels in a hypomethylated, early-flowering line of flax (Linum usitatissimum)
P29	L.G.L. Richardson* and M.D. Smith	University of Waterloo, (M.D.S.) Wilfrid Laurier University	Structure-function analysis of the Arabidopsis Toc159 family acidic domains
P30	R.L. Hood*, M.A. Bernards, and S.E. Kohalmi	Department of Biology, The University of Western Ontario	Relative ADT expression patterns in response to heat and cold treatments in Arabidopsis thaliana
P31	K. Dahal* and N. Hüner	Department of Biology, The University of Western Ontario	Photosynthetic Response of Winter and Spring Cereals to Elevated CO_2
P32	A.K. Derks*, S. Vasiliev, G. Shen, D.A. Bryant, D. Bruce	Department of Biological Sciences, Brock University, (G.S, D.A.B) Department of Biochemistry and Molecular Biology, Pennsylvania State University	CpcB lyase null mutations disrupt, but do not necessarily prevent, phycocyanin chromophore function in the cyanobacterium Synechococcus sp. PCC 7002
P33	D. Levac* and V. De Luca	Department of Biological Sciences, Brock University	Probing the structure of oligomers of Catharanthus roseus 16-hydroxytabersonine-16-O-methyltransferase by molecular modeling, site directed mutagenesis and kinetic analysis
P34	M.A. Gabriel, A. Pajak and F. Marsolais	Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, London, (M.A.G). Department of Biology, the University of Western Ontario	Structure and function relationship of plant asparaginases
P35	A. Senatore, C. Trobacher, C. Holley, L. Munsie and J.S. Greenwood	Molecular and Cellular Biology, University of Guelph	Precursor protease vesicles predict programmed cell death during anther dehiscence in tomato

P36	F.C. Guinel, B.J. Ferguson, M.A. Held, A.P. Morse, A.N. Pepper, L. L. Sloetjes, & E.M. Wiebe	Biology, Wilfrid Laurier University	R50 (sym16): a pea nodulation mutant with a shoot not responding to cytokinins?
P37	T. Humphrey, S. Lumba, J. Patel, and D. Bonetta	Dept of Cell and Systems Biology, University of Toronto, (D.B.) Faculty of Science, University of Ontario IT	FRIABLE1 is a previously unknown mediator of cell adhesion in Arabidopsis
P38	M.J. Iqbal, E.D. Leonardos and B. Grodzinski	Department of Plant Agriculture, University of Guelph	Biotron – Plant Productivity Module
P39	P. Janakirama*, U. Sajja, Q. Wang and V. Grbic	Department of biology, University of Western Ontario	Functional characterization of HUA2 protein
P40	L.K. Koziol*, B. Vanderbeld and W.A. Snedden	Department of Biology, Queen's University	Expression analysis of a subfamily of calmodulin-like genes in Arabidopsis
P41	M. Margaritis* and R. Menassa	Department of Biology, The University of Western Ontario, (R.M.) Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada	Cellulose degradation in Medicago sativa from increased accumulation of Cel5-CBM6 and Cel6B cellulases with an ELP tag
P42	M. Krol, A.G. Ivanov, A. Mattoo, I. Booij-James, D. Rosso, N.P.A. Huner, P.V. Sane	Department of Biology and The Biotron, University of Western Ontario(A.M., I.B-J) Henry A. Wallace Beltsville Agricultural Research Center, USDA /ARS USA	The absence of LHCII proteins alters the structure and function of PS II reaction centres in Chlorina F2 barley mutant
P43	I. Molina, J.B. Ohlrogge, M. Pollard	Department of Plant Biology, Michigan State University	Lipid polyester deposition and localization in developing seeds of Brassica napus and Arabidopsis thaliana
P44	H. Murray* and S.M. Macfie	Department of Biology, the University of Western Ontario	Bioavailability of metals in urban garden soils: a proposal

P45	R.M. Subasinghe*, M. Micallef , B. Grodzinski, and B.J. Micallef	Department of Plant Agriculture, the University of Guelph	High-resolution monitoring of stem extension allows for very early detection of exposure to Fusarium oxysporum f.sp. radicis-cucumerinum in Cucumis sativus
P46	B. Hendy* and R. Menassa	Department of Biology, The University of Western Ontario, (R.M.) Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada	Protein purification in plants utilizing small molecule-dependent inteins and elastin-like polypeptides
P47	T. Wei and A. Wang	Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada	Involvement of cellular membrane traffic proteins in the formation and translocation of the Soybean mosaic virus replication complex in plants
P48	S. Mosher, W. Moeder, K. Yoshioka	Department of Cell and Systems Biology, Center for the Analysis of Genome Evolution and Function, University of Toronto	Investigating possible SA and ABA crosstalk in the lesion mimic mutant cpr22
P49	J.P. Simpson*, R. Di Leo, P.K. Dhanoa, W.L. Allan, S.M. Clark, R.T. Mullen and B.J. Shelp	Department of Plant Agriculture, University of Guelph, Guelph, (P.K.D and R.T.M) Department of Molecular and Cellular Biology, University of Guelph,	Identification and characterization of a plastid-localized Arabidopsis glyoxylate reductase
P50	Y. Sun* and B.R. Glick	Department of Biology, University of Waterloo	Methods of plant growth promotion by Burkholderia phytofirmans PsJN
P51	C.P. Trobacher*, C. Holley, J.S. Greenwood	Department of Molecular and Cellular Biology, University of Guelph	A precursor protease vesicle-localized cysteine proteinase, SlCysEP, is expressed during post-germinative programmed cell death of tomato endosperm
P52	W. Urquhart, J. Baxter, K. Chin, D. Gupta, W. Moeder, and K. Yoshioka	Department of Cell and systems Biology, University of Toronto and Center for the Analysis of Genome Evolution and Function, University of Toronto	The utility of the constitutively active cyclic nucleotide gated ion channel, ATCNGC11/12, in elucidating functionally important residues in CNGCs

P53	Z. Yadegari, K.P. Pauls	Department of Plant Agriculture, University of Guelph	Molecular mapping of genes involved in the phenylpropanoid pathway in common bean (Phaseolus vulgaris L.)
P54	Q. Hazraty, M.T. Hopkins and S.J. Lolle	Department of biology, University of Waterloo	Correlating phenotype with mRNA and protein expression levels in Arabidopsis hothead mutants
P55	F. Shahmir and K.P. Pauls	Department of Plant Agriculture, University of Guelph	Isolation and identification of gene responsible for microspore embryogenesis in Brassica napus L.
P56	G. Tian*, P. Pauls, Z.M. Dong, L. Reid and L.N Tian	Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, (G.T.) Department of Biology, University of Western Ontario, (P.P.) Department of Plant Agriculture, University of Guelph, (Z.M.D.) Department of Biology, St. Mary's University, (L.R.) Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada	Corn (Zea mays) plant growth promotion by nitrogen fixing bacterium Gluconacetobacter diazotrophicus
P57	F.A. Meerja*, L. Tian, S. Sibbald	Department of Biology, the University of Western Ontario, Agriculture and Agri-Food Canada	Improvement of European plum (*Prunus domestica* L.) regeneration and transformation
P58	W.L. Allan, J.P. Simpson, S.M. Clark, and B.J. Shelp	Department of Plant Agriculture, University of Guelph	Manipulation of γ-hydroxybutyrate and redox levels in Arabidopsis by abiotic stress is associated with induction of glyoxylate reductase isoforms
P59	M. Perry and P. Krishna	Faculty of Law and Faculty of Science, (P.K.) Faculty of Science, the University of Western Ontario	The Use of Material Transfer Agreements in Biotechnology in Canada
P60	T. Rahman* and P. Krishna	Department of Biology, the University of Western Ontario	Brassinosteroid, abscisic acid and calcium: determining the connection
P61	A. Silva*, M. Columbus, and D.D. Lefebvre	Department of Biology, Queen's University	Phytoremediation of a contaminated groundwater site

P62	M. Columbus*, A. Silva, and D.D. Lefebvre	Department of Biology, Queen's University	Characterization of transpiration rates of poplar and willow trees
P63	M. Haggitt* and M.A. Bernards	Department of Biology, The University of Western Ontario	Biosynthesis of potato (*Solanum tuberosum* L.) suberin: The w-hydroxylation of fatty acids
P64	H.T. Tran* and W.C. Plaxton	Department of Biology, Queen's University	Purification and characterization of three secreted purple acid phosphatase isoforms from phosphate-starved Arabidopsis thaliana suspension cells
P65	B.A. Hurley*, S.K. Rao, W.A. Snedden and W.C. Plaxton	Department of Biology, Queen's University	Functional analysis of AtPAP12 and AtPAP26, the predominant intracellular and secreted purple acid phosphatases upregulated by phosphate-deprived Arabidopsis thaliana
P66	B. Szyszka* and N.P.A. Hüner	Department of Biology, the University of Western Ontario	Identification of phosphorylated thylakoid membrane proteins of *Chlamydomonas raudensis* UWO 241
P67	B. O'Leary*, Y-M. She and W.C. Plaxton	Departments of Biology and (Y-M.S.)Chemistry[2], Queen's University	In vivo multi-site phosphorylation of bacterial-type phosphoenolpyruvate carboxylase from developing castor oil seeds
P68	S.K. Rao, W.A. Snedden and W.C. Plaxton	Department of Biology, Queen's University	Heterologous expression and characterization of recombinant bacterial- and plant-type phosphoenolpyruvate carboxylases from developing castor oilseeds
P69	B. Karas*, L. Ross, J. Murray, K. Nowakowski, L. Amyot, C. Johansen, and K. Szczyglowski.	Department of Biology, University of Western Ontario, (B.K., L.R., J.M., K.N., L.A., C.J., K.S.) Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London,	Cellulose synthase-like D protein is required for root hair morphogenesis and root nodule symbiosis in L. japonicus.

P70	U. Sajja* and V. Grbic	Department of Biology, the University of Western Ontario	HUA2 interacts with FCA and is required for accumulation of FCA-γ transcript
P71	S. Kosuta, M. Held, C. Johansen, B. Karas, A. MacGillivary, G. Morieri, A. Downie, G. Oldroyd and K. Szczyglowski	Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, (G.M., A.D., G.O.) John Innes Centre	SYMRK receptor kinase reveals the evolutionary roots of symbiosis
P72	M. Held*, S. Kosuta, L. Amyot, and K. Szczyglowski	Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, (M.H., K.S.) Dept. Biology, the University of Western Ontario	Role of cytokinin and NIN transcriptional regulator in colonization of roots by nitrogen fixing bacteria
P73	A.G. Ivanov, M. Krol, E. Selstam, P.V. Sane, D. Sveshnikov, Y.-I. Park, G. Öquist, N.P.A. Huner	Department of Biology and The Biotron, University of Western Ontario (E.S., D.S.,G. Ö) Department of Plant Physiology, University of Umeå, (Y.-I.P.) Department of Biology, Chungnam National University	Induction of isiA by iron deficiency in Synechococcus sp. PCC7942 is associated with alterations in carotenoid and lipid composition
P74	A Kalinina, D. Cuppels, A. Zoina, D.C.W. Brown	Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada	*Prunus persica*: "who has the problem with transformation, *Agrobacterium* or the researcher?"
P75	A.J. Reid and R. Menassa	Agriculture & Agri-Food Canada, Southern Crop & Food Research Centre, London, ON	Production of recombinant interleukin-24 in tobacco plants and BY-2 cells
P76	A. Kaldis and R. Menassa	SCPFRC, Agriculture and Agri-Food Canada	Trafficking and degradation of recombinant IL-10 in plants
P77	F. Liu, I.J. Tetlow, M.J. Emes	Department of Molecular and Cellular Biology, University of Guelph	Protein-Protein Interactions between Starch Synthases and Branching Enzymes in Maize Endosperm Amyloplasts
P78	A. Rochon*, K. Hahn, P.R. Fobert, and C. Després	Department of Biology, Brock University; (K.K. and P.R.F.) National Research Council of Canada, Plant Biotechnology Institute, Saskatoon,	Redox regulation of Arabidopsis TGA1 by a novel glutaredoxin

P79	D.R. Fingrut*, W. Li, and D.P. Maxwell	Department of Biology, the University of Western Ontario	A non-coding RNA regulates molybdenum cofactor biosynthesis in *Chlamydomonas reinhardtii*
P80	W.J. Bjornsson* and C.G. Trick	Department of Biology, The University of Western Ontario, (C.G.T) Schulich School of Medicine and Dentistry, University of Western Ontario,	Regulation of extracellular toxins in *Heterosigma akashiwo*: nutrient limited chemostat studies
P81	K.L. Iglic* and C.G. Trick	Department of Biology, The University of Western Ontario, (C.G.T) Schulich School of Medicine and Dentistry, University of Western Ontario,	Methyl Viologen: An appropriate tool to measure oxidative stress in *Symbiodinum* sp. (Freudenthal) with fluorescent probes?
P82	J.A.J.A. Ramanauskas, J. Veerman*, G.D. Paton , S. Vassil'ev and D. Bruce	Department of Biological Sciences, Brock University	Photoprotection, photoinhibition and recovery in *Parmelia sulcata:* A fluorescence study
P83	S. Goel* and P. Krishna	Department of Biology, The University of Western Ontario	Identification of novel putative cochaperones of Hsp90 in *Arabidopsis* using bioinformatics analysis

Abstracts

Legend:
K - Keynote Speakers
CP - Contributed Papers (Oral)
P - Posters

K1

Expecting the unexpected: lessons learned from the flavonoid pathway

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Flavonoids are a well-known class of plant metabolites that are responsible for much of the red, blue and purple color of flowers and leaves. Over the years these compounds have factored into Mendel's work on inheritance, McClintock's discovery of transposable elements, and more recent work uncovering the phenomenon of cosuppression. Flavonoid biochemistry also provided one of the earliest examples of metabolic engineering in plants. However, engineering flavonoid biosynthesis is not always straightforward, as evidenced by the elusive blue rose and efforts to enhance the levels of flavonoid and isoflavonoid phytonutrients in edible plants such as tomato. Despite more than a century of work on the biochemistry and physiology of this system, it continues to offer up new surprises and challenges. The work in our laboratory has focused on understanding the intracellular organization of the flavonoid pathway as a multienzyme complex. We recently reported that the first two enzymes of the flavonoid pathway reside not only at the cytoplasmic face of the ER, but also in the nucleus of many Arabidopsis cells. Analysis of the physical interactions of these two enzymes, together with studies of the determinants of their dual localization, is providing evidence for previously-unsuspected functions for these proteins. Efforts to identify interacting partners for these enzymes have simultaneously uncovered new physical connections with proteins outside of the central flavonoid pathway, indicating that this system resides within a much larger metabolic network. These findings suggest that models for the regulation of metabolic flux and the deposition specialized metabolites within the plant cell must be considered in a new light

K2

How Plants Evolve the Ability to Make So Many Scent Compounds

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Plant volatiles serve various ecological roles from attraction of pollinators to defense again herbivores and pathogens. Synthesis of plant volatiles involves the removal of hydrophilic moieties and oxidation/hydroxylation, reduction, methylation and acylation of various plant metabolites. Some biosynthetic enzymes of plant volatiles produce multiple products from a single substrate or act on multiple substrates. Plant species often vary in the set of volatile compounds that they synthesize, indicating that the genes encoding the enzyme for volatile biosynthesis are constantly changing and genes (and enzymes) for new volatiles constantly arise. Genes for the biosynthesis of new volatiles can evolve by duplication of genes that specify the synthesis of either existing volatiles or non-volatile metabolites, followed by divergence. Changes in preferred substrate or resultant product of such enzymes may occur through minimal changes of critical residues. Convergent evolution is often responsible for the ability of distally related species to synthesize the same volatile. In this talk I will describe specific examples we have observed in the recently discovered family of phenylpropene synthases that illustrate many of these processes.

K3
Cytokinins in symbiosis: new insights into old hypothesis.

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Legumes have evolved a specific response to *Rhizobium*-encoded lipochito-oligosaccharide signalling molecules, known as nodulation factors (NFs). Instead of evoking plant defences, NF signalling leads to the establishment of a symbiotic relationship. In addition to playing an essential role in the mechanism of selective recognition which allows the symbiotic bacteria to enter the host root, the NF-dependent pathway acts to stimulate the organogenesis of symbiotic organs, root nodules. Ultimately, the nodules will host the symbiotic bacteria within intracellular compartments, called symbiosomes, which assures an appropriate environment for biosynthesis and maintenance of bacterially-encoded nitrogenase, the enzymatic complex that converts atmospheric N_2 to ammonium. It is becoming increasingly clear that NFs operate as exogenous elicitors of an environmental (biotic) root response pathway, which, in turn, acts to modify the intrinsic plant developmental machinery. Recent findings provide unequivocal support for the existence of cross-talk between NF and cytokinin during initiation of nodule primordia organogenesis[1-2]. Acting downstream from NF perception, cytokinin appears to be the key differentiation signal for nodule organogenesis but may also participate in locally and systemically operating mechanisms that regulate root susceptibility to bacterial infection.

1. J. Murray et al., Science 315, 101 (2007).
2. L. Tirichine et al., Science 315, 104, (2007).

CP-A1

Expression of Therapeutic Proteins in Tobacco BY-2 Cell Suspension Culture

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Numerous recombinant proteins have been expressed in plant platforms for the production of therapeutic pharmaceuticals including cytokines, antibodies, recombinant enzymes and human vaccines. The levels of accumulation of these proteins in their respective plant systems are variable. Through the use of transgenic tobacco BY-2 cell suspension cultures, stable *in vivo* production of secreted Interleukin-10 (IL-10) has been established, thus providing a viable model for the expression and suitable accumulation of plant-made pharmaceutical proteins. We have previously shown ER-targeted IL-10 accumulated to 50 ng/mg of total soluble protein (TSP) in tobacco leaves. The same construct produced 250 ng/mg TSP when expressed in BY-2 cell suspension culture. In an effort to simplify downstream purification, minimize plant tissue processing and optimize cytokine stability, we created constructs that would secrete IL-10 into the culture medium. Several IL-10 constructs intended for secretion were produced including fusions to GFP for subcellular visualization, elastin-like polypeptide (ELP) for stable accumulation and *Strep*II for ease of downstream purification, of which results shall be discussed. Tobacco BY-2 cell suspension lines producing secreted IL-10 may provide a system to investigate the feasibility of plant-made therapeutic pharmaceutical production in a controlled and predictable environment.

CP-A2

Phenotypic analysis of flowering time and segregation distortion in a novel mutant of the *MADS-AFFECTING FLOWERING 2* gene of *Arabidopsis thaliana*

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The *MADS-AFFECTING FLOWERING* LOCUS (*MAF*), a tandem quadruplication of MADS-box genes (*MAF2-5*) on chromosome five of *Arabidopsis thaliana* (L.)Heynh., is involved in flowering repression. Repression by *MAF2* is observed as an inhibition of flowering after an intermediate cold treatment (~16days). A novel mutant, *maf2-3*, was investigated to provide further insight into the functionality of *MAF2*. Both WT *MAF2* and the mutant *maf2-3* demonstrate cold-sensitive alternative splicing. Phenotypic analysis shows the *maf2-3* allele behaves similarly to the T-DNA insertion line in 'Colombia' and is a naturally occurring null. A distortion of Mendelian segregation favoring the mutant allele was also observed in *MAF2*/*maf2-3* segregating lines. Segregation distortion was found to be suppressed by intermediate periods of cold and by *FLOWERING LOCUS C*, *FLC*. Developmental transcript profiling suggests segregation distortion may be associated with seed development. To acquire further functional insight, occurrence of the allele was investigated in a large accession collection. The insertion mutation is ubiquitous in Eastern Eurasia, suggesting an intense population bottleneck. Segregation distortion favoring the *maf2-3* allele may have contributed to the rapid expansion of plants with this allele during the post-glacial colonization of Europe by *A. thaliana*. Association of major floral repressors with segregation distortion and seed development indicates these 'floral repressors' may be more accurately referred to as 'developmental repressors'.

