Requested	Abstr. #	First Author (Presenting Author)	Assigned	Comments
Oral	1	Dev Mani Pandey		Requeted Travel Grant (PHOTOSYNTHETIC ENZYMES AND METABOLITES IN ANOECTOCHILUS FORMOSANUS)
Poster	2	Ute Roessner-Tunali		ELUCIDATING BORON TOLERANCE IN BARLEY USING A COMBINED METABOLOMIC AND PROTEOMIC APPROACH
Both	3	Raveender Vannela		ROLE OF EXOPEPTIDE SECRETION IN METAL REMOVAL BY CYANOBACTERIUM NOSTOC CALCICOLA
Poster	4	R.A. Khabari-Nejad (Elham Attaran)		Evaluation of Anthocyanin contents under salinity (NaCl) Stress in Bellis perennis L.
Oral	5	Jan Hazebrouek		METABOLOMIC ANALYSIS OF LOW PHYTIC ACID MAIZE KERNELS
Poster	6	Rebecca Thompson		THE EXPRESSION OF YEAST HEXOKINASE IN MAIZE ENDOSPERM TO ENHANCE THE STARCH BIOSYNTHESIS PATHWAY
Oral	7	Kazuki Saito		Functional genomics through integration of transcriptomics and metabolomics in Arabidopsis thaliana
Poster	8	Quang Phan Nguyen		CHARACTERIZATION OF A PUTATIVE ARABIDOPSIS SORTING NEXIN
Oral	9	Manfred Beckmann		Food, Substantial Equivalence, Metabolomics: As Good As It Gets?
Oral	10	Will Allwood		METABOLOMIC FINGERPRINTING AND PROFILING OF PATHOGENIC INTERACTIONS INVOLVING BRACHYPODIUM DISTACH
Oral	11	Jyoti Shah		SYSTEMIC ACQUIRED RESISTANCE: THE LIPID CONNECTION
Poster	12	Shivkumar P. Devaiah		PROFILING OF MEMBRANE LIPID SPECIES IN ARABIDOPSIS TISSUES
Oral	13	Ruth Welti		LIPIDOMICS: ESI-MS/MS-BASED PROFILING TO DETERMINE THE FUNCTION OF GENES INVOLVED IN METABOLISM OF CO
Poster	14	Aiko Barsch		COMPREHENSIVE METABOLITE PROFILING OF SINORHIZOBIUM MELILOTI AND ITS HOST PLANT MEDICAGO TRUNCATULA
Poster	15	A. Moing		QUANTITATIVE METABOLIC PROFILING BY PROTON NMR FOR GENETIC AND FUNCTIONAL STUDY OF TOMATO FRUIT QUA
Poster	16	Y. M. Tikunov		METABOLOMICS OF TOMATO FRUIT VOLATILE COMPOUNDS
Poster	17	Hilary Major		A METABOLOMIC APPROACH TO THE IDENTIFICATION OF TRAITS IN TOMATO INTROGRESSION LINES USING TOF-MS
Oral	18	John Draper		HIERARCHICAL METABOLOMICS AND MACHINE LEARNING FOR PHENOTYPING AND FUNCTIONAL GENOMICS
Oral	19	E. Urbanczyk-Wochniak		PROFILING OF DIURNAL PATTERNS OF METABOLITE AND TRANSCRIPT ABUNDANCE IN POTATO LEAVES REVEALS SPEC
Oral	20	David Parker		METABOLOMICS OF HOST-PATHOGEN INTERACTIONS IN BRACHYPODIUM DISTACHYON
Poster	21	Ei-Ichiro Fukusaki		A ceramics supported soil-less cultivation system for Arabidopsis thaliana and its application for metabolomics.
Oral	22	Kanokwan Jumtee		Requeted Travel Grant (THE EFFECT OF GROWING SYSTEMS ON METABOLITE VARIATIONS OF ARABIDOPSIS THALIANA)
Oral	23	Kazuo Harada		Requeted Travel Grant (IN VIVO 15N-ENRICHMENT OF METABOLITES IN ARABIDOPSIS CULTURED CELL T87 AND ITS APPLIC
Poster	24	Tapasree Roy Sarkar		Requeted Travel Grant (Cloning, Expression and Characterization of a Putative Flavonoid Glucosyltransferase from Grapefruit (Citrus
Oral	25	Claudia Birkemeyer		LARGE SCALE INVESTIGATION ON THE RECOVERY OF METABOLITES ACCESSIBLE BY VARIATIONS OF GC-MS PROFILING
Poster	26	Zhiqiang Pan		CLONING AND CHARACTERIZATION OF CYTOCHROME P450S FROM SORGHUM POTENTIALLY INVOLVED IN THE BIOSYNT
Oral	27	Belinda Townsend		TRITERPENOID SAPONIN GLYCOSYLTRANSFERASES IN OATS
Poster	28	John Lokvam (Thomas A. Kursar)		Evolution of secondary metabolites in a Mimosoid legume, the genus Inga
Poster	29	Yuqin Jin		Reverse Genetic Analysis of Wax Synthases in Arabidopsis
Poster	30	Xu Li		Molecular and genetic characterization of the two paralogous genes coding for the BCCP subunit of the heteromeric acetyl-CoA carbo
Oral	31	Raoul J. Bino		also presenting author on abstract # 32 (THE FURTHER DEVELOPMENT OF METABOLOMICS AS A FUNCTIONAL GENOMICS T
Oral	32	Ingrid van der Meer (Raoul J. Bino)		Bino also author on astract # 31 (METABOLIC PROFILING OF A MUTANT MEDICAGO POPULATION OVER-EXPRESSING ARAB
Poster	33	Kevin Walker		Expression and Characterization of a Phenylalanine Aminomutase Functioning in Taxol Biosynthesis