CP-A3

A novel expression platform for the production of diabetes-associated autoantigen human glutamic acid decarboxylase (hGAD65)

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Chloroplast transformation of plants has been increasingly employed as an alternative approach over nuclear transformation for the expression of therapeutically valuable proteins because of its unparalleled advantages, including very high levels of foreign protein expression, increased transgene containment because inheritance of plastids are mainly uniparental maternal, lack of gene silencing, position and pleiotropic effects, and undesirable foreign DNA). Additionally, the presence of chaperones and enzymes within the chloroplast helps to assemble complex multi-subunit proteins and correctly fold proteins containing disulfide bonds, thereby drastically reducing the costs of in vitro processing. Here, we report the use of the chloroplast of the single-celled eukaryotic alga, *Chlamydomonas reinhardtii* as a novel expression platform for the production of a diabetes-associated autoantigen, human glutamic acid decarboxylase (hGAD65). The DNA cassette encoding full-length human GAD65, under the control of the *C. reinhardtii rbc*L5'- and 3'-UTRs, was constructed and introduced into the chloroplast genome of *C. reinhardtii* by biolistic bombardment. Integration of the human gene into the chloroplast genome of *C. reinhardtii* was confirmed by PCR. Transcriptional expression of GAD65 was demonstrated by RT-PCR. Western blot analysis verified the expression and accumulation of the recombinant protein. The immunogenicity of algal-derived rhGAD65 was demonstrated by its specific reactivity with diabetic sera by ELISA as well as by its ability to induce proliferation of splenic T cells from NOD mice, an animal model of human autoimmune diabetes. The accumulation level of rhGAD in transgenic algae was calculated to be 0.5 % of the total protein. These results demonstrate the potential of using transgenic chloroplasts of single-celled green algae for rapid production of large quantities of valuable biopharmaceuticals.

CP-A4

Common Bacterial Blight of *Phaseolus vulgaris*: Towards the Identification of a Resistance Gene

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Infection by plant pathogens is a significant limitation to the yield and quality of common dry bean (*Phaseolus vulgaris* L.), an important pulse crop in Canada, cultivated throughout the central and Prairie Provinces. Common bacterial blight (CBB) presents itself as brown lesion on seeds, leaves and pods, and is caused by *Xanthomonas axonopodis* pv. phaseoli. Recently, a CBB-resistant cultivar, OAC-Rex was developed from a cross between *P. vulgaris* and a CBB-resistant accession of *Phaseolus acutifolius*. OAC-Rex represents the first CBB resistant cultivar released in North America, however the genes responsible for this resistance not yet been identified. Binary-bacterial artificial chromosome (BiBAC) libraries were created to aid in the identification of the CBB-resistance genes in OAC-Rex and HR67, a second CBB resistant line. The libraries were screened with CBB resistance-associated molecular markers identified by previous studies, and the identified clones were analyzed using a gel-based restriction fingerprinting method for assembly into contigs. The fragments at the extreme ends of the contigs were used to re-probe the libraries and expand the coverage of the contig. The unique clones will be transiently expressed in susceptible bean lines using *Agrobacterium tumefaciens,* and the plants infected with *X. axonopodis*. Clones containing genes for CBB resistance should cause a significantly reduced *X. axonopodis*-induced lesion. The inserts will be sequenced and analyzed to identify potential resistance genes.

CP-A5

Two arabidopsis plant u-box (atpub) e3 ubiquitin ligases may function redundantly as regulators of plant signalling pathways during gametophytic transmission

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The ability of plants to sense and respond to environmental and endogenous signals is essential to their growth and development. Ubiquitin-mediated proteolysis has emerged as an important process involved in how plant signalling pathways can be regulated in response to environmental or developmental cues. The involvement of the Ub-26S system during self-incompatibility was established by the characterization of the *Brassica* ARC1 protein, an E3 ubiquitin ligase that targets substrates presumed to be needed for compatible pollinations for degradation. In self-compatible A*rabidopsis thaliana*, there exists a 17-member sub-family with similar domain organization to *Brassica* ARC1 that may function in a manner analogous to ARC1. Phylogenies based on domain sequence similarities have shown that AtPUB18 and AtPUB19 consistently form a clade. To alleviate the difficulty of functionally characterizing redundant proteins, crosses between *pub18* and *pub19* lines were performed. No *pub18/pub19* plants were ever observed among the F_2 generation. Interestingly, selfed *PUB18/pub18 PUB19/pub19* plants also never yielded *PUB18/+ PUB19/+* progeny, but instead showed a segregation ratio that did not reflect independent assortment of the genes. As a consequence of these results, the distorted segregation is being further investigated to assess how the *AtPUB18* and *AtPUB19* alleles are involved in transmission through the male and female gametophytes.

CP-A6

The role of IAP1 in intercellular salicylic acid accumulation during Age-Related Resistance

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Age-Related Resistance (ARR) has been observed in numerous plant species, resulting in increased disease resistance as the plant matures. The induction of the ARR pathway is currently believed to lead to the accumulation of salicylic acid (SA) in the intercellular space, which is thought to be acting as an ant-microbial agent. Study of an ARR defective mutant, iap1-1, (important in the ARR pathway,) demonstrated that intercellular addition of SA improved resistance to Pseudomonas syringae pv. tomato (Pst) two-fold, and intercellular SA levels were reduced compared to wild type, suggesting that IAP1 lies upstream of SA accumulation during ARR. Genes involved in SA accumulation and genes up-regulated during ARR were expressed to similar levels in response to Pst in iap1-1 and wild type suggesting that IAP1 acts downstream of these genes in the ARR pathway. Additionally, it has been hypothesized that IAP1 may be involved in the transport of SA to the intercellular space. Identification and characterization of iap1-1 will provide important insights into the ARR defense pathway.

CP-B1

Interaction of brassinosteroid with other plant hormones in stress responses of *Arabidopsis* seedlings.

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Brassinosteroids (BRs) are a group of plant steroids that are essential for proper plant development. Without BRs, plants are dwarfs and infertile. We have demonstrated that *Arabidopsis thaliana* and *Brassica napus* seedlings grown in the presence of 24-epibrassinolide (EBR) are more resistant to a variety of abiotic stresses (heat, drought, cold and high salt). To understand how BR promotes this broad-range stress tolerance, we analyzed genes differentially expressed in EBR-treated seedlings *vs* untreated seedlings under non-stress and stress conditions by use of ATH1 arrays. A large proportion of BR-regulated genes identified in this study are associated with abiotic and biotic stress tolerance, and some genes with the biosynthesis or signaling of other plant hormones. Thus, in addition to possible direct effects of BR on stress-related gene expression, interaction of BR with other plant hormones appears to also contribute to BR-mediated stress tolerance. To address this, we have compared the physiological and molecular effects of EBR on wild type *Arabidopsis* seedlings with mutants that are either deficient in or insensitive to salicylic acid, ethylene, jasmonic acid and abscisic acid. Our results indicate that EBR has independent effects, as well as those involving salicylic acid, ethylene, and abscisic acid. Furthermore, heat and salt stress phenotyping of knock out mutant lines of select genes identified in the microarray screen, has unveiled novel stress-related genes, which appear to be maximally regulated by either BR or abscisic acid. This study is the first to highlight the involvement of other plant hormones in BR-mediated stress tolerance.

CP-B2

Increased air temperature during simulated autumn conditions affects electron transfer and energy dissipation in *Pinus banksiana*

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The seasonal development of trees of the boreal forest might be affected by climate change, as the trees will experience naturally decreasing daylength during autumn, while warmer air temperature will maintain photosynthesis and respiration. Working with controlled environments, we used a factorial design to assess the impact of photoperiod and temperature on energy partitioning and physiological acclimation of the photosynthetic apparatus in Jack pine (*Pinus banksiana*). Control treatments of plants grown under 16-h photoperiod and 22˚C (representing warm summer conditions) and plants grown under 8-h/7˚C (cool autumn) were compared to plants grown under warm autumn (8-h/22˚C) and cool summer conditions (16-h/7˚C). Although fully functional, the xanthophyll cycle does not appear to be a major contributor to balance the electron flow in the warm autumn treatment as it was the case in the other three treatments. Instead these plants rely on conformational changes in the light harvesting complex for safe dissipation of excess energy, resulting in a form of NPQ that is fast relaxing. Under warm autumn conditions one limiting factor of the electron transport rate is the transfer of electrons from Cyt b_6f to PSI. We observed a drastic increase in the amount of PTOX, which could act as a mediator to poise the redox state of the plastoquinone pool.

CP-B3

Growth and development under elevated CO_2 for transgenic lines of *Arabidopsis thaliana (L.) Heynh*. having altered dark respiratory rates

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Arabidopsis lines showing increased rates of dark respiration due to antisense repression of mitochondrial pyruvate dehydrogenase kinase (mtPDHK) also show an increase in seed oil content and specific seed weight. These data point to the role of mtPDH in both catabolic and anabolic processes. We hypothesize that *Arabidopsis* lines having increased respiratory rates and altered mtPDHK expression will show enhanced growth rates and productivity under elevated CO_2 levels due to increased sink capacity. An extensive growth analysis was carried out by growing control and transgenic lines having either constitutive or seed-specific expression of antisense mtPDHK under ambient (380ppm) or high (700ppm) CO_2. A significant increase in plant productivity was observed in transgenic lines compared to controls under elevated CO_2 and interestingly this improvement in productivity was most pronounced in the constitutive line YA5-3^1 having a moderate increase in its dark respiratory rate. YA5-3^1 excelled in both vegetative and reproductive growth producing a larger inflorescence which corresponded to greater number of siliques and seeds and an increase in its total seed weight and aerial tissue dry mass. Endogenous levels of mtPDHK transcript are being quantified using mRNA-PCR to characterize seed-specific lines. In addition, the possible effect of antisense repression of mtPDHK on branched-chain-α-ketoacid dehydrogenase activity is being investigated.

CP-B4

Rubisco and the response of black spruce to climate warming

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Black spruce (*Picea mariana* (Mill.) B.S.P.) is a dominant North American boreal species and its response to climate warming will affect the boreal forest. We studied how elevated growth temperatures affect photosynthetic processes in seedlings grown at cool (22/16°C) and warm (30/24°C) day/night temperatures to determine what limits photosynthetic carbon gain above the thermal optimum. Seedlings grown at high temperatures were smaller and shorter than cool-grown seedlings, with lower net CO_2 assimilation rates at their growth temperature. Below 30°C, warm-grown seedlings had lower net and gross CO_2 assimilation rates than cool-grown seedlings, due to reduced maximum carboxylation rates of Rubisco (V_{cmax}) and maximum electron transport rates (J_{max}). Above 30°C, warm-grown seedlings had higher net CO_2 assimilation rates than cool-grown trees, but similar gross CO_2 assimilation rates because respiration in warm-grown seedlings acclimated. At 40°C, we found no evidence for either electron transport limitations or triose phosphate use limitations, but the initial slope of the CO_2 response curve was 50% lower than predicted by photosynthetic models in cool-grown seedlings and 75% lower in warm-grown seedlings, indicating that Rubisco activation was limiting carbon assimilation above the thermal optimum. Higher growth temperatures will therefore reduce the carbon gain and growth rate of black spruce through Rubisco deactivation and could lead to the collapse of this dominant boreal forest tree in a warmer climate.

CP-B5

Dynamics of mycorrhizal symbiosis: getting to the roots of metal-stress tolerance

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Environmental pollutants, such as heavy metals (HM), pose significant risks to ecosystems and human health. In this regard, plants have been shown to remove HM pollutants from contaminated environments through a process known as phytoremediation. As an integral component of ecosystem function, the arbuscular mycorrhizal (AM) symbiosis is an association between the roots of most plant species and Zygomycetes fungi which is recognized for benefitting plants subjected to biotic and abiotic stress factors, including soil-HM pollution. Using statistical meta-analysis and experimental root-organ culture systems, we investigated HM phytoremediation processes and the inherent role of AM symbiosis as a key factor of plant growth and metal-stress tolerance. We determined that AM fungi benefit plants by (1) enhancing metal uptake under low soil-HM conditions, and (2) decreasing metal uptake via metal-binding under high soil-HM conditions thereby enhancing plant growth. Subsequent *in vitro* analyses indicated that both the 'enhanced uptake' and 'metal-binding' phenomena occur simultaneously and that the resulting profiles of metal-uptake and plant growth can be associated to their combined overall effects. Furthermore, it is suggested that plants may invest in mycorrhizal symbiosis in an extrinsic stress avoidance strategy, complementing other intrinsic stress resistance mechanisms, to circumvent the effects of HM toxicity. From the framework of statistical meta-analysis, this study has developed new research models having fundamental and practical implications in environmental remediation, meanwhile serving as an insightful steppingstone for experimental studies.

CP-B6

Cytokinin Profiles during the infection of corn with *Ustilago maydis*

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The dimorphic smut fungal pathogen *Ustilago maydis* requires growth within its host plants to complete its sexual cycle. *U. maydis* growth within the corn plant (*Zea mays*) induces developmental changes including the formation of conspicuous galls on the above ground portions of the plant. Plant growth hormones like cytokinins [CKs] and auxins [IAA] involved in cellular differentiation as well as plant defence responses hormones like absicic acid [ABA] may play a role in the infection and formation of disease symptoms in corn smut. Bioassays completed in the 1940's and 70's suggest the presence of CK like activity in corn, smut tumors of corn and *Ustilago* cultures. However, this work was never confirmed by physiochemical hormone extraction, analysis and quantification. Also more recent work has indicated that diffusible compounds which induce the differentiation of maize cells in culture are also involved in the mating process and phenotypic switch of *Ustilago* from saprophyte to pathogen. In this study hormonal profiles of CKs, IAA and ABA for various disease symptoms as well as haploid and dikaryotic fungal cultures are being analyzed with mass spectrometry (LC-MS/MS). Elevated CK levels were identified in leaves of *Ustilago* infected corn compared to uninfected controls. Moreover, tumors contained distinct CK profiles that matched those produced by free axenic dikaryon *Ustilago* cultures. By extracting hormones and analyzing them in this way, the specific metabolic forms involved in the infection process can now be elucidated.

CP-C1

Genotype-specific transcriptome changes to osmotic stress and stress recovery within *Zea mays* roots

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Genetic variation for abiotic stress responses among varieties of a species may be explained in part by differences in gene transcription that may be detected in microarray experiments involving several such varieties. We have investigated genetic variation in gene transcriptional profiles on roots in a set of seven maize inbred lines (*Zea mays* L.) that differ in their behaviour upon exposure to as well as recovery from osmotic stress. Similarities and differences in transcription profiles among these lines, as detected using 84 long-oligonucleotide microarrays, will be discussed with reference to the potential of microarrays to provide information not only on the molecular response to abiotic stress in a general sense but more specifically on the genotype-specific molecular response. This study provides insight into understanding genetic variation of root responses to water stress through transcriptional profiling.

CP-C2

Expression profile of *Brassica napus* inoculated with the plant growth-promoting rhizobacteria *Pseudomonas putida* UW4

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Plants often live in association with growth promoting bacteria, which provide several benefits to their hosts. One such benefit is the lowering of plant ethylene levels through the action of the bacterial enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase that cleaves the immediate biosynthetic precursor of ethylene, ACC. The hormone ethylene is responsible for root initiation and elongation in the seedling, but under stressful conditions, ethylene inhibits root elongation and auxin transport. The ACC deaminase-containing bacterium *Pseudomonas putida* UW4 was isolated from the rhizosphere of reeds and has proven to be a potent growth-promoting strain in several studies. In order to study the effects of this bacterium on the plant, as well as the role of the enzyme ACC deaminase in plant growth promotion, we used an *Arabidopsis thaliana* oligonucleotide microarray to provide a transcriptional profile of both shoot and root tissues of *Brassica napus* plants inoculated on the roots with bacteria. The results indicate that plants in association with this bacterium show differential regulation of genes involved in auxin signaling, nitrogen metabolism and stress response; while plants inoculated with a strain of the bacterium where the ACC deaminase gene has been knocked out show stronger defense and stress responses.

CP-C3

The cDNA library screen and functional characterization of host proteins associated with Plum pox virus (PPV) infection

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Plum pox virus (PPV) is a regulated virus in Canada. It is considered as one of the most threatening pathogens to the stone fruits and ornamental *Prunus* species. Currently, there is no natural resistance to PPV that can be introduced into elite cultivars through conventional breeding. Despite extensive research and study on PPV interaction with its host, the exact viral replication mechanism remains unclear. Like other positive sense RNA viruses, PPV has a small genome and relies on host gene products (host factors) for translation and replication. To isolate host factors of PPV, a PPV infected peach cDNA library was constructed and used in a yeast two-hybrid screen. Two PPV-encoded viral proteins, NIb (viral RNA polymerase) and VPg (viral protein genome linked), were cloned as baits. The library screening was conducted at three different temperatures to include temperature sensitive interactions. A total of 215 host candidates were identified and based on their predicted functions, four of these candidate genes were selected for further functional analysis. *Arabidopsis thaliana* T-DNA insertion lines of corresponding ortholog genes are being screened for PPV infection in order to determine if these genes are required for PPV infection.

CP-D1

Co-immunopurification of phosphorylated bacterial- and plant-type phospho*enol*pyruvate carboxylases with the plastidial pyruvate dehydrogenase complex from developing castor oil seeds

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Phospho*enol*pyruvate carboxylase (PEPC) is a ubiquitous and tightly regulated cytosolic enzyme situated at the core of plant primary C-metabolism. The PEPC interactome of developing castor oil seed (COS) endosperm was assessed using co-immunopurification (co-IP) followed by proteomic analysis. Earlier studies suggested that immunologically unrelated 107-kDa plant-type and 118-kDa bacterial-type PEPCs (p107/PTPC and p118/BTPC, respectively) are subunits of an unusual 910-kDa hetero-octameric Class-2 PEPC complex of developing COS. The current results demonstrate that a tight physical interaction occurs between p118 and p107, since p118 quantitatively co-IP'd with p107 following elution of COS extracts through an anti-p107-IgG immunoaffinity column. No PEPC activity or immunoreactive PEPC polypeptides were detected in the corresponding flow-through fractions. Although BTPCs lack the N-terminal phosphorylation site characteristic of PTPCs, the co-IP'd p118 appeared to be significantly phosphorylated. The co-IP of p118 with p107 was not influenced by their phospho-status. A 110-kDa PTPC co-IP'd with p118 and p107 when photosynthate-deprived COS was used. As well, the plastidial pyruvate dehydrogenase complex (PDC_{pl}) was identified as a novel PEPC interactor. Subcellular fractionation indicated that p118 and p107 are strictly cytosolic, whereas PDC_{pl} is targeted to both the cytosol and leucoplast. Thus, a putative cytosolic metabolon involving PEPC and PDC_{pl} could function to channel carbon from phospho*enol*pyruvate to acetyl-CoA and/or to recycle CO_2 from PDC_{pl} to PEPC.

CP-D2

The 'surfaceome of *Catharanthus roseus* is enriched for monoterpenoid indole alkaloid biosynthesis: Cloning and characterization of loganic acid methyltransferase from epidermis enriched cDNA library of *Catharanthus roseus*.

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Catharanthus roseus (Madagascar periwinkle) is the sole commercial source of the monoterpenoid indole alkaloids (MIAs), vindoline and catharanthine that are components of the commercially important anticancer dimers, vinblastine and vincristine. Carborundum abrasion (CA) technique has been used to extract leaf epidermis enriched mRNA and random sequencing of the derived cDNA library has established 3,655 unique ESTs, composed of 1,142 clusters and 2,513 singletons. Virtually all known MIA pathway genes were found in this remarkable set of ESTs, while only 4 known genes were found in the publicly available 12, 627 *Catharanthus* EST dataset. Several new MIA pathway candidate genes were identified, as demonstrated by the cloning and functional characterization of *loganic acid O-methyltransferase (LAMT)* involved in secologanin biosynthesis. *LAMT* is a member of the SABATH family of methyltransferases, this family contains no active site residues but rather provides a hydrophobic binding pocket in which the substrate can attack the methyl group of S-adenoyl-L-methionine. *LAMT* has a relatively high K_M 14.6 mM for loganic acid as compared to other members in this family which is reflective of its polar nature. *LAMT* shows strong product inhibition with a Ki of 215 μM for loganin which may provide evidence for why iridoids are a limiting factor in MIA biosynthesis. Together these results suggest that the remaining MIA pathway and regulatory genes are very likely represented within the *Catharanthus* 'surfaceome' library and that EST sequencing of surfaceomes could be of great value to harvest pathways for secondary metabolism in other plant species.

CP-D3

Hydroponics induces apoplastic flow to the xylem in *Arabidopsis*

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Roots take up nutrients from the soil solution and deliver them to the xylem for long distance transport to the shoot. There are two possible routes for radial solute transport in roots: the apoplastic and symplastic pathway. Nutrients in the apoplast move through the cell walls of the epidermis and cortex up to the endodermis where Casparian bands (CBs) form an apoplastic barrier, forcing ions to cross the plasma membrane and enter the vascular cylinder symplastically. This CB function has been demonstrated in mature root segments as the accumulation of metal ions and fluorescent tracers on the cortical side of the endodermis. However, there are no CBs at the root tip or at the base of young lateral roots and, at these locations, apoplastic bypass pathways could exist in roots. We tested the permeability of entire Arabidopsis root systems using the apoplastic tracer PTS (3-hydroxy-5,8,10-pyrene trisulfonate) and found that there is no direct apoplastic pathway to the vascular cylinder in young, growing, unmanipulated roots. PTS was excluded from the transpiration stream in wild-type (WT) and the *scarecrow* mutant but was transported to the leaves in the *short-root* mutant, demonstrating that only in the absence of CBs can solutes enter the stele apoplastically. Furthermore, no PTS was observed at the root tip or in the xylem elements at lateral root junctions in undisturbed roots indicating that apoplastic bypass pathways do not exist at these locations. We found that only roots from hydroponically-grown plants are permeable to PTS, which demonstrates that this growth technique induces apoplastic bypass pathways. This fact should be taken into consideration in studies utilizing plants grown in hydroponics.

CP-E1

Dual targeting of the tRNA nucleotidyltransferase in plants – not just the signal

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In plants, protein synthesis takes place in the cytosol, mitochondrion and plastid. Each of these compartments requires functional transfer RNAs (tRNAs) bearing a 3'-terminal cytidine-cytidine-adenosine sequence which is generated by the enzyme tRNA nucleotidyltransferase. As only one isoform of tRNA nucleotidyltransferase has been identified in plants, we explored how signals contained on this enzyme allow it to be distributed to multiple cellular compartments. We show that this protein contains amino-terminal organellar targeting information and that its cellular destination is dependent on which of multiple in-frame start codons is used for translation. In addition, we demonstrate that the mature domain of tRNA nucleotidyltransferase plays a role in enzyme distribution. Our data implicate not only amino-terminal targeting signals, but also additional factors within the protein or the cell in the regulation of localization of tRNA nucleotidyltransferase.

CP-E2

A functional genomics approach to dissect the pathogenicity of *Sclerotinia sclerotiorum*.

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Sclerotinia sclerotiorum (Lib.) de Bary is among the world's most damaging fungal plant pathogens with an expansive host range. Its economic importance flags *S. sclerotiorum* as an exemplary model of a soilborne necrotrophic plant pathogen. The 2005 release of the draft genome sequence provides an invaluable resource for dissecting the biology of this disease. To exploit this resource, methods of genetic transformation and subsequent recovery of monocaryotes, need to be improved. Recently, we developed a protocol for targeted, *Agrobacterium tumefaciens*-mediated gene disruption transformation of *S. sclerotiorum.* Fungal ascospores were transformed with a gene-specific disruption construct using the hygromycin B resistance gene as a selectable marker. Gene disruption frequencies were defined as 6 transformants out of 10^5 ascospores. Transformants were confirmed by PCR and Southern analysis. Results revealed that 100% of the transformants contained a single-copy targeted integration of the T-DNA to the *S. sclerotiorum* genome in the way of gene replacement. This approach circumvents the laborious and time-consuming methods of protoplast transformation and readily yields single-copy T-DNA transformants. Considering its efficiency, we are currently using this approach to disrupt genes that have been described in other fungi to be required for pathogenicity, and genes that are potentially involved in the perception of the host. Current data from this study will be presented.

CP-E3

Identification of a *Phytophthora sojae* avirulence effector that elicits cell death in soybean plants carrying *Rps3a*

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Phytophthora sojae (Kaufmann & Gerdemann) causes root and stem rot in soybean (*Glycine max* (L.) Merr.). To protect soybean crops from this devastating disease, resistance (*R*) genes have been introgressed from wild-germplasm into commercial cultivars. These *R* genes confer immunity to *P. sojae* isolates expressing cognate avirulence (*Avr*) genes. A combination of bulked-segregant-analysis and expression profiling was used identify *P. sojae* transcripts that are associated with the avirulence traits: *Avr3a*, *Avr5*, and *Avr3c*. Analysis of the microarray data revealed a transcript whose expression shows 100% association with the *Avr3a/5* trait. This marker resides in a region with a high density of avirulence gene homologs (*Avh*). The expression of a nearby *Avh* gene, *Avh92*, is also associated with the *Avr3a/5* trait; and the gene itself is physically linked to the *Avr3a/5* locus. *Avh92* alleles of other *P. sojae* races were sequenced and three alleles were identified. A comparison of the three alleles revealed *Avh92* has undergone diversifying selection typically associated with avirulence genes. In a particle bombardment assay, an allele of *Avh92*, expressed by avirulent races, was shown to induce cell death in the leaves of soybean plants carrying *R* gene *Rps3a*. This data suggests that *Avh92* encodes the *Phytophthora sojae* avirulence effector *Avr3a*.

CP-E4

TF989, a novel myb-like transcription factor, acts as activator for *CHS8* gene expression in soybean

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We have previously shown that *Chalcone Synthase 7* (*CHS7*) and *CHS8* genes play important roles in isoflavonoid synthesis in soybean (*Glycine max*). To identify the factors that regulate the expression of *CHS7* and *CHS8* genes in soybean seeds, we obtained a 1662 bp genomic DNA fragment 5' upstream of *CHS8* gene using genome walking strategy from four GenomeWalker libraries. Computational analysis of transcription factor binding sites (TFBS) in *CHS8* promoter region predicted 171 potential TFBSs. A total of 45 of these TFBSs were confirmed by DNase I footprint analysis. Out of 45 TFBSs, 11 sites contained binding sequence specific for MYB familiy of transcription factors (TFs). A list of candidate MYB TF genes were made based on our previous microarray data and DFCI soybean expressed sequence tag database. A total of 21 MYB TFs were cloned and co-expressed with CHS8promoter:GUS in arabidopsis protoplast using transient expression system. The expression of GUS reporter gene expression in response to different MYB TFs were measured. The results indicated that CHS8 promoter driven GUS gene expression was consistently and significantly increased to a much higher level when co-expressed with TF989 as compared other TFs and control. Binding of TF989 to the *CHS8* promoter region was further confirmed by *in vitro* gel shift assay. A detail characterization of TF989 has been conducted and specific sequences in the *CHS8* promoter regions identified. Results will be discussed.

CP-E5

Plant recombinant antibodies for the prevention of foot-and-mouth disease virus

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Foot-and-mouth disease (FMD) is the most significant constraint to international trade in live animals and animal products. Outbreaks have a devastating effect on the economy of the affected country, due to the cost of disease control measures and trade embargos. While current whole-virus-based vaccines are protective against FMD, there are several limitations restricting their usefulness as prophylactic treatment or to control outbreaks: (1) current measures to tell infected from vaccinated animals are inadequate, (2) vaccinated animals can develop sub-clinical carrier state during outbreaks, and (3) vaccination cannot always protect susceptible animals fast enough. As a consequence most Western countries do not vaccinate against FMD and outbreaks are controlled by mass culling of all infected and suspected animals. Passive immunization, using a mixture of low-cost recombinant antibodies to create a synthetic polyclonal, is an attractive alternative to protect susceptible animals in infection zones. Our research has focused on developing a cost-effective and scalable production system of recombinant anti-FMDV antibodies in plants. Virus-neutralizing monoclonal antibodies specific for the three major FMDV serotypes were developed. The genes corresponding to antibody variable regions were identified and cloned to generate recombinant single chain antibody fragments (scFvs). Vector design was optimized to facilitate high accumulation of scFvs in our biosafe low-alkaloid tobacco platform. Novel purification methods for recombinant scFvs were established and plant-made scFvs were shown to bind FMDV *in vitro*. Virus neutralization and small animal trials will be carried out in the near future to demonstrate passive immunization.

CP-F1

A predicted interactome for *Arabidopsis*: expanding the Bio-Array Resource's collection of large-scale data sets available for hypothesis generation

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We have entered the post-genomic era, where technological advances have made the generation of data about the levels and states of all biological molecules – transcripts, proteins, metabolites – in a cell or organism increasingly high-throughput and cost-effective. These data can provide a wealth of information to lab-based researchers, if the data are "mined" appropriately. The complex cellular functions of an organism frequently rely on physical interactions between proteins. A map of all protein-protein interactions, an interactome, is thus an invaluable tool. We present an interactome for *Arabidopsis thaliana* predicted from interacting orthologs in yeast, worm, fruit fly and human. The *in silico* validation of these predicted interologs was established via expression and subcellular localization data. There was significant co-expression of genes whose proteins were predicted to interact. As well, interacting proteins were also significantly more likely to be found within the same subcellular location and significantly less likely to be found in conflicting localizations than randomly paired proteins. These predictions can aid researchers by extending known complexes and pathways with candidate proteins. Similarly, the ability to perform so-called "electronic Northerns", i.e. querying expression data sets with a gene of interest to see how it is responding across all treatments in the database, has proved to be of enormous utility, especially in the context of non-redundancy within gene families. In this vein, the international AtGenExpress Project has generated gene expression data sets from representative experiments in Arabidopsis and has made them available to the community. We have developed tools, available as part of the Bio-Array Resource at http://bar.utoronto.ca, for exploring these and other data, to allow deeper insights into biological questions and to help guide lab-based research.

CP-F2

Impact of mycorrhiza and growth rate on vascular maturity in roots

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It has long been accepted that the distance behind the tip at which the tracheary elements become mature is positively correlated with the rate of root growth. However, how the presence of a mycorrhizal association impacts this has received litter consideration. As mycorrhiza tend to produce smaller, and therefore potentially slower-growing root systems, our hypothesis was that mycorrhizal root systems would induce tracheary elements to mature closer to the tip. In the current experiment, both ectomycorrhizal roots (*Pinus banksiana*) and roots associated with arbuscular mycorrhizal fung (AMF); (*Zea mays*, *Lycopersicon esculentum* and *Fragaria virginiana*) were investigated. In each system, anatomical maturity was determined via chlorazol black E staining to reveal lignification, and functional maturity was assessed by cellufluor conductivity. In the case of the ectomycorrhizal roots, growth of mantle-bearing roots was absent, and the tracheary elements matured much closer to these tip than in their non-mycorrhizal counterparts. Conversely, AMF status had no impact on the distance behind the root tip at which the tracheary elements matured. In considering the rate of root growth alone, there was generally no correlation within a particular species between root growth and xylem maturity. However, when multiple species were analyzed together, a significant positive correlation between root growth rate and a greater distance between the tip and the mature tracheary elements was observed. Impacts of these findings on nutrient acquisition will be discussed.

CP-F3

Genome instability in *hothead* mutants: Outcrossing is not the only answer.

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Arabidopsis plants harboring mutations at the *HOTHEAD* locus inherit DNA sequences not present in the genome of the parents. This mode of non-Mendelian inheritance has been coined "restoration" since data indicate that sequences return to a previously existing ancestral state. We postulate that such a mechanism would allow inbreeding plants to maintain greater genetic variation, providing an adaptive advantage, for example, in times of stress. Although outcrossing could explain these finding, we have demonstrated that cross-pollination events do not occur at sufficiently high rates in these self-pollinating lines to account for our results. In addition to systematically determining outcrossing frequencies for hth mutants, experiments have been undertaken to definitively establish the rate at which genetic restoration occurs. Based on these experiments, we show that the inheritance of ancestral sequences takes place independent of outcrossing and at frequencies well above these rates alone.

CP-F4

Redox Modulation of Starch Biosynthesis

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Starch is a glucose polymer composed of amylose and amylopectin. ADP-glucose is the primary precursor for starch synthesis and is made by ADP glucose pyrophosphorylase (AGPase). Glucan chain elongation is carried out by starch synthases and the branch points are generated by starch branching enzymes. AGPase is heterotetrameric, consisting of two large subunits and two small catalytic subunits. In monocotyledonous plants possess two forms of AGPase, a plastidic and a cytosolic form. Posttranslational modification of AGPase by redox modulation, involving thioredoxin and formation of intramolecular disulfide bonds between the two small subunits has been demonstrated (Fu et al. (1998). AGPase activity increases in response to light and sugar levels, and at night AGPase is converted to an inactive dimer (Hendricks et al, 2003). Evidence is presented showing the plastidic form of wheat AGPase is subject to redox modulation and work is currently being performed to investigate if the cytosolic form is also redox modulated.

References:

Fu Y., Ballicora M.A., Leykam J.F., Preiss J. (1998) Mechanism of reductive activation of potato tuber ADP-glucose pyrophosphorylase. Journal of Biological Chemistry 273: 25045-25052

Hendricks J.H.M., Kolbe A., Gibon Y., Stitt M., Geigenberger P. (2003) ADP-glucose pyrophosphorylase is activated by post-translational redox-modulation in response to light and to sugars in leaves of *Arabidopsis* and other plant species. Plant Physiology 133: 1-12

CP-F5

Methodologies for the quantification of ferric and cupric reductase activities by iron-limited algal cells. H.G. Weger, M.B. Fink and C.N. Walker. *Department of Biology, University of Regina, Regina, Saskatchewan, S4S 0A2, Canada.*

The colorimetric Fe^{2+} indicators BPDS (bathophenanthroline disulfonic acid) and FZ (3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine) are routinely used to assay for plasma membrane ferric reductase activity in iron-limited algal and plant cells. Ferric reductase assays using these colorimetric indicators must take into account the fact that Fe^{3+} chelators can also in general bind Fe^{2+} and may therefore compete with the colorimetric Fe^{2+} indicators. Conversely, the presence of BPDS or FZ may also facilitate the reduction of Fe^{3+}-chelates, potentially leading to overestimation of ferric reduction rates. Lastly, both BPDS and FZ have non-negligible affinities for Fe^{3+} in addition to their well-known affinities for Fe^{2+}; this leads to potential difficulties in ascertaining whether free and/or chelated Fe^{3+} are potential substrates for the ferric reductase. In this work we describe an oxygen electrode-based assay for both ferric and cupric reductase activities. Using this assay system, we demonstrate that the plasma membrane metal ion reductase activity of iron-limited algal cells reduced complexed Fe^{3+} (i.e. Fe^{3+}-chelates) but did not reduce free (non-chelated) Fe^{3+}, and also reduced free Cu^{2+} to Cu^{+}, but did not reduce Cu^{2+} that is part of Cu^{2+}-chelates. These results suggest that an O_2 electrode based metal reductase assay system has some specific advantages compared to the traditional colorimetric assay system.

P1

***Populus* drought transcriptome: intra-specific variation in transcriptome activity**

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Trees of the genus *Populus* have both high economic and ecological value in temperature regions the world over. The productivity of *Populus* species is attributable to genetic factors and growing conditions, notably water availability, and the interplay between those two parameters. Our research aims to develop a mechanistic understanding of the interplay between genotype and environmental effects, like water availability, and the role that they play in productivity of trees in the genus *Populus*. Specifically, we are testing the hypothesis that intra-specific differences in biomass accumulation (growth rate) are influenced by environmental challenges such as water deficit, and that these differences are underpinned by genotype-dependent variation in transcriptome activity. To test this hypothesis, we have analysed the transcriptome activities of six different *Populus balsamifera* clones that vary in their growth rate, under both well-watered and water-deficit conditions. Transcriptome activity was assessed by bioinformatic analyses of Affymetrix GeneChip microarray data. Transcriptome activity differs among the various *Populus balsamifera* clones in both well-watered and water-deficit conditions, with varying degrees of correlation with biomass accumulation. This work has implications for our understanding of variation in transcriptome activities within a species, and how this might relate to productivity of an industrially important species.

P2

A central regulator of the plant transpiration stream

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Plant transpiration is a major determinant of both global carbon fixation and terrestrial water cycles. Transpiration makes water available for photosynthesis and determines the rate of water loss via evaporation from leaves. Transpiration is largely influenced by the dynamic regulation of stomata, the formation of xylem, and root system architecture. Previously we showed that the R2R3-MYB transcription factor, *At*MYB61, was both necessary and sufficient to control the diurnal changes in the stomatal aperture. Here we show that this same transcription factor directly controls the other major facets of the plant transpiration stream, xylem formation and root system architecture. *AtMYB61* is expressed in differentiating xylem cells and emerging lateral root primordia. Loss of *AtMYB61* function results in a decrease in xylem formation, alterations in xylem cell structure, and a decrease in lateral root formation. *AtMYB61* expression in differentiating xylem is contingent on the availability of photosynthate, providing a link between the establishment of the transpiration stream and photosynthesis itself. *At*MYB61 regulates a suite of downstream target genes, which in turn determine the extent and features of xylem cell formation and root system architecture. Thus, *At*MYB61 is the first transcription factor defining a genetic module directly linking the components of the plant transpiration control system, a key innovation in the evolution of terrestrial life.

P3

Transcriptional regulation of *AtMYB61* expression by nutritional cues

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The transcription factor *At*MYB61 plays important roles in controlling facets of the plant transpiration stream. Here we show that *AtMYB61* is regulated in a novel manner by nutritional stimuli, including sugars and amino acids. Diurnal changes in *AtMYB61* transcript abundance are dampened in mutants impaired in diel carbohydrate cycling. Pharmacological analyses suggest that *AtMYB61* transcript abundance is influenced by soluble sugars, through a pathway that appear to require sugar metabolism, but does not directly involve hexokinase signalling. This hypothesis is supported by the finding that *AtMYB61* expression remains sugar responsive in the hexokinase (*HXK1*) loss-of-function mutant *gin2*. *AtMYB61* transcript abundance increases to the greatest extent in response to the major product of photosynthesis, sucrose, and is repressed in response to two major products of photorespiration, glutamate and glycine. *AtMYB61* expression is de-repressed by soluble sugars in a mechanism that involves intragenic sequences. Phylogenetic footprinting, bioinformatic, and biochemical analyses suggest a role for intron sequences in the regulation of *AtMYB61* expression. Based on these and other data, we present a model that explains the diurnal de-repression of *AtMYB61* expression by diurnal fluctuations in nutritional cues.

P4

Dissection of the *AtMYB61* regulatory circuit by chemical genetics

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Arabidopsis thaliana MYB61, a member of the R2R3-MYB family of transcription factors, regulates several key aspects of plant growth and development, including vascular architecture, secondary root formation, stomatal aperture, and flowering time. Though *At*MYB61 has been shown to play an important role in the control of these traits, the components of the regulatory circuitry that reside upstream and downstream of this transcription factor remain to be elucidated. Recently, we employed a chemical genetics approach involving a screen of over 2000 different compounds that differentiated between wild-type *A. thaliana* seedlings relative to seedlings containing a *myb61* loss-of-function mutation. Five different chemicals belonging to the sulfonamide family of compounds had a deleterious effect on dark-mediated elongation growth in wild-type seedlings, while the *myb61* T-DNA insertion mutants were relatively unaffected by the presence of the sulfonamides. Here we report on the initial chemical screen, and the progress we have made toward using the screen to characterise additional components of the *At*MYB61 regulatory circuit. We propose that sulfonamide insensitivity found in the *myb61* knockout seedlings can be used as a starting point to dissect the molecular components involved in both the upstream regulation and downstream manifestation of *At*MYB61 activity.

P5

Drought and the model tree: a genomics approach

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Exposure to episodic drought can impinge significantly on forest health and the establishment of productive tree plantations. There is therefore great interest in understanding the mechanisms of drought response in the ecologically dominant and economically important genus *Populus*. Affymetrix GeneChip microarray analysis of two commercially important *Populus* clones was used to identify genes characterising the drought response for each genotype, and to relate whole organism responses to changes in the transcript abundance of genes over a diurnal period. One clone, *P. deltoides* X *P. nigra* (DN34), had a pronounced and adverse response to drought. Despite having a pronounced phenotypic response to drought, the transcript abundance patterns of only a very few genes were altered in response to water limitation in DN34. In contrast, the *P. nigra* X *P. maximowiczi* (NM6) clone had a much more subtle phenotypic response to drought, but many more genes exhibit differences in diurnal patterns of transcript abundance in response to water limitation, suggesting that this genotype may buffer its response to drought by deploying more complex gene networks than DN34. Our studies provide insights into the variety of genetic mechanisms underpinning the *Populus* drought response, and provide candidates for future experiments aimed at understanding this response across this economically and ecologically important genus.