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Poster	34	Mattias Rantalainen		Requeted Travel Grant (1H NMR METABOLIC FINGERPRINTING FOR GENOTYPE CLASSIFICATION)
Poster	35	Albert Koulman		also presenting author on abstract # 36 (VARIATIONS IN THE PHENYLPROPANOID METABOLISM OF ANTHRISCUS SYLVESTRI
Oral	36	Albert Koulman		also presenting author on abstract # 35 (NON-DESTRUCTIVE METABOLIC PROFILING OF THE RHIZOSPHERE USING SOLID-PH
Oral	37	Nicolas Schauer (Joachim Kopka)		MASS SPECTRAL LIBRARIES FOR METABOLITE IDENTIFICATION AND CHARACTERIZATION IN COMPLEX GC-EI-MS PROFIL
Oral	38	C. H. R. de Vos		SCREENING TOMATO FRUIT FOR FLAVOUR AND HEALTH USING INTEGRATED METABOLOMICS APRROACHES
Poster	39	Yasutaka Nishiyama		ELUCIDATION OF ANTHOCYANIN ACCUMULATION MECHANISM BY OMICS APPROACH USING <I>PAP1</I> OVER-EXPRESS
Poster	40	M. Ann D. N. Perera		BIOLOGICAL SYNTHESIS OF ALKENES: A CASE STUDY OF THE APPLICATION OF METABOLOMICS IN PATHWAY DISCOVER
Both	41	Ivelin Rizov		Requeted Travel Grant (METABOLOMIC CLASSIFICATION OF TOBACCO PLANT ENGINEERED WITH POTATO VIRUS Y CP DN
Poster	42	Takayuki Tohge		Elucidation of anthocyanin accumulation mechanism by "omics" approach using <I>PAP1</I> over-expressing mutants (1) -Integration
Oral	43	Daniel J Kliebenstein		Metabolomics and QTL Mapping What are the genes controlling metabolic variation?.
Poster	44	C. C. Periappuram		FUNCTIONAL GENOMICS OF SOYBEAN COMPOSITION
Poster	45	Peifen Zhang (Seung Yon Rhee)		MetaCyc and AraCyc: Metabolic Pathway Databases for Plant Research and Application
Poster	46	O. J. F. Vorst (R. D. Hall)		AN UNSUPERVISED APPROACH FOR THE DIFFERENTIAL ANALYSIS OF MULTIPLE LC/MS-DERIVED METABOLIC PROFILES
Poster	47	Miyako Kusano (Thomas Moritz)		METABOLIC CHANGES IN LEAVES DURING PHOTOPERIODICALLY INDUCED GROWTH CESSATION
Poster	48	Harin Kanani		Requeted Travel Grant (METABOLIC PROFILING OF THE SHORT TERM Arabidopsis thaliana RESPONSE TO ENVIRONMENTAL
Oral	49	Derek Stewart		METABOLIC PROFILING: PLATFORM TECHNOLOGIES TO DETECT UNINTENDED EFFECTS FOLLOWING GENETIC MODIFICA
Poster	50	Hideyuki Suzuki		High throughput metabolite profiling of the suspension-cultured Arabidposis thaliana T87 cells by combination of various mass spectro
Oral	51	Johan Trygg		CHEMOMETRIC ANALYSIS FOR UNDERSTANDING COMPLEX BIOLOGICAL SYSTEMS
Poster	52	Jane L. Ward		THE UK NATIONAL CENTRE FOR PLANT AND MICROBIAL METABOLOMICS
Both	53	Jennie Lewis		CONSISTENCY IN PLANT GROWTH: THE KEY TO SUCCESSFUL METABOLITE PROFILING
Oral	54	Srinivasa Rao Uppalapati		Requeted Travel Grant (GENE EXPRESSION AND METABOLITE PROFILING OF TOMATO LEAF TISSUE TREATED WITH THE P
Poster	55	Suh-Yeon Choi		Metabolite profiling during leaf development in transgenic and wild type Arabidopsis
Oral	56	Gordon R. Gray		CHANGES IN THE METABOLOME OF Arabidopsis IN RESPONSE TO LOW TEMPERATURE: ENVIRONMENTAL STRESS VERSU
Poster	57	Heather L. Babka		Targeting of ATP-Citrate Lyase to the Plastids of Arabidopsis thaliana
Poster	58	Mathew L. Hillwig		Metabolomic Profiling of Hypericum Species
Oral	59	Waleerat Banyai		Requeted Travel Grant (PLANT CHARACTERISTICS AND ARTEMISININ PRODUCTION IN TRANSGENIC ARTEMISIA ANNUA WI
Poster	60	Allison Gordon		METABOLOMIC PROFILING OF LAMB BLOOD PLASMA BY GC/TOF-MS
Poster	61	Andrea Raab		Requeted Travel Grant (FORMATION OF ARSENIC-PHYTOCHELATIN COMPLEXES BY PLANTS IN RESPONSE TO ARSENIC ST
Poster	62	Junping Chen		IDENTIFICATION OF METABOLITES ASSOCIATED WITH FREEZING TOLERANCE IN CONSTITUTIVELY FREEZING TOLERANT
Oral	63	Lankun Wu		Metabolomics Approach to Analysis of Bioactive Constituents of Echinacea
Oral	64	Vinod Goyal		Not sure of coming (Impact of interaction of salt and water stress in brassica juncea)