P6

***Populus* drought transcriptome: spatial and temporal effects on transcriptome activity**

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Trees of the genus *Populus* provide an excellent opportunity to investigate questions related to the interplay between a given individual's environment and its capacity to respond to external stimuli. Specifically, we are interested in determining whether identical *Populus* clones propagated in different nurseries differ in their capacity to respond to an environmental insult – in this case, drought. It is hypothesised that nursery source influences drought transcriptome activity in a specific manner, and that these changes may be affected by yearly variations in the environmental conditions experienced at each nursery. As a first step in testing this hypothesis, three commercially favoured hybrid *Populus* clones ("DN34", "Walker" and "WP69") were each obtained from two different locations (Alberta, Manitoba or Saskatchewan) and grown for six weeks under common garden conditions in a growth chamber. Water was withheld from half of each population, and at the onset of a significant change in leaf water potential, leaves were harvested for transcriptome analysis using Affymetrix whole genome poplar GeneChip microarrays. The data from these experiments provide insights into the interplay between genotype and environment, and hint at the potential role played by epigenetic phenomena in this interaction.

P7

Gene expression and isoflavone concentrations in soybean sprouts treated with chitosan

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This study was undertaken to investigate whether chitosan treatments of sprouts of three soybean cultivars, 'AOC Champion', 'AC Orford', and 'AC Proteina' can increase not only isoflavones concentration in sprouts but also the transcript levels of genes encoding enzymes at key points of the phenylpropanoid pathway: phenylalanine ammonia lyase (PAL, EC 4.3.1.5), chalcone synthase (CHS, EC 2.3.1.74) and chalcone reductase (CHR, EC 2.3.1.170), and at branch point enzymes in isoflavone biosynthesis [isoflavone synthase (IFS, EC 1.14.13.86)]. Compared to untreated sprouts, chitosan treatment caused an up-regulation of three of four target genes (*PAL*, *CHS* and *IFS*) in only 'AC Orford', a-low isoflavone seed content. Significant down regulation of *CHS* and *IFS* was detected in 'OAC Champion' and 'AC Proteina', a medium and high isoflavone seed contents, respectively. Response however depended on the molecular weight of chitosan and treatment frequency. Chitosan treatments had limited effect on isoflavones concentrations in all treatments only reducing glycitein in 'OAC Champion' by 38%. No correlation was found between gene expression and isoflavone concentrations. Differences in isoflavone concentrations were observed between sprouts of the cultivars; 'AC Proteina' had the highest isoflavones concentration and 'AC Orford' the lowest. Results indicate that although isoflavones concentration and genes expression varied with cultivar, chitosan treatment is not a viable option for increasing isoflavone content in sprouts of cultivars evaluated.

P8

Photoperiodic injury in tomato is linked to diel N metabolism and the circadian clock

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Photoperiodic injury (PI) of vegetative tissues occurs under either extended photoperiods or non-24 h light/dark cycles in a number of plants including tomato. We have identified and characterized genetic variation for susceptibility of tomato to PI. Use of these genetically-distinct lines has shown that PI is linked both to circadian-controlled processes and to N metabolism as evidenced by nitrite accumulation under light/dark cycles that elicit PI. Genetic analysis of populations segregating for PI indicate that the trait is controlled by two genes through a suppressive-epistatic interaction. Analysis of diel activities of nitrate reductase (NR) and nitrite reductase (NiR) under either a 12 h or 24 h photoperiod for lines either tolerant or susceptible to PI showed that nitrite accumulation occurred when the NR/NiR activity ratio approached a value of one or more; this only occurred in 24 h light for lines susceptible to PI. A line tolerant to PI did maintain similar diel rhythms for NR activity state, NiR activity, and levels of amino acids under either a 12 h or 24 h photoperiod; this was not the case for a line susceptible to PI. Collectively, the data provide evidence that the circadian clock is important for regulating N metabolism including the relative activities of NR and NiR.

P9

Vitality stains reveal senescence pattern of *Eriophorum vaginatum* corm

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Eriophorum vaginatum is a tussock forming sedge that thrives in cold, nutrient poor environments. The perennial nature of *E. vaginatum* depends on the ability of the corm to store nutrients and survive the winter. The corm senesces after flowering and seed production as its apical meristem undergoes change from being vegetative and producing leaves to becoming generative to produce inflorescence. Senescence refers to the final step leading to death; it is a complex, highly regulated process with crucial developmental function ongoing throughout the life of the plant. Because of the compacted growth pattern it and formation of an elevated tussock, it has been suggested that senescence of the individual corms progresses from the distal region and through the apex of the corm. Cell vitality was determined by taking free-hand sections of the corm below the apex, the mid-section and above the distal region of the corm, and stained with Evan's Blue and Fluorescein. In the sections from the apical region of the corm, Evan's Blue was excluded from the cells indicating that the cells are viable. However, the cells from the mid and distal parts of the corm retained Evan's Blue, indicating these cells are undergoing senescence. The same results were obtained with Fluorescein that confirmed the senescence of the individual corms is initiated in the distal region and progresses upwards. Therefore, the elevated habit of the *E. vaginatum* tussock is due to the progressive growth of new corms, which are connected to the senescencing older corms that experience little decay in cold anoxic wetlands.

P10

Characterization of *Eriophorum vaginatum* seed tissues and seedling growth.

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Eriophorum vaginatum L. is a sedge that has been noted as a colonizer of disturbed sites. Its persistence in metal polluted wetlands near Sudbury, Ontario, suggests potential use as a phytoremediator. It is unknown whether the presence of *E. vaginatum* tussocks in metal contaminated wetlands is due to *E. vaginatum* propagation from seed. Therefore, it is important to determine germination and growth characteristics of *E. vaginatum* seeds. In this study we describe *E. vaginatum* fruit structure, its permeability to water and germination characteristics. *E. vaginatum* inflorescences were collected in June of 2007 from sites near Sudbury, Ontario. The achene is shed form the inflorescences with attached filamentous bristles. The achene has a hard pericarp covering the seed. The seed coat is composed of compressed epidermis with a waxy cuticle. Underlying the seed coat is one layer of aleurone parenchyma cells with thin primary cell walls. The endosperm is filled with starch and oil bodies, acting as the primary nutrient source for the developing embryo. Despite their cuticle cover, the seeds are permeable to water and are fully imbibed after 24h. Peak germination (84%) occurs when seeds are exposed to light at 27.4 +/- 1°C for 10 days. Future research will focus on the effects of metals on *E. vaginatum* germination and seedling establishment in determining its perspective use for revegetation of contaminated wetlands.

P11

The extracts from sweet fern (*Comptonia peregrina*) have high antioxidant and antibacterial properties.

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Sweet fern (*Comptonia peregrina*) grows abundantly in northern Ontario because it has a unique ability to form symbioses with N_2-fixing actinomycetes (*Frankia)* and invades nitrogen-poor soils. The use of this plant by Aboriginal people has its long history. The antioxidant capacity of several sweet fern (*Comptonia peregrina*) extracts was determined using the oxygen radical absorbing capacity (ORAC) method. The basis of this analysis was the measurement of the fluorescent decay of fluorescein in the presence of a free radical generator, AAPH. The rate of fluorescent decay was inhibited by either a known concentration of a standard antioxidant, TROLOX, or a sample extract, which act to protect the fluorescein molecules from free radical damage. The sweet fern extracts used in this study consisted of a crude water extract, a butanol extract, an ethyl acetate extract, and sweet fern essential oil. The antioxidant capacity of the various extracts ranged from 25.097 ± 0.104% to 53.952 ± 0.856% µM TROLOX equivalents per 0.01 g/L of extract, with crude water extract having the lowest antioxidant activity and ethyl acetate extract having the highest. The ethyl acetate and butanol extracts negatively affected growth of *Bacillus subtilis* (gram positive) and *Alcaligenes faecalis* (gram negative) indicating an antibacterial properties of sweet fern. Furthermore, HPLC analysis of ethyl acetate revealed 21 peaks indicating that there are at least 21 different substances. Further fractionation of those peaks could lead to identification of specific compound with potent antioxidant properties in sweet fern.

P12

Development of a Genome Information Resource for the Identification of Genes Involved in Plant Cuticular Wax Biosynthesis

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All land plants are coated with a waxy "skin" called the cuticle that serves as a protective barrier against uncontrolled water loss, UV light, microbial pathogens, and insects. The cuticle is synthesized by epidermal cells and is comprised primarily of lipids (cutin and waxes). Intracuticular waxes are embedded in the three-dimensional cutin matrix and epicuticular waxes, often in the form of crystals, cover the outer surface. We are taking advantage of a large number of wax-deficient (*cer*) mutants of *Arabidopsis* to identify and functionally characterize gene products involved in wax biosynthesis and deposition. One goal of the present work is to develop genomic tools to rapidly identify uncloned *CER* genes. A large collection of genes that are specifically expressed in the epidermis have been identified using DNA microarray analyses (1). We have correlated the physical positions of these epidermal-specific genes with the approximate physical positions of uncloned *CER* genes as determined by genetic linkage analyses. These nearby epidermal-specific genes are good candidates for the *CER* genes and these are currently being investigated by analyses of T-DNA insertion lines. We have also characterized by scanning electron microscopy the plant surface morphology of known *cer* mutants and a set of uncharacterized wax-deficient lines in search of novel mutants. The wax chemical profiles of these new mutants have also been characterized by gas chromatography revealing distinct disruptions in the wax metabolic pathways. Molecular identification of these *CER* genes, aided by the above genome resource, will provide important insights into wax biosynthetic pathways and provide tools for the genetic engineering of cuticular wax composition to generate drought and pathogen resistant plants.

(1) Suh M.C., Samuels, A.L., Jetter, R., Kunst, L., Pollard, M., Ohlrogge, J., and Beisson, F. (2005). Cuticular Lipid Composition, Surface Structure, and Gene Expression in Arabidopsis Stem Epidermis. Plant Physiology 139:1649-1665.

P13

The identification of transcript and protein products arising from the *apt1* locus in *Arabidopsis thaliana*

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In plants, adenine phosphoribosyltransferase (APT) is the primary pathway by which adenine is metabolized to adenosine monophosphate in a one step reaction. In a similar manner, APTs interconvert active cytokinin bases into inactive nucleotide forms. Initial research on APT genes in *Arabidopsis* indicated that one isoform, APT1, provided the majority of activity and deficiency of this enzyme resulted in male sterility due to aberrant pollen production. Recent annotations of the *Arabidopsis* genome reveal the presence of an additional *apt1* transcript containing an extra exon. This study intends to identify the transcript products of the *apt1* gene, their functional roles, and whether they arise from alternate splicing or two distinct promoters. The use of recombinant proteins will identify the activity possessed by each isoform and differences in affinity for adenine or cytokinins. Further, artificial microRNA lines will be developed to silence individual transcripts in order to define the contributions of each to activity and fertility. This insight will guide future endeavors as to the purpose of multiple transcripts coding for similar protein products and further identify undiscovered differential transcripts arising in *apt* loci of closely related species.

P14

The iridoid glucoside, antirrhinoside, from *Antirrhinum majus* L. has differential effects on two generalist insect herbivores

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Defensive plant secondary metabolites can be found distributed within the plant according to the optimal defence theory (ODT). The iridoid glucoside, antirrhinoside, is constitutively distributed throughout *Antirrhinum majus* L. in a manner consistent with ODT but there is no evidence that this compound has a defensive function with respect to insect herbivory. To address this question two generalist herbivores, *Lymantria dispar* L. (gypsy moth) and *Trichoplusia ni* Hübner (cabbage looper) were fed excised whole leaves of *A. majus* and antirrhinoside-artificial diet assays. In leaf excision feeding trials 4th instar gypsy moth rejected, without sampling, the leaves of *A. majus* regardless of what node the leaf was excised from. In contrast, 4th instar cabbage looper readily fed on the excised leaves and antirrhinoside was not found in their bodies or feces (frass) as determined by thin layer (TLC) and high pressure liquid chromatography (HPLC). A second major leaf iridoid in *A. majus*, antirrhide, was found in both cabbage looper and gypsy moth frass. In diet feeding assays the growth of gypsy moth and cabbage looper were not inhibited by methanol extracts, iridoid fractions or pure antirrhinoside at concentrations of 0.6% in diet but cabbage looper growth was enhanced. At an antirrhinoside concentration of 3.3% in diet gypsy moth growth was reduced whereas cabbage looper growth again increased significantly relative to the control. It is therefore likely that antirrhinoside does function as defence against herbivory for one generalist insect herbivore but also, at low concentrations, enhances the growth of another.

P15

Differential accumulation of cadmium in durum wheat: the roles of low molecular weight organic acids.

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Cadmium is a non-essential toxic metal that readily accumulates in plants. Its entry into the food chain via anthropogenic sources including power stations, nickel-cadmium batteries and phosphate fertilizers has led to known neurotoxic, mutagenic and carcinogenic effects. To understand the mechanisms of uptake and distribution of cadmium in plants, two pairs of near-isogenic lines of durum wheat (*Triticum turgidum var durum*) that differ in cadmium accumulation are being studied. The cultivar pairs can be distinguished into 'high' and 'low' accumulating near-isogenic lines; the 'high' line typically translocates twice as much cadmium into the leaves and grain as compared to the 'low' line. We will use a two-fold approach to understand this differential pattern of cadmium accumulation. (1) We will investigate the relative proportions of cadmium in the apoplast and symplast in the root tissues to test the hypothesis that cadmium is retained in the root apoplasm of the 'low' lines. (2) Information provided on the distribution of cadmium within the plant will be correlated with the types and concentrations of organic acids in the root exudates and plant tissues in order to elucidate the putative roles of organic acids as chelators. Organic acids may either retain cadmium within a tissue or facilitate transport of cadmium between tissues.

P16

Overlapping, but non-redundant roles for multiple tomato GABA transaminases

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Gamma-aminobutyrate (GABA) is a ubiquitous non-protein amino acid that is implicated in stress metabolism and signaling. Recently, we demonstrated that recombinant *Arabidopsis* GABA transaminase (GABA-T) exhibits both pyruvate- and glyoxylate-dependent activity. A bioinformatics screen of the tomato plant EST database enabled identification of three novel GABA-T isoforms (LeGABA-T 1, 2 and 3). Each GABA-T cDNA was fused to green fluorescent protein and transiently expressed in tobacco BY-2 suspension cells using particle bombardment. Epifluorescence microscopy of transformed cells revealed that *Le*GABA-T1 was localized exclusively to mitochondria, whereas *Le*GABA-T2 and *Le*GABA-T3 were localized to cytosol and plastids, respectively. Soluble recombinant expression of each isoform was achieved via removal of the N-terminal targeting domains as was appropriate and co-expression of the full-length or truncated genes with the GroES/EL chaperone complex in *Escherichia coli*. All three isoforms contained both pyruvate- and glyoxylate-dependent activity, and the mitochondrial form had specific activity similar to that found for the *Arabidopsis* enzyme, but the specific activity for cytosolic and plastidial forms was two orders of a magnitude lower. Quantitative real time PCR analysis revealed that the three isoforms had similar expression patterns in leaf or root tissue with development, but were different in flower or fruit tissue, suggesting that the three isoforms play non-redundant roles in reproductive organs.

P17

Subcellular localization of *Arabidopsis thaliana* ADT1-GFP fusion proteins

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The final step in phenylalanine biosynthesis involves the dehydration of arogenate to phenylalanine by arogenate dehydratases (ADTs). Six ADTs have been identified in *Arabidopsis thaliana* and *in silico* analyses predict that the gene sequences code for an N-terminal transit peptide to direct the ADTs to specific subcellular organelles. At least three of the peptides are predicted to direct localization of the protein to the chloroplast. To date, transient expression assays have been performed in onion root cells and *Nicotiana tabacum* for ADT1. These assays introduced green fluorescent protein (GFP)-ADT1 fusion constructs into onion and tobacco by particle bombardment and *Agrobacterium* infiltration, respectively. The ADT component of the fusion constructs either represent the full length (FL) sequence including the putative transit peptide or a mature (M) sequence lacking the putative transit peptide. The data show that the presence of the transit peptide is required for localization to proplastids in onion roots or to the outer membrane of tobacco chloroplasts. This unique expression pattern in tobacco suggests that the *Arabidopsis* transit peptide is sufficient to target ADT1 to the chloroplast but lacks species specific components required for the successful transport through the chloroplast membrane.

P18

The Proline-rich, Extensin- like Family of Receptor Kinases Play a Role in Cell Elongation

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Receptor kinases play a fundamental role in intercellular signaling and comprise the largest receptor family in plants with over 600 predicted genes in the *Arabidopsis* genome. Receptor kinases are located at the plasma membrane and have an external ligand-binding domain as well as an intracellular kinase domain. The extracellular domain of plant receptor kinases can vary considerably and we are focusing on the Proline-rich Extensin-like Receptor Kinase (PERK) family, which has an extracellular domain similar to that of extensins. The PERKs are a 15 member gene family of receptor kinases. The aim of our research is to identify the biological function of the ubiquitously expressed perks through analysis of mutant phenotypes and chemical response assays. In *Arabidopsis*, single T-DNA knockout lines for each of the PERKs shows no mutant phenotypes. Double and triple knockouts were produced and the resulting mutants have been screened for chemical response phenotypes. Analysis of the *perk*8,9,10 triple knockout has shown increased sensitivity to the actin depolymerising drug Latrunculin B. *Perk*8,9,10 roots displayed an altered sensitivity in the presence of auxin hormone indoleacetic acid (IAA) when compared to wild type Columbia seedlings. Both phenotypes appear to be caused by underlying cell elongation defects. We propose that the PERKs are involved in cell elongation processes and that the auxin response may be mediated through the PERKs.

P19

***In Silico* and *In Planta* Analyses of Promoters from Soybean Seed Lectin Homologues**

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The genome of soybean *Glycine max* (L.) Merr., is thought to contain 40-60,000 genes, each individually controlled by regulatory sequence elements in the adjacent promoter. Multi-gene families are useful model systems for molecular mechanisms of differential gene expression. The soybean legume lectin family has at least four expressed members. The seed lectin, *Le1*, is specifically located in seeds. We cloned the promoters from two *Le1* gene homologues, *Le2* and *Le3* and show that the three promoters drive differential *gusA* (GUS) reporter gene expression in transgenic *Arabidopsis thaliana*. The results indicate that *Le3* drive gene expression predominantly in vegetative tissues, including root, whereas *Le2* drive expression in all tissues examined, excluding roots. The *Le1* and *Le3* promoters also show complementary expression profiles in germinating seedlings: as the effect of the *Le1* promoter decreases, the effect of the *Le3* promoter increases. Using bioinformatics techniques, regulatory motifs (using the entries from the PLACE database) were identified in the promoters, corroborating the *in planta* results that the three different lectin promoters are distinct and drive gene expression in different tissues.

P20

Genetic Analysis of Arabidopsis-Spidermite Interaction.

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In response to herbivore attack, plants have evolved a variety of mechanisms to deter herbivore feeding, which prevent the herbivores from jeopardizing the plant's health, reproduction, and ultimately survival. The two-spotted spider mite *Tetranychus urticae* is a generalist herbivore that sucks out the plant cell's contents leaving small light-coloured punctures or spots on their leaves. Furthermore, the spider mite is a newly developed model organism that will, in the near future, provide tools for molecular analysis of its development, physiology and metabolism. The main objective of my research is to characterize the differential resistance of Col and L*er* Arabidopsis accessions to spider mite damage. My aim is to characterize damage on Arabidopsis plants upon spider mite feeding, to determine the genetic bases of this differential response, and to map genes responsible for the variation in susceptibility between L*er* and Col. A genetic dissection of mite-plant interactions could thus provide insights into the signalling and transcriptional basis of plant defences used against herbivores. In addition, genome-wide sequences of spider mites will lend themselves to the analysis of the transcriptome's response to host-plant defensive compounds, providing an opportunity for making an important contribution to our understanding of plant-herbivore interactions.

P21

Two arabidopsis plant u-box (atpub) e3 ubiquitin ligases may function as regulators during transmission

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Ubiquitin-mediated proteolysis has emerged as an important process involved in how plant signalling pathways can be regulated in response to environmental or developmental cues. The involvement of the Ub-26S system during self-incompatibility was established by the characterization of the *Brassica* ARC1 protein, an E3 ubiquitin ligase that targets substrates presumed to be needed for compatible pollinations for degradation. In self-compatible A*rabidopsis thaliana*, there exists a 17-member sub-family with similar domain organization. Based on their conservation of E3 ligase activity, these UND-containing AtPUB-ARM proteins appear to function in a manner analogous to ARC1. Phylogenies based on domain sequence similarities have shown that AtPUB18 and AtPUB19 consistently form a clade. General analysis of *pub18* or *pub19* T-DNA insertion lines for growth defects did not yield any distinct phenotypes, possibly due to the probability that both proteins operate redundantly. To alleviate the difficulty of functionally characterizing redundant proteins, crosses between *pub18* and *pub19* lines were performed. No *pub18/pub19* plants were ever observed among the F_2 generation. But interestingly the progeny from a selfed *PUB18/pub18 PUB19/pub19* plant differed from the expected Mendelian segregation ratio. As a consequence of these results, the progeny from selfed *PUB18/pub18* and *PUB19/pub19* plants were screened to assess how the individual *AtPUB18* and *AtPUB19* alleles might be involved in transmission through the male and female gametophytes.

P22

Functional analysis of the chloroplast protein import GTPase receptors using fluorescence spectroscopy

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Translocons at the outer envelope membrane of chloroplasts (TOC complexes) are responsible for the targeting and import of chloroplast-destined proteins. Previous research shows that there are structurally distinct TOC complexes composed of members of two gene families found in *Arabidopsis thaliana* which may be responsible for the import of different types of proteins. TOC159 (atTOC159/132/120) and TOC34 (atTOC34/33) gene families encode GTPases and have been hypothesized to interact with specific members from the other family to form structurally distinct TOC complexes. In particular, it has been shown that atTOC159 and atTOC120/132 represent structurally and functionally distinct TOC complexes that also preferentially contain atTOC33 and atTOC34, respectively. The current project is designed to test the hypothesis that the associations among members of these distinct TOC complexes are mediated by specific interactions between the GTPase domains of the TOC159 and TOC34 families members. Furthermore, we hope to map the putative transit peptide binding site(s) on the GTPase receptors using fluorescence spectroscopy. Fluorescence spectroscopy has been shown to be a powerful tool to test protein-protein interactions. To map the transit peptide binding sites, molecular techniques will be used to manipulate the location of fluorescent amino acids within the protein and fluorescence will be monitored. The rationale for the project, preliminary fluorescence data, and future experimental approaches will be presented.

P23

Protein trafficking and localization in leaves

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One of the major difficulties facing recombinant protein production in plants is insufficient accumulation of proteins. The primary organ for protein synthesis and storage is the seed. Within seeds, proteins are accumulated in protein storage vacuoles (PSVs). In contrast, seed storage protein (SSP) expression is repressed in vegetative tissues and PSVs are greatly reduced in number and size. We have initiated a study of SSP trafficking and localization in leaves of an *Arabidopsis thaliana* mutant. The mutant showed highly upregulated seed storage protein expression in leaves. The mutation was shown to disrupt a chromatin remodeling enzyme which functions to repress the expression of SSPs in wild type *Arabidopsis* leaves. The expression of SSPs in mutants will be detected by immunoblotting and protein accumulation will be localized using confocal microscopy and electron immunogold microscopy of translational SSP fusions with green fluorescent protein (GFP). The abundance and morphology of PSVs will be detected by staining with toluidine blue and by co-localization of a fluorescent PSV marker with translational SSP fusions with GFP. Finally, the developmental pattern of SSP expression will be studied. The research will further our understanding of protein trafficking and storage in plants and the results may translate into the potential for producing high levels of recombinant proteins in leaves.

Study of plant eIF4E genes for *Plum pox virus* (PPV) resistance

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Plum pox virus (PPV) is the causal agent of plum pox disease for stone fruit species (*Prunus* spp.). Since viruses depend on host factors for their infection, identifying and manipulating host factors that are involved in virus infection can be an approach for developing strategies for virus resistance. Our research has focused on studying translation initiation factors eIF4E and eIF(iso)4E, which may be a type of host factor involved in PPV infection. We cloned 4E and iso4E genes from plum. Sequence analysis indicates that plum 4E and iso4E genes both have 5 exons and 4 introns which are the same as those in *Arabisopsis thaliana.* The identity of eIF4E and eIFiso4E coding regions is 54.7% at the nucleotide level and 47.9% at the amino acid level. The identity levels between different isoforms of eIF4E are similar to those in *Arabidopsis*. The similarity of the same isoform of 4E between species is higher than that of different isoforms in the same species. We have introduced 4E and iso4E via hair-pin design into plum to silence the plum endogenous 4E and iso4E genes. The transgenic plants will be evaluated for PPV resistance.

The *Arabidopsis* Cyclic Nucleotide-Gated Ion Channels, ATCNGC11 and ATCNGC12 influences cation stress responses

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Cyclic nucleotide gated ion channels (CNGCs) are non-selective cation channels that were found to play an important role in visual and olfactory signal transduction in animals. They constitute six putative transmembrane domains with a pore region between the fifth and sixth domains. Their N- and C- termini extend into the cytosol and are believed to bind calmodulin and cyclic nucleotides, respectively. Recently, CNGCs have been discovered in plants where they are believed to possess a slightly different structure in which the C-terminus binds both calmodulin and cyclic nucleotides. The *Arabidopsis thaliana* genome encodes twenty CNGC subunits and studies have so far revealed some of their ability to selectively transport cations that play a role in mediating environmental stresses. Our research has focused on investigating the roles of ATCNGC11 and ATCNGC12 during ion stress by utilizing suppressor mutants of the constitutively active chimeric ATCNGC11/12 (*cpr22*) channel as well as knockout mutants for ATCNGC11 and 12. Heterologous expression of ATCNGC11 and ATCNGC12 in cation-uptake deficient yeast first revealed their ability to transport Ca^{2+} and K^{+}. Secondly, ion sensitivity assays involving germination and root growth using the above mentioned mutants showed that ATCNGC11 and ATCNGC12 were involved in ion stress responses.

P26

***Arabidopsis thaliana (L.) Heynh.* having altered expression of mitochondrial pyruvate dehydrogenase kinase show enhanced growth and development under elevated CO_2**

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Relative activities of mitochondrial pyruvate dehydrogenase kinase (mtPDHK) and phosphatase determine the phosphorylation status of mitochondrial pyruvate dehydrogenase (mtPDH) and there by its activity; mtPDH catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA and acetyl-CoA is a central metabolite for both catabolic and anabolic reactions. We hypothesize that having antisense repression of mtPDHK will allow *Arabidopsis* transgenics to show enhanced growth rates and productivity under elevated CO_2 due to greater sink strength. Wild-type, constitutive and seed-specific transgenic lines having antisense mtPDHK expression were grown under ambient (380ppm) and high (700ppm) CO_2, and plant development was monitored over time. Vegetative growth rate and rosettes size of all plant lines grown under elevated CO_2 were significantly greater than those grown under ambient CO_2. The transition from vegetative to reproductive phase occurred earlier in transgenics both under ambient and high CO_2. Height and total length span of inflorescences, the total number of siliques and seeds and the average weight per 100 seeds was increased in transgenics under elevated CO_2 compared to controls**.** In constitutive lines a correlation between mtPDH activity and enhancement in vegetative and reproductive growth could be seen. Results of this study will provide insight into mechanisms by which plant productivity can be optimized under elevated CO_2 levels.

P27

Identification of a null mutant for cytosolic fructose-1,6-bisphosphatase in *Flaveria linearis*

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An oxygen- and carbon dioxide-insensitive mutant of *Flaveria linearis* is linked to a low activity of fructose-1,6-bisphosphatase (cytFBPase) (Micallef and Sharkey, 1996). Further analysis of this mutant has shown that no cytFBPase activity can be detected using partially purified extracts. The lack of activity is not due to a down regulation in either enzyme transcription or translation. Instead, a comparison between the mutant and the wildtype cDNA sequences shows a 69bp internal deletion in the mutant. This deletion is deduced to include amino acids involved in substrate binding (Hur et al., 1992), which causes the null cytFBPase phenotype. This is the first report of a null mutant for an enzyme in the sucrose synthetic pathway in plants.

P28

Increased *LEAFY* transcript levels in a hypomethylated, early-flowering line of flax (*Linum usitatissimum)*

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The expression of two genes, the floral pathway integrator gene *LEAFY (LFY)* and *TERMINAL FLOWER1 (TFL1)*, which encodes an antagonist of *LFY*, was investigated as an initial step towards locating the gene(s) that regulate the early-flowering phenotype of a 5-azacytidine-induced flax line, RE2. In addition to differing in a range of phenotypic characteristics, RE2 has hypomethylated DNA and flowers 8-10 days earlier than its control line, RC. The hypotheses for the study were that: a) as seen in other species, *TFL1* would be strongly expressed and *LFY* would be weakly expressed in the shoot tips of young plants and that as the plants aged the expression would reverse, and b) RE2 would either over-express *LFY* or under-express *TFL1* relative to RC. A sharp increase in *LFY* transcript accumulation was observed in the shoot tips of RE2 at the onset of flowering. This increase was not seen in RC. The increase in *LFY* transcript accumulation is probably related to the early-flowering phenotype of RE2, but, at this time, there is no evidence linking this to its hypomethylation. A putative homologue of *TFLI* in flax was detected in the genomic DNA, but its expression was not detected in the shoot tips.

P29

Structure-function analysis of the Arabidopsis Toc159 family acidic domains

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Nuclear-encoded chloroplast proteins are translated in the cytosol with an N-terminal transit peptide, and are recognized by receptor components of the translocon at the outer membrane of chloroplasts (Toc). The Toc159 family of receptors are the primary chloroplast preprotein receptors in Arabidopsis, and are denoted atToc159, atToc132 and atToc120; where the number represents their molecular weight in kilodaltons. Primary protein structural analysis of the Toc159 receptors reveals a tripartite structure consisting of an N-terminal acidic (A-) domain, a central GTPase (G-) domain and a C-terminal membrane anchor (M-) domain. The Arabidopsis Toc159 receptors are approximately 65% identical across their G- and M-domains, but their A-domains share about only 20% sequence identity. Recent evidence has led to a working hypothesis that the Toc159 receptors show functional specificity; atToc159 preferentially recognizes photosynthetic preproteins, while atToc132 and atToc120 recognize plastid house-keeping proteins. The overall goal of the current research project is to gain insight into the function of the A-domains of the Toc159 family of receptors. First, we will investigate the overall secondary structure of the A-domains of atToc159 and atToc132, respectively, using CD spectroscopy. This structural information will be important for making inferences about A-domain function, and may contribute to our understanding of how the Toc159 receptors are able to recognize specific groups of chloroplast preproteins. Our most recent data will be presented.

P30

Relative *ADT* expression patterns in response to heat and cold treatments in *Arabidopsis thaliana*.

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Arogenate dehydratases (ADTs) catalyze the final step of the arogenate pathway of phenylalanine (phe) biosynthesis. The *Arabidopsis thaliana* genome encodes six different *ADT*s at unlinked loci. Currently, it is unknown why *Arabidopsis* needs six ADT isoforms to fulfill its requirement for phe synthesis. However, phe is required for several pathways and can either be used directly for protein synthesis or as a precursor in the synthesis of lignin, flavonoids, and many other secondary metabolites. Therefore, it may be possible that these diverse needs are met by individual *ADT*s transcribed in response to different intracellular and environmental cues. To determine how environmental alterations affect *ADT* gene expression, mature plants were subjected to either a cold (5ºC) or heat (38ºC) shock treatment for 24 hours, followed by a 24 hour recovery period at 20ºC. Rosette leaves were sampled at various time points including prior to stress (0 h), during the stress treatment (0.5 h, 1 h, 3 h, 6 h, 12 h, 24 h), and following 24 h (48 h) post-stress recovery. Samples were used to determine relative *ADT* expression levels using real-time PCR. Findings suggest that the six *ADT*s respond differentially, which is consistent with the idea that the expression of at least some *ADT*s respond to environmental stresses.

P31
Photosynthetic Response of Winter and Spring Cereals to Elevated CO_2"

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Photosynthesis is the principal physiological mechanism through which terrestrial plants sense and respond directly to, rising atmospheric CO_2 concentrations and changing temperatures. Cold tolerant species are able to acclimate to low temperatures. Acclimation of cold tolerant herbaceous plants grown at cold temperatures is accompanied by an increased capacity for carbon metabolism. In short term the rate of photosynthetic CO_2 assimilation (A) per unit leaf area is stimulated following shift to an elevated CO_2. Long-term exposure to higher CO_2 concentration leads to variety of acclimation effects that directly or indirectly influence the photosynthetic capacity of the plant. The overall objective of this research is to identify the effect of short and long term elevated CO_2 (700ppm) on the temperature-induced changes in photosynthesis and total biomass production in winter and spring wheat and rye cultivars. We observed growth coefficients of 1/3 for plants grown at 5 OC compared to plants grown at 20 ^{O}C for all cultivars; however dry weight to fresh weight ratio and specific leaf weight were higher in cold acclimated winter genotypes. Short term elevated CO_2 stimulated the light saturated rate of photosynthetic capacity by 50-60 % in all cultivars grown at 20 ^{O}C. However, the stimulation started to decline after 36 hours of CO_2 elevation. Photosynthetic efficiency and carboxylation efficiency were higher in plants shifted to elevated CO_2. We did not observe significant changes in respiration rates following short term CO_2 elevation in all cultivars. We will further study the effect of short and long term CO_2 elevation on growth kinetics, biomass accumulation, light saturated rate of photosynthesis, chlorophyll fluorescence, quantity and activities of photosynthetic genes in winter and spring wheat and rye cultivars combined with low (5 ^{O}C) and high (40 ^{O}C) temperatures.

P32
CpcB lyase null mutations disrupt, but do not necessarily prevent, phycocyanin chromophore function in the cyanobacterium *Synechococcus* sp. PCC 7002

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Phycobilisomes are the major light harvesting complexes for cyanobacteria and phycocyanin is the primary phycobiliprotein of the phycobilisome rod. Phycocyanobilin chromophores are covalently bonded to the phycocyanin β subunit (CpcB) by specific lyases which have been recently identified in the cyanobacterium *Synechococcus* sp. PCC 7002. Surprisingly, we found that mutants missing the CpcB lyases were nevertheless capable of producing pigmented phycocyanin when grown under low light conditions. 10K absorbance measurements revealed the energy states of the β phycocyanin chromophores to be slightly shifted and 77K steady state fluorescence emission spectroscopy showed that excitation energy transfer involving the targeted chromophores was disrupted. This evidence indicates that the position of the phycocyanobilin chromophore within the binding domain of the phycocyanin β subunit had been modified. We hypothesize that alternate, less specific lyases are able to add the chromophore, with varying effectiveness, to the β binding sites. Our data is discussed in the context of the role of chromophorylation in phycobilisome assembly, function, and stability.

P33

Probing the structure of oligomers of *Catharanthus roseus* 16-hydroxytabersonine-16-O-methyltransferase by molecular modeling, site directed mutagenesis and kinetic analysis

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Small molecule *O*-methyltransferases (OMTs) (E.C. 2.1.1.6.x) catalyze the transfer of the reactive methyl group of *S*-adenosyl-*L*-methionine to free hydroxyl groups of various acceptor molecules. Plant OMTs unlike their monomeric mammalian homologues exist as functional homodimers. While the biological advantages for dimer formation with plant OMTs remain to be established, studies with OMTs from the benzylisoquinoline producing plant, *Thalictrum tuberosum* showed that co-expression of 2 recombinant OMTs produced novel substrate specificities not found when each rOMT was expressed individually [Plant J. (1999) 17: 329-339] . These results were used to suggest that different OMTs could form heterodimers that confer novel substrate specificities not possible with the homodimer alone. The present study has describes a molecular model, based on the known X-ray structure of isoflavone OMT [Nature Structural Biology (2001) 8: 271-279], of the 16-hydroxytabersonine-*O*-methyltransferase (16OMT) from *Catharanthus roseus* that is involved in vindoline biosynthesis. Based on this model, site specific mutagenesis has been used to modify the substrate specificity and/or to inactivate 16OMT. Specific mutants were produced that could be used to study the biochemical properties of homodimers and heterodimers. Experimental evidence is provided to show that active sites found on OMT dimers do function independently and that bifunctional heterodimeric OMTs may be formed in vivo to produce a broader and more diversified range of natural products in plants.

P34

Structure and function relationship of plant asparaginases

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L-asparaginases are hypothesized to play an important role in nitrogen supply to sink tissues, especially in legume developing seeds. Two recombinant L-asparaginases have been characterized from *Arabidopsis thaliana*, corresponding to two subtypes of activity present in plants: a K^+-independent enzyme with dual activity for asparagine and β-aspartyl dipeptides, and a K^+-dependent enzyme strictly specific for asparagine. Kinetic analysis of two K^+-dependent L-asparaginases from *Phaseolus vulgaris* revealed that K^+ stimulates V_{max}, but also raises K_m for asparagine. Structural determinants of K^+-activation and substrate specificity will be studied by different approaches, including crystal structure determination, molecular modeling, analysis of chimeric enzymes and site-directed mutagenesis. Based on the crystal structure of a K^+-independent L-asparaginase from lupine, all residues mediating subunit interactions and participating in the active site are strictly conserved between the two *A. thaliana* enzymes. Experiments with chimeric enzymes will evaluate the function of a variable extension at the C-terminal of the α-subunit, which may restrict substrate accessibility at the active site. Candidate residues located at the active site that may bind the K^+ ion have also been identified.

P35

Precursor protease vesicles predict programmed cell death during anther dehiscence in tomato

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A variety of cysteine proteinases are common to programmed cell death (PCD) in plants, and some of these proteinases are compartmentalized within novel organelles, the precursor protease vesicles (PPVs), in an inactive state until they are required. A novel *Solanum lycopersicum* cysteine proteinase, SlCysEP , was identified and its gene cloned, sequenced, and characterized. *SlCysEP* exhibits all of the hallmarks of C1A cysteine proteinases. In addition, it has a C-terminal KDEL motif that mediates retrieval of peptides to the ER. SlCysEP exhibited strong *in vitro* proteolytic activity and underwent self-hydrolysis producing mature enzyme when subjected to an acidic pH. *SlCysEP* transcript and protein were detected at high levels in stamens of pre-dehiscent flower buds and dehiscing flowers but not in any other floral whorl. The pre-pro peptide was present as a zymogen in earlier stages prior to dehiscence and as a truncated mature enzyme during dehiscence. SlCysEP localized primarily to the stomium and the sporophytic cell layers surrounding the locules. Electron microscopy immunogold labelling revealed that cells expressing SlCysEP were undergoing developmental PCD and SlCysEP was compartmentalized within ER-derived PPVs which burst at later stages of PCD to release the enzyme into the cytoplasm.

P36

R50 (*sym16*): a pea nodulation mutant with a shoot not responding to cytokinins?

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R50 is a mutant of pea (*Pisum sativum* L. "Sparkle") with a high content of cytokinins (CKs) as measured by liquid chromatography / tandem mass spectrometry; the CK levels increase especially in the shoots as the plant ages. This CK accumulation is caused by a low activity of CK oxidase (the only enzyme known to degrade plant CK), the expression of which is altered in time and place when compared to the wild-type "Sparkle". R50 shoots exhibit a partial de-etiolation phenotype; furthermore, they display no response to ethylene (C_2H_4) whereas R50 roots are as sensitive to it as "Sparkle" roots. As well, R50 nodule organogenesis is responsive to C_2H_4 as nodulation is restored with C_2H_4 inhibitors. R50 nodulation phenotype is unique: the nodule primordium is restricted to only few periclinal divisions of the progenitor cells and the infection threads are meandering in the cortex as if they had lost their sense of direction. As a result, only few nodules have completed their development 21 days after inoculation. In this study, all the known traits of R50 are discussed in relation to the high levels of CK present in the shoots of a mature plant. We propose that R50 is a pea mutant, the shoots of which are insensitive to cytokinins.