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Oral	65	Hye Kyong Kim		Requeted Travel Grant (CLASSIFICATION OF ILEX SPECIES BASED ON METABOLOMIC FINGERPRINTING USING 1H NMR AN
Oral	66	Joelene K. Smith		THE IMPACT OF SAFENERS IN ARABIDOPSIS USING METABOLIC FINGERPRINTING WITH GC/MS-TOF
Poster	67	Beth Fatland (Beth Fatland & Joel Schmidt)		Reverse Genetic Analysis of the Metabolism of Cytosolic Acetyl-CoA
Oral	68	Young Hae Choi		Requeted Travel Grant (METABOLOMIC DISCRIMINATION OF CATHARANTHUS ROSEUS LEAVES INFECTED BY PHYTOPLASM
Poster	69	HJohn J. Ross		THE SEARCH FOR FLOWERING HORMONES IN PEA
Poster	70	C. Kristensen (M. Morant)		Requeted Travel Grant (Insertion of high flux pathways in Arabidopsis thaliana without impact on plant morphology, metabolome and t
Poster	71	T. Koobkokkruad W. De-Eknamkul		A New TLC-Densitometric Technique for Simple Artemisinin Analysis
Poster	72	Grit Laue (Ernst Gassmann)		Supervised and Automated Unsupervised GC/MS Metabolomics for the Diagnosis of Agrochemical Targets
Oral	73	Daekeun Hwang (Sunghwa Choe)		Gene Discovery via Metabolite Fingerprinting of Arabidopsis Activation Tagging Lines
Oral	74	Hikaru Seki (Toshiya Muranaka)		DEVELOPMENT OF A NOVEL TECHNOLOGY PLATFORM FOR ROOT CULTURE SYSTEMS FOR FUNCTIONAL GENOMICS
Poster	75	Jon Moorby		FTIR AND GC-TOF-MS ANALYSIS OF BLOOD OF GRAZING LAMBS WITH DIFFERING GROWTH RATES
Oral	76	Baichen Zhang (Colin Turnbull)		PUSHING THE LIMITS OF METABOLOMICS: DEVELOPMENT OF MS PROCEDURES FOR SCREENING OF NOVEL LOW ABUND
Oral	77	Susanne Wiklund (Henrik Antti)		MONITORING OF PLANT DEVELOPMENT USING HR/MAS NMR AND MULTIVARIATE DATA ANALYSIS
Oral	78	Vladimir Tolstikov		LC/ESI-MS ANALYSIS FOR PLANT METABOLOMICS: METHOD DEVELOPMENT AND DATA MINING
Poster	79	Boris Jankowski		ANALYSIS OF THE METABOLITE PROFILE OF ARABIDOPSIS THALIANA DURING DEVELOPMENTAL AND DIURNAL CYCLES
Both	80	Michael Gibson		SPECTRUM-BASED CHROMATOGRAM ALIGNMENT
Oral	81	G. Dobson		APPLICATION OF METABOLITE AND FLAVOUR VOLATILE PROFILING TO STUDIES OF BIODIVERSITY IN SOLANUM SPECIES
Poster	82	T. Shepherd		Profiling of Metabolites and Volatile Flavour Compounds from Solanum Species using Gas Chromatography-Mass Spectrometry
Poster	83	Jie Li		MetNetDB: a database for Arabidopsis systems biology
Poster	84	Geng Ding		Reverse genetic analysis of novel biotin proteins in plants
Poster	85	L. Li		STARCH GRANULE METABOLISM AND ACETYL-COA UTILIZATION
Poster	86	D. F. Cortes		METABOLITE PROFILING OF THE MUTANT LINES OVEREXPRESSING AtIAMT1 AND AtJAMT1, TWO MEMBERS OF THE SABA
Poster	87	Camille Dubois		Requeted Travel Grant (PACLITAXEL STABILITY AND CONVERSION THROUGH METABOLITE ANALYSIS IN TAXUS CELL SUSP
Oral	88	Michael C. Naill		Requeted Travel Grant (CORRELATION OF METABOLITE PROFILING AND GENE EXPRESSION IN PACLITAXEL-ACCUMULATI
Oral	89	Lee Tarpley		also presenting author on abstract # 94 (MARKER METABOLITES CAPTURING THE METABOLITE VARIANCE PRESENT IN A PLA
Poster	90	Y. Abu-Nada (A. C. Kushlappa)		PMETABOLIC PROFILING OF POTATO CULTIVARS VARYING IN LEAF RESISTANCE TO LATE BLIGHT, PHYTOPHTHORA INFE