P37

FRIABLE1 is a previously unknown mediator of cell adhesion in *Arabidopsis*.

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We have identified an *Arabidopsis* mutant, *friable1* (*frb1*), which exhibits defective cell adhesion. The reduced cell adhesion in *frb1* mutants leads to cell dissociation, and sometimes even organ dissociation. Paradoxically, ectopic cell adhesion leading to organ fusion is also observed in *frb1* seedlings. Although these phenotypes are often seedling lethal, a proportion of seedlings recover and grow to adulthood. In contrast to other cell adhesion defective mutants isolated so far, there is no obvious reduction in the pectic fraction of isolated cell walls. Measurement of total cell wall sugars indicates that there is not a significant change in the monosaccharide composition of both cellulose and matrix components. In addition, using comprehensive microarray polymer profiling (CoMPP) we show that the majority of the pectic epitopes in *frb1* walls are unchanged compared to wild-type. We have cloned *FRB1* and it encodes a membrane bound, plant specific protein with no similarity to known proteins. The subcellular location of the FRB1 protein is predominantly in the Golgi apparatus, suggesting that its molecular function might be enzymatic.

P38

Biotron – Plant Productivity Module

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The Biotron funded by Canadian Foundation for Innovation includes state-of-the-art environmental research facilities developed at the University of Western Ontario and the University of Guelph. The Plant Productivity Module is located at the University of Guelph. The research conducted in this module concerns the elucidation of the mechanisms underlying plant plasticity with respect to acclimation to and biomass production under environmental stress conditions typically associated with climate change such as increased CO2 concentrations, broad temperature fluctuations, drought, and poor nutrition. The knowledge obtained can be used not only to develop crops with enhanced capacity to adjust to and resist the stress effects of climate change but also to develop crops that exhibit the potential for higher yields under stress conditions.

The facility includes custom-designed, computer controlled environment growth chambers capable of providing temperatures ranging from - 20 to +40ºC, irradiance from complete darkness to 80% full sunlight, and CO2 from sub-ambient to 5000 µmol m-1. Unique features of these growth chambers are the presence of leaf and whole plant gas exchange systems, designed and developed by the Guelph group, which enable the continuous and non-invasive measurement of photosynthesis, growth and biomass production. In addition radio/mass isotope labelling capabilities permit studying carbon metabolism/partitioning. Contiguous with this facility is a central laboratory for biochemical analyses using instrumentation for separation and quantification of metabolites. The system design and development will be discussed.

P39

Functional characterization of HUA2 protein

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Coordinate control of processes that occur at the transition from vegetative to reproductive phase in plants are not well understood. *HUA2* has been shown to positively regulate two MADS box genes *FLOWERING LOCUS C (FLC)* affecting flowering time and *AGAMOUS (AG)* affecting floral patterning, thus having implications for the coordinated control of induction and maintenance of floral state. Our lab has previously demonstrated that the natural variant *HUA2-Sy-0* is unique, uncoupling the effects on *FLC* and *AG*. This suggests that *HUA2* affects *FLC* and *AG* responses via different mechanisms. To gain more insight into HUA2 function, potential HUA2 interacting proteins were identified by performing yeast two-hybrid screens. UBP1, RBP45 and AtPrp40 were identified to interact with HUA2. These interacting proteins are known to participate in eukaryotic splicing, suggesting that HUA2 functions inside the nucleus as a pre-mRNA processing factor. We have demonstrated that HUA2 affects splicing and 3' end processing of the *AG* primary transcript. My current aim is to characterize the interaction between HUA2 and its potential interacting proteins.

P40

Expression analysis of a subfamily of calmodulin-like genes in *Arabidopsis*

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Ca^{+2} is an important second messenger that regulates many aspects of plant development and stress response. Ca^{+2} is sensed by calcium-binding proteins, such as calmodulin (CaM). Plants also contain a multi-gene family (~50 members in *Arabidopsis*) of CaM-like proteins (CMLs). Many of these CMLs have been shown to be upregulated during stress response. We have created expression profiles using promoter-reporter fusion constructs of three CML genes (CML 37, 38 and 39) during both development and stress. Additionally, we have created 5' promoter deletion constructs in order to find *cis*-elements that are responsible for the unique expression patterns of these three genes. Our results indicate that the 5' promoter regions of these three genes contain regulatory elements that function during both development and stress.

P41

Cellulose degradation in *Medicago sativa* from increased accumulation of Cel5-CBM6 and Cel6B cellulases with an ELP tag

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Bioethanol production from high-yield, perennial crops is an area of great interest today, with the increasing demand for renewable fuel resources. Research is ongoing to explore and determine the most favourable way to degrade plant cellulose for efficient ethanol production. In contrast to past research where cellulase accumulation occurred in the chloroplast and cytosol, our intention is to accumulate cellulase in the endoplasmic reticulum. By incorporating an ELP tag and a KDEL retention signal in the transgene, more cellulose could accumulate than has previously been achieved. As well, expressing Cel6B exoglucanase *in plantae*, which has not been done before, may cause a synergistic effect with Cel5-CBM6 endoglucanase, increasing hydrolytic activity during cellulose degradation. Transient expression of Cel5-CBM6 and Cel6B in tobacco, as well as stable expression in alfalfa (*Medicago sativa*), should reveal any increased cellulase accumulation. The long-term goal of increasing cellulase production in high-yield forage crops, such as alfalfa, will benefit the biofuels and agriculture industries. As well, recombinant cellulase production in alfalfa will aid digestion of cellulose in ruminants, such as cattle, reducing nutrient and biomass waste.

P42

The absence of LHCII proteins alters the structure and function of PS II reaction centres in *Chlorina F2* barley mutant

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The *Chlorina F2* mutant of barley is severely impaired in Chl *b* synthesis and accumulation of Lhcb1 and Lhcb6 proteins. However, the abundance of PSII reaction center D1 protein is comparable to that in the WT. Immunoblots with a commercial phosphothreonine antibody indicated that D1 protein was phosphorylated in the WT. In contrast, this phosphothreonine antibody did not detect any phosphorylation of the D1 polypeptide in the *F2* mutant. However, *in vivo* and *in vitro* radiolabeling studies demonstrated that the D1 protein is indeed phosphorylated in both the WT and *F2* mutant. In addition, the D1 of the *F2* mutant was less susceptible to protease treatment than that of WT. Functionally, thermoluminescence measurements of PSII revealed that the ΔT_M for $S_2/S_3Q_B^-$ and $S_2/S_3Q_A^-$ charge recombinations was lowered from 16ºC for the WT to 3ºC for the *F2* mutant. This indicates a significant reduction in the activation energy for charge recombination in *F2* PSII reaction centres. We suggest that threonine phosphorylation sites of D1 in the *F2* mutant are not accessible to the phosphothreonine antibody but are accessible to labeling with ^{32}P indicating that D1 protein in the *F2* mutant undergoes either a specific folding or conformational change caused by the absence of LHCII polypeptides. The role of LHCII proteins in modulating the phosphorylation pattern of D1, its susceptibility to photoinhibition and the redox properties of PSII reaction center are discussed.

P43

Lipid polyester deposition and localization in developing seeds of *Brassica napus* and *Arabidopsis thaliana*

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Mature seeds of *Arabidopsis thaliana* and *Brassica napus* contain complex mixtures of aliphatic monomers derived from non-extractable lipid polyesters. Most of the monomers are deposited in the seed coat, and their compositions suggest the presence of both cutin and suberin layers. The location of these polyesters within the seed coat, and their contributions to seed coat permeability and other functional properties are unknown. Polyester deposition was followed over Brassica seed development and distinct temporal patterns of monomer accumulation were observed. Dissection and analysis of *Brassica* seed coats showed that suberization is not specific to the chalaza. Analysis of the Arabidopsis *ap2-7* mutant suggested that suberin monomers are preferentially associated with the outer integument. Several Arabidopsis knockout mutant lines for genes involved in polyester biosynthesis (*att1*, *fatB* and *gpat5*) were examined for seed monomer load and composition. The variance in polyester monomers of these mutants is correlated with dye penetration assays. Furthermore, stable transgenic plants expressing promoter::*YFP* fusions showed *ATT1* promoter activity in the inner integument, whereas *GPAT5* promoter is active in the outer integument. Together, the Arabidopsis data indicated that there is a suberized layer associated with the outer integument and a cutin-like polyester layer associated with the inner seed coat.

P44

Bioavailability of metals in urban garden soils: a proposal

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Very few studies have combined the effects of metal speciation, plant species and soil characteristics on the bioavailability of metals. The aim of this study is to determine the effects of a number of soil properties on metal uptake in edible crops. The goal is to assess the need for soil-specific and/or plant-specific guidelines for the acceptability of soils for agriculture. Four soils were analyzed for metal content, pH, conductivity, texture, and organic matter content. Concentrations of Pb were more than 2 times the CCME (Canadian Council of Ministers of the Environment) limit in two of the soils; one of these contained 6 times the limit for Cu while the other contained a slight excess of Zn. In the third soil, Cd was 1.6 times the CCME limit. Concentrations of all measured elements in the fourth soil were below the CCME limits. In addition to determining the relationships between soil characteristics and the uptake of metals into crops, the organic matter in these soils will be manipulated to better assess the importance of this variable to metal bioavailability. While this project alone will not result in a complete model for the uptake of metals by plants, it will contribute to the research of MITHE-SN (Metals in the Human Environment Strategic Network) under the theme of Soils and Plants.

P45

High-resolution monitoring of stem extension allows for very early detection of exposure to *Fusarium oxysporum f.sp. radicis-cucumerinum* in *Cucumis sativus*

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The causal agent causing stem and root rot in cucumber *(Cucumis sativus* L.*)* is *Fusarium oxysporum f.sp. radicis-cucumerinum* (FORC). This disease has become a major root disease in greenhouse cucumber particularly where re-circulating hydroponics systems have been implemented. Reliance on the wilting symptom, the major visual symptom of the disease, often is not adequate in disease control since the disease may be well established before this symptom first appears. The objective of this study was to determine if very early detection (i.e. within a day after exposure) of this pathogen is possible. Physiological parameters including stem length and leaf area were monitored over time, and the stem elongation rate (SER) was monitored using high-resolution rotary sensors to determine how quickly these parameters are affected after exposure to FORC culture and culture filtrate. Data were collected beginning after two-week-old plants were exposed to the pathogen or culture filtrate until severe wilting symptoms become evident. There was a significant decrease in the SER after only six hours for plants inoculated with FORC culture, and after only eight hours for plants exposed to culture filtrate only. Thus, very early detection for FORC exposure in cucumber is possible. Also, a compound in the culture filtrate can elicit the decrease in SER.

P46

Protein purification in plants utilizing small molecule-dependent inteins and elastin-like polypeptides

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Traditional protein purification methods are prohibitively expensive at the industrial scale. As therapeutic peptides demonstrate their potency against human disease, a strong impetus towards commercialized production emphasizes the development of novel purification methods. Elastin-like polypeptides (ELP) are biopolymers composed of unique pentapeptide repeats of Val-Pro-Gly-Xaa-Gly, where Xaa can be any amino acid excluding proline. This unique hydrophobic pentapeptide can undergo inverse phase transitioning; exceeding a specific temperature causes ELPs to form highly-structured insoluble polypeptides. This reversible process presents a chromatographic alternative to initially separate a targeted protein from the total soluble protein. Inteins are protein splicing elements that can catalyze their self-excision from a precursor polypeptide. Modified inteins abolish the requirement for costly proteases used to cleave protein purification tags from a targeted protein. Both intein and ELP functionality have been separately demonstrated in plants, which are being developed as affordable bioreactors for the production of valuable therapeutic proteins. A plant model system is proposed that simultaneously utilizes ELPs and small-molecule dependent inteins to isolate and release the targeted protein, leading to high-yield commercial production of therapeutic proteins.

P47

Involvement of cellular membrane traffic proteins in the formation and translocation of the Soybean mosaic virus replication complex in plants

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Virus replication in plant cells takes place in a so-called virus replication complex (VRC). To study virus replication mechanisms, the *Soybean mosaic virus* (SMV) 6K2 membrane protein, a putative critical protein of VRC was fused with the fluorescent protein. The fusion proteins were found to be associated with the endoplasmic reticulum (ER) network and vesicular compartments. The 6K2-induced vesicles were 2–10 μm in diameter and motile within cells. At the later stage after transfection, these vesicles were also found in adjacent epidermal cells, raising a possibility that the 6K2 vesicles in the primary cells may have ability to move to adjacent cells through plasmodesmata. The core components of the cellular vesicle trafficking machinery including ADP-ribosylation factor 1 (ARF1) and Sec22, were co-localized with the 6K2 vesicles, suggesting that the ARF or Sec22 proteins may play a role in anchoring the VRCs to the ER, and therefore, may be involved in virus replication. Furthermore, the formation of the 6K2 vesicles was blocked by ARF1 inhibitors, brefeldin A, and by mutations that hamper Arf1-GTPase activity GDP. Their motility was arrested by inhibitors of filamentous actin, but not by inhibitors of microtubules. These data may help identify the cellular pathways associated with the formation and translocation of VRC in plants.

P48

Investigating possible SA and ABA crosstalk in the lesion mimic mutant *cpr22*

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The *cpr22* mutant is an *Arabidopsis* lesion mimic mutant exhibiting spontaneous cell death. The *cpr22* plant constitutively expresses the pathogenesis related genes *PR-1*, *PR-2* and *PR-5*, has elevated levels of salicylic acid (SA) and displays an enhanced resistance to *Hyaloperonospora parasitica* Emco5. *cpr22* was mapped and shown to have a deletion in a cluster of cyclic nucleotide-gated ion channel genes, resulting in an in-frame chimeric fusion of the genes *AtCNGC11* and *AtCNGC12*. When *cpr22* plants were grown in high relative humidity, all the above phenotypes were suppressed. Crosses between *cpr22* and nahG transgenic plant revealed that these suppressed phenotypes were SA dependant. To investigate this environmental sensitivity further, transcriptome analyses were conducted to assess changes in gene expression after a 24 hour shift from a high relative humidity of 95 percent to a lower relative humidity of 65 percent. Abscisic acid (ABA) biosynthetic genes and early signaling genes were up-regulated in response to this abiotic stress, but downstream ABA-inducible marker genes were down-regulated. Furthermore, we have shown that *cpr22* has an impaired ability to respond to ABA induced dormancy in germination assays, have an accelerated loss of fresh weight during dehydration, and fail to express the ABA and drought/cold stress marker gene *RD29A* during drought conditions.

P49

Identification and characterization of a plastid-localized *Arabidopsis* glyoxylate reductase.

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Recently, recombinant expression of a cytosolic enzyme from *Arabidopsis* (designated as glyoxylate reductase 1 or *At*GR1) revealed that it effectively catalyzes the *in vitro* reduction of both glyoxylate and succinic semialdehyde (SSA). Through web-based bioinformatics we identified a second putative GR cDNA (designated as *AtGR2*), which is 57% identical on an amino acid basis to GR1. Fluorescence microscopic analysis of tobacco suspension cells transiently transformed with GR1 or GR2 linked to the green fluorescent protein revealed that GR1 was localized to the cytosol, whereas GR2 was localized to plastids via targeting information contained within its N-terminal 45 amino acids. Subsequent production and purification of soluble recombinant GR2 protein in *Escherichia coli* required deletion of its putative targeting signal and co-expression with the molecular chaperones GroES/EL. Kinetic analysis revealed that recombinant GR2 catalyzed the conversion of glyoxylate to glycolate (K_m glyoxylate = 34 μM), and SSA to γ-hydroxybutyrate (K_m SSA = 8.96 mM) via an essentially irreversible, NADPH-based mechanism. GR2 had a 350-fold higher preference for glyoxylate than SSA. The identification and characterization of distinct plastid and cytosolic glyoxylate reductase isoforms should contribute to our understanding of aldehyde detoxification and the plant stress response.

P50

Methods of plant growth promotion by *Burkholderia phytofirmans* PsJN

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Bacterium *Burkholderia phytofirmans* PsJN has been previously shown to promote plant growth. Here we examine several methods of plant growth promotion to identify the mechanisms utilized by PsJN. More specifically, we show that this bacterium produces siderophores, IAA, and the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Following isolation of the ACC deaminase gene (*acdS*), an AcdS deficient mutant of PsJN was generated. This mutant lost a portion of its ability to promote plant growth as observed in growth pouch assays with canola seeds. Interestingly, the $AcdS^-$ mutant synthesized a decreased level of siderophores and an increased amount of IAA. The ability of wild-type PsJN and its $AcdS^-$ mutant to promote plant growth in a variety of different conditions is currently being examined.

P51

A precursor protease vesicle-localized cysteine proteinase, SlCysEP, is expressed during post-germinative programmed cell death of tomato endosperm

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The post-germinative death of endosperm cells following reserve mobilization in seeds of *Ricinus communis* L. and *Solanum lycopersicum* L. cv. 'Glamor' has been characterized as being genetically programmed. In both instances, programmed cell death (PCD)-specific organelles, ricinosomes or precursor protease vesicles (PPVs), form in cells where death is imminent. These organelles are derived from the endoplasmic reticulum and accumulate a KDEL-tailed cysteine proteinase, known as CysEP or SLCysEP. Upon vacuolar collapse as the cell dies, the cytosol becomes acidified causing the enzyme to be released into the cytoplasm and autocatalytically activated. The enzyme then degrades any remaining protein in the cell corpse, facilitating recycling of amino acids to the growing seedling. Tomato SlCysEP (Accession EU122386) is involved in several instances of developmental PCD in the plant. Here, both the expression and accumulation of SlCysEP in germinating and post-germinative tomato seeds was characterized. SlCysEP is contained in PPVs prior to endosperm cell death, and both the accumulation of the enzyme and cell death are promoted by gibberellic acid, cytokinin, and ethylene in the endosperm. The presence of the putative mature form of the enzyme is a reliable marker for some instances of PCD in tomato. This work sets the stage for a determination of the regulatory mechanisms controlling PCD in plants.

P52

The utility of the constitutively active cyclic nucleotide gated ion channel, ATCNGC11/12, in elucidating functionally important residues in CNGCs

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Cyclic nucleotide gated ion channels (CNGCs) are non selective cation channels which were first identified in animal retinal photoreceptors and olfactory sensory neurons. CNGCs in the *Arabidopsis* genome make up a large family consisting of 20 members. The large size of this gene family in plants suggests CNGCs play a diverse and important role in many physiological functions. In addition to the 20 wild type CNGC genes, a chimeric CNGC, *ATCNGC11/12*, has been identified in the mutant *cpr22*. This channel is constitutively active, resulting in striking mutant phenotypes. We have utilized the novel chimeric CNGC to investigate not only the cause of the chimeric's aberrant function but also the structural and functional characteristics of CNGCs in general. Here we show the utility of a suppressor screening of the *cpr22* mutant and site directed mutagenesis of ATCNGC11/12 to identify functionally important residues in this channel. Through bioinformatics, protein modeling, hypersensitive response analysis, and mutant yeast complementation experiments, we have demonstrated the requirement of several amino acids responsible for ATCNGC11/12's aberrant activity as well as for general CNGC function.

P53

Molecular mapping of genes involved in the phenylpropanoid pathway in common bean (*Phaseolus vulgaris* L.)

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Previous genetic analyses identified 15 genes that control seed coat pattern and color in common bean (*Phaseolus vulgaris* L.). Some of these genes have been positioned on the common bean linkage map. It has been hypothesized that genes involved in the phenylpropanoid pathway correspond to some of the classical seed coat color genes in bean. In a previous study we cloned and sequenced fragments of thirty-five phenylpropanoid pathway genes from common bean. The purpose of the current work is to map the positions of these genes on the common bean linkage map and determine whether their position correspond to any of the loci for classical seed coat color genes. The mapping population that was used consisted of recombinant inbred (RI) lines derived from a cross between 'BAT 93' and 'Jalo EEP558'. Polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLP) were identified for the phenylpropanoid gene sequences between parental lines. The segregation patterns of 20 phenylpropanoid pathway genes have been analysed in the RI population and their locations in the bean linkage map were determined by a JoinMap analysis. The additional genes in this pathway will be mapped in a similar way and cosegregation between phenylpropanoid and classical seed coat color genes will be tested.