Requested	Abstr. #	First Author (Presenting Author)	Assigned	Comments
Poster	91	Vidya Iyer		METABOLIC FLUX ANALYSIS OF SUCROSE METABOLISM INTO PROTEIN, OIL AND STARCH IN SOYBEAN SEEDS
Poster	92	Tripty A. Hirani		ASP-295 IS IMPORTANT FOR PHOSPHORYLATION IN THE ACTIVE-SITE LOOP OF PYRUVATE DEHYDROGENASE E1alpha BY
Oral	93	A. Daniel Jones		CLASSIFICATION OF UNKNOWN METABOLITES USING LC/MS: HOW MUCH CAN WE LEARN USING NOMINAL MASS ANALYS
Poster	94	Lee Tarpley		REFERENCING IN METABOLITE SPACE THROUGH LARGE-SCALE GENERATION OF PREDICTIVE FUN
Oral	95	Lloyd W. Sumner		METABOLOMICS AND INTEGRATED FUNCTIONAL GENOMICS OF MEDICAGO TRUNCATULA RESPONSES TO BIOTIC AND A
Poster	96	Masami Yokota Hirai		ELUCIDATION OF A WHOLE ADAPTIVE PROCESS TO NUTRIENT DEFICIENCY BY INTEGRATION OF METABOLOMICS AND T
Poster	97	S. M. Saqlan Naqvi		NECTARIN-4 IS A HOMOLOGUE OF XYLOGLUCANSE INHIBITORY PROTEIN
Oral	98	Zegrar Ahmed		Late Submission, Requeted Travel Grant (Biologic diversity of ecosystems forest and consistent of the dforestation by remote sensing
Poster	99	Elizabeth Winters		OVEREXPRESSION OF ATP CITRATE LYASE IN ARABIDOPSIS
Oral	100	Jerome R. Faulkner		Late Submission (EPICHLOE AMARILLANS IN ASSOCIATION WITH AGROSTIS HYEMALIS PRODUCES ONLY N-ACETYL NORL
Poster	101	Kathrin Wenzel		Late Submission (GCTOF METABOLITE PROFILING OF ARABIDOPSIS MUTANTS AND ECOTYPE ACCESSIONS UNDER HIGH L

ABSTRACT # 1

PHOTOSYNTHETIC ENZYMES AND METABOLITES IN *ANOECTOCHILUS FORMOSANUS*

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Photosynthetic enzymes (Rubisco, ribuloase, 1,5-bis phosphate carboxylase/oxygenase; PEPCase, phosphoenolpyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase and NADP-MDH, NADP malate dehydrogenase) and metabolites (OAA, oxaloacetic acid; malate pyruvate, and NADP) in day period were studied on six months old *in vitro Anoectochilus formosanu*s plantlets. *A. formosanu*s were cultured in MS medium supplemented with 0.5 mg l^{-1} N6-benzyladenin, 0.5 g activated charcoal, 0.07% agar and 20 gl^{-1}sucrose. Cultures were maintained at 25 ± 2 ^{0}C under cool while fluorescent tubes (40 µmol m^{-2} s^{-1}) with 16h photoperiod. After three months larger shoots were subcultured in the rooting media for another three months. Rubisco and PEPCase at the pH 8.0 while, PEPCK and NADP-MDH at the pH of 6.7 and 7.5 respectively, showed maximum catalytic activity. PEPCK activity was higher as compared to NADP-MDH, PEPCase and Rubisco activity. Similarly, pyruvate content was higher as compared to oxaloacetic acid, malate, and NADP. Results suggest that increased PEPCK activity catalyzes conversion of OAA into PEP while, higher malate content as compared to OAA could be explained by the increased activity of NADP-MDH which facilitated conversion OAA into malate.

ABSTRACT # 2

ELUCIDATING BORON TOLERANCE IN BARLEY USING A COMBINED METABOLOMIC AND PROTEOMIC APPROACH

Ute Roessner-Tunali, J.H.Patterson, R.Chapman, M.G.Forbes, K.L.Ferguson, G.B.Fincher[+], A.Bacic
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Boron (B) is an essential plant micronutrient, although the complete suite of physiological roles for B in plant growth and development remains unclear. Excessive B levels are toxic, and ultimately lead to significant reductions of crop yield. Indeed, excess soil B is regarded as a major constraint to crop production in many low-rainfall regions, including southern Australia, West Asia and North Africa. The development of B tolerant barley cultivars represents a practical strategy to overcome this problem. In order to elucidate the role of B within plant growth and metabolism, complementary metabolomic and proteomic approaches are being employed. Using this approach the mechanisms responsible for B tolerance/resistance will be determined.

We are examining the differential responses of a B tolerant barley landrace, Sahara 3771 and the intolerant Australian cultivar Clipper in the presence of varying B concentrations. In addition, a selection of interesting doubled-haploid lines from a cross between the intolerant and tolerant parental lines with differences in their B stress behavior will be analysed in detail with respect to their metabolic complement and proteome. The resulting metabolic and proteomic data will be used to identify the regulatory networks that control plant development under different B concentrations. The regulatory networks described should identify physiological processes, which may be exploited to further increase B tolerance of agriculturally important plants.