P54

Correlating phenotype with mRNA and protein expression levels in *Arabidopsis hothead* mutants

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The ***Arabidopsis*** organ fusion gene, *HOTHEAD (HTH),* was initially defined by 11 mutant alleles. Plants harboring any of these 11 recessive alleles show contact-meditated organ fusion, which is often symptomatic of plants that in some way perturb epidermal function. Contrary to expectation, previous work demonstrated that the wild type *HTH* gene is expressed ubiquitously. Evidence from reporter gene analyses, however, suggests that expression is much more limited and is predominantly epidermal. In an effort to resolve this issue and expand upon these findings, we have initiated a series of experiments to look at the (1) phenotypic variation between mutants, (2) expression of both *hth* mRNA and (3) Hth protein in mutant and wild-type alleles. Preliminary analyses reveal that the introduction of a stop codon results in mutant plants with markedly reduced germination rates. Preliminary protein analyses suggest that no detectable Hth protein is made in these mutants. Introduction of a splice junction mutation, however, appears to result in the synthesis of multiple protein variants. Subtle amino acid changes, on the other hand, give rise to mutants that show only weak organ fusion. Using these molecular and genetic approaches we hope to gain a better understanding Hth protein function.

P55

Isolation and identification of gene responsible for microspore embryogenesis in *Brassica napus* L.

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Double haploid production from microspore (pollen) culture has greatly enhanced the efficiency of plant breeding for a large number of species including corn and canola. However, genotypes within these species vary in their ability to produce embryos from microspores. Thus, an understanding of the key processes that drive embryogenesis will enhance the utility of double haploid production for plant breeding. Gene expression studies conducted at the University of Guelph have already identified a number of genes that were significantly up-regulated in responsive embryogenic cells (Chan 2006; Pauls et al, 2006). An unknown function gene with high level of expression in embryogenic microspores has been isolated from buds and microspore culture of *Brasicca napus*. The protein coded by this gene has a C2 domain (Ca2+-dependent membrane) and has been shown by previous work to be targeted to chloroplast thylakoid membrane. The complete gene was isolated and sequenced from *Brassica naups*. Polymorphisms in the sequences suggest that this gene is present in at least two forms in *B. napus*. Over-expression and RNA silencing will be used to examine the function of the gene in *B. napus*.

P56

Corn (*Zea mays*) plant growth promotion by nitrogen fixing bacterium *Gluconacetobacter diazotrophicus*

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Corn (*Zea mays*) is an important crop cultivated world wide. *Gluconacetobacter diazotrophicus* is an endophytic diazotroph which can fix nitrogen and produce plant growth regulators. In this study, we evaluated the colonization and plant growth promotion effect after the introduction of *G. diazotrophicus* into corn plant. Seventeen Canadian grain corn varieties and ten sweet corn varieties were inoculated with *G. diazotrophicus* strains PAL5 and *nifD::aph* mutant MAD3A. Colonization under different nitrogen fertilization conditions was evaluated. *G. diazotrophicus* colonized nineteen corn varieties after inoculation. The colonization efficiency varied among different corn varieties. Plant growth promotion was observed in one grain corn variety and two sweet corn varieties. There was no significant difference between the PAL5 inoculated plants and MAd3A inoculated plants, indicating plant growth promotion was not a result of nitrogen fixation and probably by plant growth regulators produced by the bacterium. Nitrogen fixation in corn plant by *G. diazotrophicus* is being studied in the laboratory and in fields with the management of N supply.

P57

Improvement of European plum (*Prunus domestica* L.) regeneration and transformation

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European plum is one of the commercially important *Prunus* (or stone fruit) species. In this research work, regeneration protocol is optimized for European plum cultivars to improve transformation. The developed regeneration efficiency for hypocotyls in this work was 43.6%. The optimized regeneration protocol was later used for transformation. An average of 6.12% transformation efficiency was achieved by the use of newly developed regeneration system. This is a significant improvement in the transformation efficiency as previously reported efficiency was only 1.2%. The regeneration and transformation obtained in the study may be applied to other European plum cultivars to improve transformation efficiency.

P58

Manipulation of γ-hydroxybutyrate and redox levels in Arabidopsis by abiotic stress is associated with induction of glyoxylate reductase isoforms

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Cytosolic and plastid glyoxylate reductase isoforms from *Arabidopsis* (designated hereinafter as *At*GR1 and *At*GR2, respectively) catalyze the *in vitro* conversion of succinic semialdehyde to γ-hydroxybutyrate (GHB), as well as glyoxylate to glycolate, via NADPH-dependent reactions. In the present report, the responses of GHB and related amino acids, as well as $NADP^+$ and NADPH, and *GR1* and *GR2* transcripts were monitored in rosette leaves from *Arabidopsis* plants subjected to various abiotic stresses (i.e., submergence, salinity, drought, cold and heat). Metabolite levels were measured by high performance liquid chromatography with on-line detection by either fluorescence or mass, whereas pyridine nucleotides were measured via an enzyme-cycling assay. Time course experiments revealed that GHB accumulated in response to stress, and that this accumulation was generally accompanied by higher (-aminobutyrate and alanine levels, higher NADPH/$NADP^+$ ratio, and lower glutamate level. Furthermore, analysis of gene expression by quantitative real-time reverse transcription-polymerase chain reaction revealed that the relative abundance of *GR1* (submergence, salinity, drought, cold and heat) and *GR2* (cold and heat) transcripts was enhanced by the abiotic stresses tested. Thus, it can be concluded that the accumulation of GHB, like (-aminobutyrate, is a universal response to abiotic stress, and that GR1 and GR2 are probably involved in SSA detoxification *in planta*.

P59

The Use of Material Transfer Agreements in Biotechnology in Canada

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One issue that is crucial to facilitate the unlocking of the potential of research in biology is the management of Intellectual Property Rights and the smooth administration of material transfers between laboratories and developers. Innovation and commercialisation, moving from the idea in the laboratory to implementation in the market, depends on a clear understanding by all parties of the rights and limitations that may be placed on research materials, and that may restrict or enable their future commercialisation and exploitation.
In an increasingly complex intellectual property landscape for biology, patent awareness and portfolio management are issues in which all researchers and commercialisation groups need more exposure. Here we show a preliminary analysis of the results of a survey of Material Transfer Agreements (MTAs) solicited from universities, government research centres, and private industry in Canada. The MTAs show considerable variation, for example: the right to commercialisation (or how to deal with arising patent rights); whether attribution to the provider of the material is required; and even prohibition of testing on human subjects. Many different terms are utilised to describe the above parameters. We illustrate comparisons between the MTAs in use. The data are also illustrative of the 'terms' and variations, of which the laboratories, innovators, and commercialisation groups need to be aware.

P60

Brassinosteroid, abscisic acid and calcium: determining the connection

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Brassinosteroids (BRs) are a new class of hormones that are essential for growth and development of plants. BRs also protect plants from various abiotic and biotic stresses, but the underlying mechanism by which BRs induce this broad-range stress tolerance is poorly understood. We have demonstrated that exogenous treatment of *Arabidopsis thaliana* and *Brassica napus* seedlings with 24-epibrassinolide (EBR), confers tolerance to a range of abiotic stresses like high and low temperatures, high salt and drought. Both targeted and global analyses of gene expression in EBR-treated and untreated seedlings have indicated that EBR upregulates the expression of several genes including calmodulin-like calcium-binding proteins, genes involved in abscisic acid (ABA) biosynthesis, and genes responsive to ABA. To further address the relationship between BR and ABA, we have studied the effect of EBR on heat stress responses of *A. thaliana* ABA-deficient (*aba1-1*) and ABA-insensitive (*abi1-1*) mutants. Our results indicate that EBR treatment promotes basic thermotolerance in both ABA-deficient and insensitive mutants, and promotes higher accumulation of heat shock proteins (hsps) in the *aba1-1* mutant *vs* wild type and *abi1-1* seedlings. Since Ca++/calmodulin appear to play a role in ABA signaling, BR biosynthesis and in sensing and transducing environmental stimuli, we are studying the stress phenotypes of T-DNA insertion mutant lines of a subset of calmodulin-like calcium-binding proteins that were identified in the microarray screen. The hormone-dependent expression of these genes and stress phenotypes of their knockout mutant lines will be presented. Studies aimed at testing the role of these genes in relation to BR, ABA and abiotic stress signaling will be discussed.

P61

Phytoremediation of a contaminated groundwater site.

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Phytovolatilization is a new and emerging technology in which contaminants are taken up into a plant and are transpired into the atmosphere where they are often degraded. The suspected carcinogen 1,4-dioxane is used as a chlorinated solvent stabilizer. It is a heterocyclic organic compound with the molecular formula $C_4H_8O_2$ that is quickly degraded by UV radiation. To investigate the removal of the contaminant, 1,4-dioxane, from a groundwater plume by *Populus balsamifera, P. deltoides x nigra, P. nigra x maximowicii* (Poplar), and *Salix nigra* (Willow), the concentration of 1,4-dioxane from the transpiration stream was determined. The amount of this chemical that is transpired per day by individual trees of each aforementioned species was calculated and is reported. Comparisons between the tree lines effectiveness at removal of 1,4-dioxane are made. These results will allow for more effective future planning of phytoremediation sites contaminated with 1,4-dioxane or similar contaminants.

P62

Characterization of transpiration rates of poplar and willow trees.

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Phytoremediation is an emerging green technology which holds promise in effective long-term remediation of contaminated groundwater plumes. In order to better develop this technology it is important to characterise transpiration abilities of various tree lines throughout a growing season to better understand which of these are best suited for effective phytoremediation. Transpiration rates of 4 year old individual poplar (*Populus balsamifera, P. deltoides x nigra, P. nigra x maximowicii*) and willow (*Salix nigra*) trees were studied using the Flow 4-DL Sap Flow Logger (Dynamax, Houston, TX) throughout one growing season. The Flow 4-DL sensors are non-intrusive collars that measure the heat carried by sap which is translated by the logger into real-time sap flow measurements. Comparisons are made between lines of trees (species and hybrids) to determine which have higher transpiration rates, and thus are more effective remediators, as well as to determine whether traits such as drought tolerance, i.e. in *P. deltoides x nigra*, confer any advantages in phytoremediation applications. While drought tolerance may confer an advantage in establishment, it may cause lower transpiration rates and thus negate its effectiveness in phytoremediation.

P63

Biosynthesis of potato (*Solanum tuberosum* L.) suberin: The ω-hydroxylation of fatty acids

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Suberin is a complex plant biopolymer deposited between the cell wall and plasma membrane in response to developmental and environmental signals (i.e. wounding). Suberin is composed of two distinct domains: a poly(aliphatic) domain derived from fatty acid metabolism; and a poly(phenolic) domain derived from phenylpropanoid metabolism. Using wound healing potato (*Solanum tuberosum* L.) tubers as a model system, we have used chemical and biochemical approaches to map out a logical biochemical route to the formation of key suberin monomers and identify critical steps. To understand better the co-ordinate regulation of suberin-associated aliphatic and phenolic metabolism, first we must characterize the key enzymes involved in suberin biosynthesis. In the poly(aliphatic) domain of potato wound suberin, 55% of monomers undergo ω-hydroxylation to produce either ω-hydroxylated fatty acids or α,ω-dioic acidsTwo Cytochrome P450 enzyme subfamilies composed of w-hydroxylases, CYP86A and CYP94A, have been described previously. Using an *in silico* approach, we identified three putative ω-hydroxylase sequences in the Potato Genomic Gene Index database (TIGR) based on consensus sequence homology: two CYP86A-type and one CYP94A-type. These were subsequently cloned from potato. Gene specific primers were designed and used to examine tissue expression of all three putative ω-hydroxylases by RT-PCR analysis. Transcripts of all three putative ω-hydroxylases were expressed in suberizing tissue to varying degrees. Currently, expression of recombinant protein in *Escherichia coli* is ongoing to help functionally characterize these enzymes. Since ω-hydroxylation in wound-healing potato tubers is a suberin specific metabolic step, understanding the regulation of the enzyme(s) that catalyzes this reaction would provide a critical tool to study the regulation of suberization as a whole.

P64

Purification and characterization of three secreted purple acid phosphatase isoforms from phosphate-starved *Arabidopsis thaliana* suspension cells

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Phosphate (Pi) is one of the least available macronutrients in most ecosystems. Most soil phosphorus exists as P-esters unavailable for root uptake until Pi hydrolysis by secreted acid phosphatase (APase) occurs. The cell culture filtrate (CCF) secretomes of Pi-fed (+Pi) versus Pi-starved (-Pi) Arabidopsis suspension cells were initially compared using a 2D-PAGE/proteomics approach. Twenty spots were upregulated ≥2-fold by the –Pi cells, including the Pi-scavenging RNase1 and various defense/detoxifying enzymes. APase activity assays and immunoblotting indicated the presence of secreted APases in the CCF of –Pi, but not +Pi cells. Three purple APase (PAP) isoforms were subsequently purified from the –Pi CCF and identified by MALDI-TOF MS. They corresponded to a homodimeric AtPAP12 (At2g27190, 65-kDa subunits), along with the unprecedented discovery of two differentially glycosylated and kinetically distinct homodimers of AtPAP26 isoforms (At5g34850, 55-kDa subnits). Each final preparation exhibited a pink colour in solution and broad substrate specificity. We hypothesize that their combined activities allows –Pi Arabidopsis to scavenge Pi from extracellular P-esters over a wide pH range. N-terminal sequences of both secreted AtPAP26s were identical to that of the vacuolar AtPAP26 of –Pi Arabidopsis (2006 Plant Physiol 142:1282). Work is in progress to address the functional and structural basis for the differentially targeted and glycosylated, and kinetically distinct, vacuolar and secreted AtPAP26 isoforms of –Pi Arabidopsis.

P65

Functional analysis of AtPAP12 and AtPAP26, the predominant intracellular and secreted purple acid phosphatases upregulated by phosphate-deprived *Arabidopsis thaliana*

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Agricultural phosphate (Pi) deficiency is alleviated by the massive, but inefficient and unsustainable application of Pi fertilizers. The projected depletion of global rock-Pi reserves by 2100 has prompted scientists to develop strategies for engineering Pi-efficient crops. This necessitates a thorough understanding of the intricate adaptations of Pi-deprived (-Pi) plants which include *de novo* synthesis of purple acid phosphatases (PAPs). PAPs are believed to catalyze Pi hydrolysis from a wide range of intracellular and extracellular (soil) P-esters. We recently identified AtPAP12 and AtPAP26 as the predominant PAPs upregulated by –Pi Arabidopsis. Both isozymes appear to be dual-targeted (to the cell vacuole and secretome/extracellular milieu) during Pi stress. To test the hypothesis that AtPAP12 and AtPAP26 are pivotal to intra- and extracellular Pi scavenging by -Pi Arabidopsis, the phenotypic, biochemical and molecular features of loss-of-function transgenic Arabidopsis are being assessed. Homozygous T-DNA insertional mutants for *AtPAP26* (GK144B01 & SALK152821) and *AtPAP12* (GK151C09) were established by their germination on selective media, followed by PCR verification of T-DNA insert location. Preliminary studies indicated that *AtPAP12* and particularly *AtPAP26* knockouts demonstrate markedly impaired growth (relative to Col-0 wild-type) when cultivated under –Pi, but not Pi-sufficient conditions. Double mutants of *AtPAP12* and *AtPAP26* will be created by crossing the knockout lines to study possible synergistic effects on –Pi seedlings.

P66

Identification of phosphorylated thylakoid membrane proteins of *Chlamydomonas raudensis* UWO 241.

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Chlamydomonas raudensis UWO 241 is a photosynthetic green alga isolated from the permanently ice-covered Lake Bonney in Antarctica. This unique psychrophile is adapted to an extremely stable environment of low temperatures and low irradiance. Previous studies have shown that *C. raudensis* UWO 241 is the first natural variant deficient in the state transition response. This Antarctic strain is locked and state I and unable to redistribute light energy among photosystem I and photosystem II. In addition, *C. raudensis* UWO 241 exhibits a unique phosphorylation profile with phosphorylation of a group of high molecular mass polypeptides on threonine residues and the absence of phosphorylation of typical photosystem II – related polypeptides. Thus, it is hypothesized that this Antarctic strain may exhibit phosphorylation of photosystem I. In order to identify these phospho-proteins and the complexes from which they originate, two dimensional blue native polyacrylamide gel electrophoresis (BN-PAGE) was used. Immunoblot analysis of the second dimension with phospho-threonine antibodies revealed the phosphorylation of a large (130-170 kDa) complex closely associated with photosystem I and a 43 kDa protein associated with the PSI core complex. To further examine whether phosphorylation is related to photosystem I, fractionation of thylakoid membrane complexes was performed by sucrose density gradients. Subsequent studies will investigate whether these phospho-proteins originate from photosystem I using a purified fraction of this complex.

P67

***In vivo* multi-site phosphorylation of bacterial-type phospho*enol*pyruvate carboxylase from developing castor oil seeds**

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Most native plant phospho*enol*pyruvate carboxylases exist as ~440-kDa homotetramers (Class-1 PEPC) that are activated by phosphorylation at a conserved N-terminal seryl residue of their plant-type PEPC subunits. The novel ~910-kDa Class-2 PEPC heterooligomeric complex of developing castor oil seeds (COS) arises from a tight interaction between Class-1 PEPC and enigmatic 118-kDa bacterial-type PEPC polypeptides (p118). Pro-Q Diamond staining and phosphate-affinity PAGE established that co-immunopurified p118 was phosphorylated at multiple sites. Conditions for its *in vitro* dephosphorylation by exogenous phosphatases were optimized. LC MS/MS of tryptic peptides identified Thr^{5} and Ser^{425} as p118 phosphosites. Neither site corresponds to known protein kinase motifs. Ser^{425} phosphorylation was recently confirmed by p118 immunoblotting with anti-(phosphosite specific)-IgG. This and analogous IgG raised against the Thr^{5} phosphosite will be used to assess the influence of COS development and photosynthate supply on p118 phosphorylation. Yet a third phosphosite was suggested by p118's cross-reaction with anti-(phospho Ser/Thr Akt)-IgG; Ser^{879} is a promising candidate as unlike Thr^{5} or Ser^{425} it is within both an Akt recognition motif, as well as a conserved motif for SNF1-related protein kinases. The discovery of multi-site p118 phosphorylation adds another layer of complexity to COS PEPC biochemistry. The *in vivo* function(s) of p118 phosphorylation is currently unknown, but is hypothesized to contribute to the control of COS photosynthate partitioning to storage proteins versus lipids.

P68

Heterologous expression and characterization of recombinant bacterial- and plant-type phospho*enol*pyruvate carboxylases from developing castor oilseeds

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The triglyceride-rich endosperm of developing castor oilseeds (COS) contains two classes of phospho*enol*pyruvate carboxylase (PEPC). Class-1 PEPC is a typical 410-kDa homotetramer of 107-kDa (p107) subunits, whereas the novel Class-2 PEPC ~910-kDa hetero-octameric complex arises from an interaction between Class-1 PEPC/p107 and enigmatic bacterial-type PEPC 118-kDa polypeptides (p118). Distinctive developmental profiles and kinetic properties led to the hypotheses that: (i) Class-1 and Class-2 PEPC respectively support PEP flux required for storage protein versus oil synthesis, and (ii) p118 functions as a regulatory subunit that desensitizes Class-2 PEPC to allosteric effectors. *In vivo* p107 and p118 phosphorylation occurs and may contribute to the control of COS photosynthate partitioning. cDNAs encoding p107 and p118 were cloned into the pET28b vector and overexpressed in *E. coli* as His-tagged fusion proteins. Immunoblotting with specific antibodies revealed presence of recombinant, non-proteolytically truncated p107 and p118 in resulting soluble and insoluble fractions. Soluble p107 and p118 both displayed PEPC activity. Further optimization of soluble p107 and p118 expression is in progress. Kinetic properties of purified recombinant PEPCs will be assessed. *In vitro* reconstitution of a Class-2 PEPC complex is being attempted, along with further characterization of the interaction of p107 with p118 using various biophysical tools. Recombinant p118 also represents a valuable substrate for determining the activity of its corresponding protein kinases in COS extracts.

P69

Cellulose synthase-like D protein is required for root hair morphogenesis and root nodule symbiosis in *L. japonicus*.