ABSTRACT # 3

ROLE OF EXOPEPTIDE SECRETION IN METAL REMOVAL BY CYANOBACTERIUM

NOSTOC CALCICOLA

RAVEENDER VANNELA[*] & S K VERMA[#]

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The various mechanisms suggested for metal tolerance in microbes include cellular exclusion of metals by the secretion of exopeptides or metal transport across the membrane extrusion of metal and immobilization at cell interior. The microbes are known to release small peptides and proteins extracellularly, when subjected to metal stress. These peptides can bind the metal in the solution thus reducing their bioavailability/ toxicity.

In the present paper we report our results on the influence of exopeptide secretion in cadmium uptake by parent and metal resistant strains (Met-R1 & R2) of cyanobacterium *Nostoc calcicola*. The growth pattern of parent and two mutant strains, Met-R1 & Met-R2 shows that a 2.0 m*M* concentration of Cd^{2+} was completely lethal for parent whereas both the mutants could grow normally under the similar conditions. A comparison of dead and viable cells of all the three strains exposed separately to Cd^{2+} showed a clear superiority of viable biomass over the dead ones. The two mutant strains with equal metal tolerance showed different behavior of metal removal; Met-R2 showing more than two fold higher cadmium removal than the Met-R1 strain. These data clearly indicate the presence of different mechanisms of metal resistance. The Met-R1 strain was found have less Cd accumulation due to retarded Cd transport. A comparison of exopeptide secretion by experimental strains with increasing Cd^{2+} concentration showed an enhancement in case of Met-R1 strain as compared to other two strains, which did not show a significant increase under similar conditions. Thus, the increased Cd^{2+} removal by Met-R1 may be partly attributed to the enhanced secretion of exopeptides. A study involving cellular partitioning was also conducted to confirm the role of various processes of Cd^{2+} removal by both the mutant strains. The metal-ion specific cellular adsorption of Cd^{2+} was found to be maximum in case of Met-R2 (250 μg Cd^{2+}/mg dry wt.) contributing about 30% of the total Cd^{2+}-removed from the solution. The same process contributed only 16% in case of Met-R1 strain metal removal process. The effect of immobilization on exopeptide secretion by parent and mutant strains was examined. A comparison of free cells and immobilized beads prepared from Met-R1 & R2 strain and exposed to the Cd^{2+} solution showed almost 1.5 fold increase in Cd^{2+} removal by immobilized beads (in both cases) thus, clearly establishing the efficiency of immobilization in Cd^{2+} removal irrespective of the exopeptide release.

ABSTRACT # 4

Evaluation of Anthocyanin contents under salinity (NaCl) Stress in *Bellis perennis* L.

Khavari-Nejad R.A, Bujar M and Attaran E.

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Flavonoids are the secondary metabolites that are presently at high level in most plant seeds and grains. These compounds play crucial roles in response to environmental stresses such as salinity (NaCl) stress which is a major factor in limiting crop production. Therefore, it is important to understand the ways of plant that responds to salinity stress and adapt to it. In this research the effects of the salinity stresses on changing the content of anthocyanin in *Bellis Perennis* L. as a kind of response to salinity stress were investigated inasmuch as most of the agricultural soils of the world are under salt stress conditions.

The investigation was done with *Bellis Perennis* L. because of the high contents of secondary metabolites in this plant. The plants were placed in separate pots, and treated with 0 mM NaCl as a control plant, 15 mM NaCl, 25 mM NaCl, 50 mM NaCl, 75 mM NaCl and 100 mM NaCl for 40 days.

After 40 days, symptoms of salinity stress were seen as signs of cloresis and reduction of leaf area; also, in 100mM NaCl, all treated plants died. However, others tolerated lower level of Salinity. After 60 days the plants were harvested and the total anthocyanin was extracted and estimated with both spectrophotometry method and HPLC method from fresh leaves of *Bellis perennis* L.

Results show that accumulation of anthocyanin increased in 15 mM NaCl and 25 mM NaCl significantly and higher levels of salinity caused the plants to decrease the content of anthocyanin in comparison with control plant.
The results indicate a kind of response of the plant to environmental stresses.

ABSTRACT # 5

METABOLOMIC ANALYSIS OF LOW PHYTIC ACID MAIZE KERNELS

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Phytic acid, or hexaphosphorylated myo-inositol, is the major storage molecule for phosphorous (P) in maize kernels. Phytate in foods or animal feeds will complex with proteins and several nutritionally important mineral cations resulting in reduced energy availability. Maize plants have been developed using classic mutation breeding that produce kernels with significantly less phytic acid. An extensive survey of the low phytate phenotype in different genetic backgrounds grown in several field locations revealed that a rise in inorganic P consistently does not compensate completely for the decline in phytic acid P. There were no quantitative differences in kernel phospholipids. Phosphorous contents of the protein and starch fractions were also evaluated. For a metabolomics approach, both mutant and wild type kernels were obtained from a single segregating ear, minimizing variability. Individual mature kernels were lyophilized and ground. Phenotype was measured using a
simple colorimetric test for inorganic P content. Kernels were extracted in aqueous methanol and partitioned into polar and nonpolar fractions. Metabolites were derivatized and subjected to GC/TOF/MS, and raw data was processed using the vendors (Leco) peak deconvolution software. Compounds were identified via coelution and/or mass spectrum matching with authentic standards. Each of these metabolites was semi-quantified by calculating the ratio of the peak area of a characteristic extracted ion against that of the internal standard, adjusted for original sample weight. P-containing metabolites were recognized easily by a prominent m/z 299. Metabolomic results are presented in context with what is known about the biochemistry of this low phytic acid trait.

ABSTRACT # 6

THE EXPRESSION OF YEAST HEXOKINASE IN MAIZE ENDOSPERM TO ENHANCE THE STARCH BIOSYNTHESIS PATHWAY

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2Pfeiffer University 4701 Park Rd., Charlotte, NC 28209

Sucrose metabolism in sink tissues results in the accumulation of high levels of free hexose sugars. Hexose sugars, including fructose and glucose, are metabolized as precursors of starch biosynthesis in maize kernels. The endogeneous hexokinases in the developing kernel, specifically fructokinase, is inhibited by increased levels of hexoses (Copeland, et.al., 1978 and Kanayama, et.al., 1998). The accumulation of hexoses may also cause feedback inhibition of the endogeneous sucrose metabolizing enzyme, sucrose synthase. The inhibition of endogeneous enzymes by elevated levels of hexoses may reduce the flux of carbon through the starch biosynthetic pathway. In an effort enhance the starch biosynthesis in developing kernels, we have produced transgenic maize plants expressing hexokinase driven by an endosperm specific promoter. We have demonstrated that the activity of the hexokinase we have expressed in plants is not inhibited by increased concentrations of fructose.

The expression of hexokinase A resulted in several changes in mature kernels, including a 10% increase in extractable starch and the absence of hard endosperm. These changes may account for the decrease in test weight that was demonstrated.

Copeland, L. et.al (1978) Plant Physiol. 62, 291-294.
Kanayama, Y. et.al (1998). Plant Physiol. 117, 85-90.

ABSTRACT # 7

Functional genomics through integration of transcriptomics and metabolomics in Arabidopsis thaliana

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Post-genomics approach through integration of transcriptomics and metabolomics can provide the clues for identification of gene function and the precise information about gene-to-metabolite and/or metabolite-to-metabolite networks. To identify the function of particular genes and to find new molecular networks under an ectopic gene expression and nutritional stress in Arabidopsis thaliana, we investigated global gene expression profile by DNA micro/macro-array and metabolite profile by combination of different mass spectrometric technologies including LC-MS, GC-MS and FT-MS.

Nutritional stress of nitrogen and sulfur resulted in global change of metabolome that could be correlated with the modulation of global gene expression, indicating the presence of several gene-to-metabolite networks. In particular, glucosinolate production was notably modified by these stresses, and thus the genes showing similar pattern of expression were identified as the candidates involved in glucosinolate metabolism. The metabolite profiles of pap1-D mutant and pap1 cDNA transgenic lines over-expressing a Myb gene in A. thaliana indicated that anthocyanin content increased specifically. The comprehensive gene expression and metabolite profiles of PAP1 gene over-expressing lines could indicate the novel candidate genes that are responsible for modification and storage of anthocyanins.

ABSTRACT # 8

CHARACTERIZATION OF A PUTATIVE ARABIDOPSIS SORTING NEXIN

NGUYEN QUANG PHAN, DIANE C. BASSHAM

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Protein transport to the vacuole takes place through the secretory pathway. A key regulatory point for protein trafficking in this pathway is the trans-Golgi network (TGN). At the TGN, vacuolar proteins are sorted from secreted proteins and packaged into vesicles for transport into the vacuole. Arabidopsis syntaxin 41 (SYP41) is a protein that resides in the TGN and shows sequence similarity to vesicle trafficking proteins. Immunoprecipitation of SYP41 co-precipitated a protein (named p65) found to be homologous to sorting nexins. Sorting nexins play a role in vesicular trafficking and contain a PX domain, that binds phosphoinositol lipids, and one or more coiled-coil regions for protein-protein interactions. The p65 protein may play a role in vesicle transport to the vacuole. Antibodies were raised against recombinant p65 protein, and the p65 protein was found to be distributed throughout the plant. A GFP-p65 fusion protein was transiently expressed in Arabidopsis protoplast and motile punctate spots were observed in the cytoplasm. We are now studying the specific localization and function of p65 in vesicle trafficking.

ABSTRACT # 9

Food, Substantial Equivalence, Metabolomics: As Good As It Gets?

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Substantial Equivalence embodies the idea that a conventional food may be used as a basis of comparison for novel foods as part of a safety assessment. This concept has sparked much political and scientific controversy centring on lack of a definitive meaning. We aim to explore metabolomics as tool to describe compositional differences between transgenic potatoes modified in fructan metabolism and conventional potato cultivars.

Comprehensive analysis of global metabolite profiles has been undertaken based on GC-MS and direct injection ESI-MS coupled with computational analysis. This should allow non-biased detection of both intended and unintended effects of a deliberate genetic change in an organism at the level of metabolism. An experimental strategy, including experiment design, data capture and statistical data analysis is described, which aims to provide a validated technology platform for determination of compositional differences between plants.

Statistical experiment design to capture systematic variability was important to enable metabolome variability to be measured and in these large experiments cumulative variance due to instrument drift has a major influence. Analyses based on either GC-MS profiling or ESI-MS fingerprinting was able to distinguish e.g. transgenic potatoes from conventional cultivars. Particularly using supervised data analysis techniques individual potato cultivars could be recognised by a range of clustering and classification methods. A small number of metabolites strongly associated with the GM lines were identified, all of which were predicted to be precursors or breakdown products of fructans thus representing expected changes in metabolome associated with transgene expression.