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In many legumes, including *Lotus japonicus*, susceptible root hairs are the primary sites for the initial physical contact between the host plant and compatible nitrogen-fixing bacteria. This leads to the initiation of root invasion and nodule organogenesis. To clarify the significance of root hairs during the *L. japonicus-Mesorhizobium loti* symbiosis, we have map-based cloned and performed detailed analysis of previously isolated *L. japonicus short (Ljsrh1)* and *variable (Ljvrh1) root hair* developmental mutants (1). In addition to root hair developmental defects, we found that both mutant lines had significantly delayed nodulation, with considerable differences in the type and number of nodules formed. The observed functional complementation during allelism tests indicated that *Ljsrh1* and *Ljvrh1-1* represented two loci. However, map based cloning revealed that both mutant lines carried lesions in the same *Cellulose Synthase-Like D* gene (*LjCslD*). This result provide for the first time an unequivocal genetic evidence for homodimerization of cellulose synthase-like D proteins. The results of follow up experiments with nine newly-identified alleles of *LjCslD* gene will be presented.

P70

HUA2 interacts with FCA and is required for accumulation of FCA-γ transcript

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Shoot development in plants progresses continuously during postembryonic development through initiation of primordia that can give rise to either vegetative or reproductive structures. Determination of primordial fate depends on both endogenous and environmental signals resulting in highly plastic shoot morphology adapted to specific environmental conditions. In *Arabidopsis thaliana* a complex network has evolved to integrate endogenous and environmental signals. *FLOWERING LOCUS C* (*FLC*), a floral repressor, integrates inputs from genes involved in vernalization, autonomous pathways and various activators of *FLC* expression that include *HUA2*. Autonomous pathway genes *FCA* and *FY*, form a complex to suppress the activity of *FLC*. It was shown previously that FCA and FY interact through FCA-WW and FY-PPLP domains. HUA2, an activator of *FLC*, has C-terminal end (CT-HUA2) with five PPLP repeats, suggesting that it may interact with proteins containing WW domain. Since HUA2 and FCA contains compatible interaction domains, and both of them affect the same downstream gene, *FLC*, we investigated whether HUA2 and FCA interact. We demonstrated that HUA2 interacts with FCA. We showed that CT-HUA2 is required and sufficient for interaction with FCA-WW domain. We further identified that *HUA2* is required for the expression of *FCA*-γ transcript. We also showed that *HUA2* is required for the late flowering of *fca-1* mutant plants. Therefore, putative interaction between HUA2 and FCA might be biologically significant.

P71

SYMRK receptor kinase reveals the evolutionary roots of symbiosis

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Plant leucine-rich repeat-receptor like kinases (LRR-RLKs) connect the plant cell to its environment; outside stimuli are perceived by the extracellular LRR domain and are transformed into cellular responses by the intracellular kinase. SYMRK is a LRR-RLK required for the symbiotic association of legumes with nitrogen-fixing *Rhizobium* bacteria and phosphate-acquiring arbuscular mycorrhizal fungi. Here, we present a new *symRK* allele with a novel symbiotic phenotype: normal nodulation and blocked or aborted rhizobial and mycorrhizal infection. The *symRK-14* mutation causes a single amino acid change, P to T, in the GDPC motif just upstream of the LRR region. To investigate the origin of the GDPC motif and its role in LRR-RLK function, we performed a comprehensive survey of LRR-RLKs from the fully-sequenced genomes available. Our phylogenetic analysis revealed that the GDPC motif is highly-conserved in LRR-RLKs of evolutionarily-divergent land plants, including moss and liverwort, but is absent from those of more distant plant relatives such as algae and volvox. Given the requirement of the GDPC motif for root endosymbiosis and the fact that mycorrhizal symbiosis coincides with the appearance of land plants, these findings indicate that the GDPC motif may have been crucial for the evolution of both arbuscular mycorrhizal symbiosis and the transition of plants to a terrestrial lifestyle. Interestingly, we found the GDPC motif in more than 1/4 of *Arabidopsis* LRR-RLKs, suggesting an additional functional significance outside of symbiosis. The evolutionary implications of this intriguing new motif and its possible role in symbiosis and LRR-RLK function will be discussed.

P72

Role of cytokinin and *NIN* transcriptional regulator in colonization of roots by nitrogen fixing bacteria.

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Symbiotic accommodation of nitrogen-fixing soil bacteria (rhizobia) is a highly-orchestrated process unique to leguminous plants. This process culminates in the formation of symbiotic root nodules, in which rhizobia reside and fix nitrogen. A presumed transcriptional regulator *NIN* (for Nodule Inception) has been shown to be one of the central elements controlling the symbiosis. Curiously, while it acts as a positive regulator of bacterial entry and nodule organogenesis, *NIN* has also been shown to negatively regulate root susceptibility to subsequent bacterial infections. The mechanism(s) by which *NIN* expression is regulated to fulfill these various function is unknown. We have shown that one aspect of this regulation involves cytokinin signaling. Cytokinin may influence root susceptibility and bacterial infection by controlling the expression of *NIN* and other transcriptional regulators through locally and systemically operating mechanisms. This presentation will discuss recent advances in our understanding of cytokinin perception and regulation of *NIN* expression in the context of mechanisms that mediate root colonization by symbiotic bacteria.

P73

Induction of isiA by iron deficiency in *Synechococcus* sp. PCC7942 is associated with alterations in carotenoid and lipid composition

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Comparative lipid analysis demonstrated reduced amount of PG (50%) and lower ratio of MGDG/DGDG in iron-stressed *Synechococcus* sp. PCC 7942 cells compared to control cells. In parallel, the monoenoic (C:1) fatty acids in MGDG, DGDG and PG increased from 46.8%, 43.7% and 45.6%, respectively in control cells to 51.6%, 48.8% and 48.7%, respectively in iron-stressed cells. This suggests increased membrane dynamics, which may facilitate the diffusion of PQ and keep the PQ pool in relatively more oxidized state in iron-stressed compared to control cells. This was confirmed by chlorophyll fluorescence and thermoluminescence measurements. Analysis of carotenoid composition demonstrated that the induction of isiA (CP43') protein in response to iron stress is accompanied by significant increase of the relative abundance of all carotenoids. The quantity of carotenoids calculated on a Chl basis increased differentially with nostoxanthin, cryptoxanthin, zeaxanthin and β-carotene showing 2.6-, 3.1-, 1.9- and 1.9-fold increases, respectively, while the relative amount of caloxanthin was increased only by 30%. HPLC analyses of the pigment composition of Chl-protein complexes demonstrated even higher relative carotenoids content, especially of cryptoxanthin, in trimer and monomer PSI Chl-protein complexes co-migrating with CP43' from iron-stressed cells than in PSI complexes from control cells where CP43' is not present. This implies a carotenoid-binding role for the CP43' protein which supports our previous suggestion for effective energy quenching and photoprotective role of CP43' protein in cyanobacteria under iron stress.

P74

***Prunus persica*: "who has the problem with transformation, *Agrobacterium* or the researcher?"**

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Agrobacterium tumefaciens, which causes crown gall in many plant species, has been studied for more then 30 years. During plant infection, *Agrobacterium* genes are transferred into the plant cell inducing tumor formation in plants. This process has been adapted for use in plant transformation. To create optimal conditions for genetic transformation in particular plant species, a wide range of non-oncogenic strains have been developed. Nevertheless, some plant species still remain strongly recalcitrant to an *Agrobacterium*-based approach. For instance, genetic transformation of *Prunus spp.* is not a routine procedure and often restricted to a few species and cultivars. To investigate *P. persica* L. (Batsch) transformation recalcitrance, stages of bacterium - plant interaction, such as host recognition, activation of virulent genes, tissue infection, T-DNA transfer and integration into the plant genome should be carefully examined. Using a wide range of *Agrobacterium* strains we determined that none of engineered strains infect peach tissues, while some pathogenic strains, isolated from peach trees, incited gall formation. Analysis of gall extracts showed that nopaline was the predominant opine for the most virulent strains. Employing disarmed derivates of these strains will provide an opportunity to improve transformation technology for the recalcitrant species.

P75

Production of recombinant interleukin-24 in tobacco plants and BY-2 cells

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Melanoma differentiation-associated gene-7/interleukin-24 (*mda-7*/*IL-24*) has come into the limelight as a new "magic bullet" for divergent cancers. Native IL-24 is localized to the endoplasmic reticulum (ER), where it is postulated that interactions between IL-24 and specific target molecules in the ER of cancer cells elicit an unfolded stress response leading to cancer cell apoptosis. Unfortunately, the high production cost of recombinant human IL-24 (rhIL-24) associated with conventional expression systems has limited clinical testing to gene therapy with non-replicating adenoviral vectors. Such gene therapy has been successful in phase I/II clinical trials but is limited in the amount of IL-24 that can be delivered to tumors. In this study, IL-24 constitutive expression vectors targeted to the ER with or without elastin-like polypeptide (ELP) or green fluorescent protein (GFP) translational fusion partners were introduced into tobacco plants and BY-2 suspension cells by *Agrobacterium*-mediated transformation. BY-2 calli which expressed IL-24 protein to a high level were not viable and those in suspension culture showed a strong and rapid decline in IL-24 expression over time. This loss of IL-24 protein expression was also observed in leaves of transgenic tobacco plants. Experiments with these constructs driven by inducible promoters are being conducted to determine if the expression of IL-24 is toxic to the plants and BY-2 cells and if higher levels of protein expression can be achieved using an inducible system. Biological activity of plant rhIL-24 will be determined using assays for cell proliferation and viability.

P76

Trafficking and degradation of recombinant IL-10 in plants

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During the past few years, many recombinant proteins have been expressed in plants with varying levels of accumulation. Levels are affected by properties inherent to these proteins such as turnover rates, and sub-cellular localization. The human interleukin-10 protein (IL-10) is a labile protein with a half-life *in vivo* of 30 minutes. It requires post-translational modifications and assembly, and is a good representative of other therapeutic proteins. However, IL-10 does not accumulate in plants to levels desirable for therapeutics production and traditional purification methods do not yield an affordable product. Trafficking and regulation of recombinant IL-10 fate in plant cells were studied in tobacco BY-2 cells. The accumulation of IL-10 was 5 times higher than levels in tobacco plants and appeared to be degraded by proteases, but not the proteasome. The sub-cellular localization of an IL-10 fusion with GFP demonstrated a reticulated pattern typical of the ER after 3 days of growth. After 6 days of growth, cleavage was observed between IL-10 and GFP in the fusion protein which resulted in the accumulation of GFP in the vacuole while IL-10::GFP fusion levels declined steadily. The cleaved IL-10 portion was not detectable. To simplify purification and reduce the cost, a third ER-targeted construct containing a fusion of IL-10 with an elastin-like polypeptide (ELP) was expressed in BY-2 cells. It accumulated to levels 60 times higher than those originally obtained in tobacco plants. With enhanced expression levels and low cost of purification, this system may provide a means of manufacturing many therapeutic proteins on a large scale and reduce the financial burden of the patients that require them.

P77

Protein-Protein Interactions between Starch Synthases and Branching Enzymes in Maize Endosperm Amyloplasts

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Starch is synthesized in the plastids of higher plants and provides the major caloric source for the human population, and is also an important industrial commodity. The pathway of starch biosynthesis is known to involve at least four groups of committed enzymes: ADP glucose pyrophosphorylase, starch synthases (SS), starch branching enzymes (SBE) and debranching enzymes. Starch synthases and starch branching enzymes are two important classes of enzymes, whose catalytic activities determine glucan chain length and branch point distribution in amylopectin, thus influencing many important physiochemical characterises of storage starches. Previous work has shown three forms of starch branching enzyme (SBEI, SBEIIa, and SBEIIb) were phosphorylated and that phosphorylation-dependent protein-protein interactions were found between isoforms of SS and SBE in amyloplasts of wheat. It is proposed that these protein complexes play an important role in the formation of the starch granule. Currently, active recombinant forms of maize SSI, SBEI, SBEIIa and SBEIIb have been expressed in *E.coli* in order to understand the process of protein complex formation. Recombinant proteins have also been attached to affinity chromatography media and used as 'bait' to identify other interacting proteins using maize endosperm amyloplasts. Current data indicate protein-protein interactions formed between SSI, SSII and SBEIIb. Analysis of the *ae* mutant of maize (lacking SBEIIb) indicates formation of novel protein complex between SSI, SSII and SBEI.

P78

Redox regulation of Arabidopsis TGA1 by a novel glutaredoxin

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Systemic acquired resistance (SAR) is a broad-range, long lasting defense pathway induced in Arabidopsis after attack by an avirulent pathogen. The induction of the SAR pathway is typified by the accumulation of salicylic acid (SA) and the induction of pathogenesis related genes. The nonexpressor of pathogenesis related gene (NPR1) protein is required for the establishment and deployment of SAR and exerts its effects through key transcription factors known as TGAs. In previous work, we have shown that TGA1's ability to interact with NPR1 is dependent on the redox status of two key cysteines. In unstimulated cells, these cysteines are oxidized and preclude TGA1-NPR1 interaction, whereas after SA treatment, these cysteines are reduced allowing these two proteins to interact with each other. A novel Arabidopsis glutaredoxin, GRX480, has recently been shown to interact with several tobacco TGAs. We have shown in Arabidopsis that TGA1 interacts with GRX480 and we propose that this novel glutaredoxin regulates TGA1's redox status, and therefore activity, in response to SA. Redox regulation of proteins involved in SAR, as illustrated by TGA1 and NPR1, appears to be an important control mechanism. Thus, understanding the novel glutaredoxins, which are unique to higher plants, is of paramount importance.

P79

A non-coding RNA regulates molybdenum cofactor biosynthesis in *Chlamydomonas reinhardtii*

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In a diverse range of organisms, molybdenum is complexed with a unique pterin compound to form the molybdenum cofactor (MoCo) which is required by specific enzymes for activity. Although the biosynthetic pathways of MoCo production are known, the regulation of this synthesis is not well understood. The green alga *Chlamydomonas reinhardtii* Dangeard was mutated by random insertion of a bleomycin-resistance cassette into the genome. One strain isolated (DB6) is unable to assimilate nitrate as a nitrogen source and specifically lacks terminal nitrate reductase activity. It was determined that DB6 is deficient in the MoCo required for this activity as DB6 also lacked activity of xanthine dehydrogenase, another Mo-enzyme. DB6 displays a 'molybdenum repairable' phenotype, meaning that growth is partially rescued by high (mM) levels of molybdate in the media. This would indicate that the defect lies in either in the step of molybdate transport or the insertion of molybdenum into the MoCo precursor molybdopterin. The insertional mutagen landed in a putative non-coding RNA but the nature of this transcript is still under investigation. Because mature MoCo is highly labile within the cell, synthesis must be tightly regulated. This research may lead to a better understanding of the regulation of MoCo biosynthesis in *C. reinhardtii.*

P80

Regulation of extracellular toxins in *Heterosigma akashiwo*: nutrient limited chemostat Studies

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The harmful algal bloom forming phytoplankter *Heterosigma akashiwo* has been implicated in fish-kills and economic losses to aquaculture operations worldwide. The putative toxic compounds produced by *H. akashiwo* include reactive oxygen species, haemolytic compounds, and an organic brevetoxin-like compound, none of which can be definitively linked to the toxicity of *H. akashiwo.* This study investigated the role of limitation of the macronutrients nitrate (N) and phosphate (P) and of the micronutrient iron (Fe) in *H. akashiwo* toxicity. Cultures of two strains of *H. akashiwo* were grown under varying N:P regimes and varying Fe concentrations and the production of H_2O_2 and haemolysins were assessed. Cells were assayed for their anti-algal properties against a model algal competitor *Rhodomonas salina*. The anti-grazer properties of *H. akashiwo* were examined using *Artemia salina* bioassays. Throughout all experiments, an isolate-specific toxin production and toxicological response signature were observed. Varying N:P ratios caused no significant differences in toxin production or toxicological effects. Toxin production decreased with increasing Fe limitation although anti-algal activity increased. Collectively, these data suggest that the toxins produced by *H. akashiwo* do not definitively play a role in anti-algal or anti-grazer toxicity; however, macronutrient and micronutrient limitation trigger different toxicological responses which may contribute to bloom formation.

P81

Methyl Viologen: An appropriate tool to measure oxidative stress in *Symbiodinum* sp. (Freudenthal) with fluorescent probes?

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In the Oxidative Theory of coral bleaching, the transfer of reactive oxygen species (ROS) from the algal symbiont, *Symbiodinium* sp., to the animal host, results in the phenomena of coral bleaching (Downs *et al.* 2002). Therefore, it is necessary to quantify the amount of ROS produced in *Symbiodinium* sp., under variable environmental conditions, to understand the link between these conditions and coral bleaching. In this study, *Symbiodinium* sp. CCMP 2432 was grown under variable methyl viologen (MV) concentrations (0,2.3,4.5 mM) to determine the effects of this ROS inducer on the alga and validate the use of the fluorescent flow cytometric probes, CM-DHDCFDA and DHE, to detect intra-cellular concentrations of hydrogen peroxide (H_2O_2) and super oxide (SO_2), respectively. An increased concentration of MV (2.3 mM and 4.5 mM) was found to generate an increasing trend in detectable H_2O_2 and SO_2 in *Symbiodinium* sp. during lag growth, however no significant difference was detected between MV treatments during both exponential and stationary growth. This inabilitliy to detect ROS during these phases of growth indicates that perhaps *Symbiodinium* sp. 2432 is less susceptible to MV treatment and that anther ROS inducer must be selected to generate detectable intracellular ROS.

P82

Photoprotection, photoinhibition and recovery in *Parmelia sulcata:* A fluorescence study

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Desiccated lichens are photosynthetically inactive, but upon re-hydration they can perform photosynthesis within seconds. Desiccation is correlated with both a loss of variable fluorescence and a decrease in overall fluorescence yield. The observed decrease in fluorescence indicates active quenching and increased 'shading' of the photosynthetic apparatus in the lichen upon desiccation. Photosystem II (PSII) represents the primary focus of fluorescence quenching, likely due to the high level of damage that PSII suffers during normal operation. Contrasting with PSII, Photosystem I (PSI) fluorescence kinetics appear unaffected, only exhibiting a decrease in amplitude. Photoprotection thus appears to involve an overall decrease in excitation of the photosynthetic apparatus ('self shading') as well as an active quenching of excitation in PSII by a novel long wavelength quencher that appears responsible for ~80% of the decrease in fluorescence yield. In addition, *P. sulcata* was less prone to photoinhibition in the desiccated state and recovered from high light treatment upon subsequent hydration more quickly than lichens which were treated with high light while hydrated. We also observed that desiccation recovery was not dependent on protein synthesis, while photoinhibition recovery appeared to progress normally in the absence of protein synthesis.

P83

Identification of novel putative cochaperones of Hsp90 in *Arabidopsis* using bioinformatics analysis

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The highly conserved and abundant molecular chaperone Hsp90 plays a key role in signal transduction networks, cell-cycle control, protein degradation and protein trafficking. Two key features regarding the mechanism of Hsp90 action are: 1) Hsp90 operates as part of a multichaperone complex, facilitating the folding of the client proteins into their stable or activatable conformations, and 2) Hsp90's functions are driven by the hydrolysis of ATP. The functional cycle of Hsp90 requires a cohort of cochaperones that regulate the ATPase activity of Hsp90 and confer specificity to its interaction with client proteins. Several Hsp90 cochaperones contain a tetratricopeptide repeat (TPR) domain, which is a degenerate 34-amino acid sequence that is often found in multiple copies and in tandem array in proteins. X-ray crystallographic structures of TPR domains in Hop in complex with C-terminal peptides of Hsp70 and Hsp90 containing the highly conserved EEVD motif, revealed that a subset of basic residues in the TPR domain form a so-called carboxylate clamp and interact with the acidic side chains on the peptide ligand. These basic amino acid positions are conserved in the TPR domains of other Hsp90 cochaperones. An *in silico* search for TPR proteins in *Arabidopsis* identified approximately 200 such proteins, which were then searched for domains with three-motif TPR and for conserved basic residues within these motifs. This search identified 23 proteins as potential Hsp90 interactors. Of these, 11 proteins are novel. These results suggest that Hsp90 may rely on TPR domain containing cochaperones much more than it was realized previously.

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