ABSTRACT # 10

METABOLOMIC FINGERPRINTING AND PROFILING OF PATHOGENIC INTERACTIONS INVOLVING BRACHYPODIUM DISTACHYON AND ARABIDOPSIS THALIANA

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Plant-pathogen interactions are particularly appropriate for metabolomic analyses as disease development involves host metabolite reprogramming and tailoring to the needs of the pathogen whilst resistance is often based on the production of toxic products. We are investigating two pathosystems involving the emerging Pooid model species Brachypodium distachyon and the rice blast fungal pathogen, Magnaporthe grisea and also Arabidopsis thaliana with the bacterial pathogen Pseudomonas syringae pathovar tomato DC3000 (Pst).

Analysis of the M. grisea interactions with B. distachyon involved challenge of three week-old seedlings of susceptible and resistant ecotypes. Early responses to fungal challenge employed Fourier-transform Infrared spectroscopy (FT-IR) for high-through put metabolomic fingerprinting. Discriminant function analysis (DFA) distinguished between disease symptoms and plant resistance. The data were validated via DFA projection analyses, which indicated that the experimental approach produced highly reproducible data.

Electrospray ionization mass spectrometry (ESI-MS) was employed for metabolomic profiling. Subtraction spectra revealed the ions separating healthy from infected material, those also present in the DFA loadings, were attributed as substances involved in early disease symptoms or the elicitation of resistance. Ions were further analyzed using ESI-MS-MS, which identified 7 phosphatidic acids and phosphatidyl glycerols. DFA projection analyses tightly clustered 8 training and 2 test replicates.

Currently we are focusing on metabolic changes in A. thaliana cell cultures on inoculation of a virulent Pst strain and the resistance eliciting Pst avrRpm1 variant.

ABSTRACT # 11

SYSTEMIC ACQUIRED RESISTANCE: THE LIPID CONNECTION

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Systemic acquired resistance (SAR) is an induced response activated in the distal organs of a plant by the prior exposure of another organ to a necrotizing pathogen. The activation of SAR confers enhanced resistance to a broad-spectrum of pathogens. There is an increase in the content of salicylic acid (SA) in the distal tissues that manifest SAR. This accumulation of SA in the distal tissue, and the key SA signaling factor, NPR1, are required for the activation of SAR in Arabidopsis.

Recently, we have shown that the Arabidopsis SFD1 gene is required for the activation of SAR (Nandi et al. Plant Cell 16: 465-477, 2004). However, unlike NPR1, SFD1 is not required for the basal resistance to bacterial pathogen, Pseudomonas syringae. SFD1 is required for the SAR-associated accumulation of SA in the distal leaves, but not in the pathogen- infected leaves. SFD1 encodes a dihydroxyacetone phosphate reductase involved in lipid biosynthesis, suggesting an important role for lipid metabolism in the activation of SAR. Previously, we have shown that a mutation in the Arabidopsis SSI2 gene, which encodes a fatty acid desaturase, constitutively activates a SAR-like defense response in the ssi2 mutant plant. A putative lipid transfer protein, DIR1is also required for the activation of SAR in Arabidopsis, providing further support for the involvement of lipids in the activation of SAR (Maldonado, et al. Nature 419: 399-402, 2002). A combination of genetic and lipid
omic approaches are underway to improve our understanding of the role of lipids in plant defense.

ABSTRACT # 12

PROFILING OF MEMBRANE LIPID SPECIES IN ARABIDOPSIS TISSUES.

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Phospholipase D (PLD) action has been implicated in a variety of cellular processes, including signal transduction, membrane trafficking, and membrane degradation, in a variety of developmental functions, including seed germination, aging, and senescence, and under a broad spectrum of stress conditions. In *Arabidopsis thaliana*, there are twelve PLD genes. In this work, we examine the role of the most abundant PLD gene product, PLDα1, in determining the basal composition of polar membrane lipid species in leaves, stems, flowers, siliques, seeds, and roots of wild-type Arabidopsis and in Arabidopsis with a knockout of the PLDα1 gene. Quantitative data on lipid molecular species, identified by head group, total acyl carbons, and total double bonds, are obtained by electrospray ionization tandem mass spectrometry. The compositional data are interpreted in light of PLDα1 protein and activity measurements that show enzyme activity to be highest in root and flower tissues. This work was supported by NSF award MCB 0110979, Kansas NSF EPSCoR, and the Kansas Technology Enterprise Corporation.

ABSTRACT # 13

LIPIDOMICS: ESI-MS/MS-BASED PROFILING TO DETERMINE THE FUNCTION OF GENES INVOLVED IN METABOLISM OF COMPLEX LIPIDS

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Lipids are structural backbones for cell membranes, sources of metabolic energy, and sources of regulators of numerous cellular functions in all organisms. Within each cell, there are hundreds of types of lipids, and the composition of lipids changes in response to signals from a cell's environment. Kansas Lipidomics Research Center Analytical Laboratory (www.ksu.edu/lipid/lipidomics) provides high-throughput profiling of complex, polar lipids, using electrospray ionization tandem mass spectrometry. Using internal standards, quantitative profiles of lipid molecular species of many head group classes are obtained from crude extracts without chromatographic separation of the components. When genomic manipulation and lipid profiling are used together, the genes and proteins involved in the generation of membrane compositions and regulators can be identified. Our goals include establishment of a database for lipidomic data. Supported by Kansas NSF EPSCoR, Kansas Technology Enterprise Corporation, Kansas Biomedical Infrastructure Network, and Kansas State University.

ABSTRACT # 14

COMPREHENSIVE METABOLITE PROFILING OF *SINORHIZOBIUM MELILOTI* AND ITS HOST PLANT *MEDICAGO TRUNCATULA* USING GAS CHROMATOGRAPHY – MASS SPECTROMETRY AND LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY

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In order to analyse the symbiosis between the soil-bacterium *Sinorhizobium meliloti* and its host-plant *Medicago truncatula* a metabolite analysis was established. Both symbiosis partners undergo several physiological changes during the establishment of the symbiosis. From the annotated *S. meliloti* genome sequence and the available *M. truncatula* EST database metabolic networks can be predicted. Aim of this work is to analyse changes in these networks in response to the host-plant or the microsymbiont.

Hydrophilic compounds analysed via GC-MS were identified by comparison with the NIST database and available standards. So far 65 and 44 compounds could be identified in the bacterial and plant extracts respectively. LC-MS analysis allowed the analysis of highly polar compounds like NAD but in general only a minor amount of metabolites was detected.

A principal component analysis (PCA) was shown to be able to distinguish *S. meliloti* cells grown on different carbon sources based on their metabolite profile, thus revealing the functionality of the established methods.

ABSTRACT # 15

QUANTITATIVE METABOLIC PROFILING BY PROTON NMR FOR GENETIC AND FUNCTIONAL STUDY OF TOMATO FRUIT QUALITY

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Metabolic profiling using quantitative one-dimensional 1H-NMR was used for two purposes: biochemical phenotyping of an entire offspring for genetic study of tomato fruit quality, and comparison of wild types and mutants for functional genomics of the relation between fruit size and fruit composition.
Proton NMR allows the detection of most proton-containing metabolites present in a tissue crude extract above a minimum threshold level (Fan 1996). 1H-NMR spectra were recorded at 500 MHz on a Bruker Avance spectrometer using a 5 mm inverse probe. A special care was taken to control sample pH and obtain quantitative data. Fifteen to eighteen compounds, corresponding to major soluble sugars, organic acids, amino acids and some secondary metabolites, could be quantified in tomato fruit samples.
For genetic studies, data from metabolic profiles were used for detection of quantitative trait loci (Paterson et al. 1988) controlling the variation of fruit composition in a BC2 progeny issued from a cross between accessions of the cultivated species Lycopersicon esculentum and the wild species Lycopersicon hirsutum. We mapped quantitative trait loci for 17 compounds. For functional genomics, comparison of the fruit composition of Condine Red mutants (pet and pa2) affected for fruit size and wild type revealed tissue specific responses.

References

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Paterson AH et al., 1988. Nature 335, 721-726.

ABSTRACT # 16

METABOLOMICS OF TOMATO FRUIT VOLATILE COMPOUNDS

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Volatile compounds are important contributors to the tomato fruit flavour. In this study, ripe fruits of 96 tomato cultivars of three types (cherry, round and beef tomatoes) have been used. These were firstly analyzed for sensory attributes by a professional panel and then used to profile the volatile compounds using an automated SPME-GC-MS approach. After optimization of the system, this approach was shown to be a highly reproducible and reliable technique for volatile compound analysis.
For an initial targeted analysis of 18 specific volatile compounds previously proposed to contribute to fragrance and taste, the ion specific peak areas were subjected to PCA. Clear correlations of known volatile compounds with the different tomato types as well as specific cultivars were identified.
For an untargeted analysis “MetAlignTM” software was used for automated baseline correction and alignment of all 203 available GC-MS datasets in one automated run. The software detected over 20,000 masses. All masses appeared to cluster into about 350 mass groups, representing volatile compounds. PCA of the group-specific masses often revealed a clustering of chemically related compounds or compounds coming from the same biochemical pathway. Besides confirming the targeted approach it allowed identifying new compounds correlated to the targeted compounds and sensory attributes.

ABSTRACT # 17

A METABOLOMIC APPROACH TO THE IDENTIFICATION OF TRAITS IN TOMATO INTROGRESSION LINES USING TOF-MS

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We are using LC-MS to investigate differences in metabolite profiles in two tomato species, Lycopersicon esculentum and Lycopersicon pennellii, and a population of introgression lines. The population comprises L. esculentum plants containing defined chromosomal introgressions from the wild relative L. pennellii, each line containing a single introgression, and covering the whole genome over 50 lines*. We are employing metabolite profiling as part of our studies investigating determinants of fruit quality and yield, with a view to mapping metabolite changes to areas of the genome and relating these changes to plant performance.

Samples were taken from the pericarp of ripe fruits, and extracted in methanol/ chloroform/ water, yielding two extracts an aqueous and a non-polar. Extracts were analysed by chromatography and ESI-TOF-MS in both electrospray positive and negative ion mode. Data were processed using Waters MarkerLynx software, which incorporates a deconvolution package that allows detection and alignment of peaks across the samples. The data were then combined into a single matrix of mass and retention time pairs with associated normalised intensities for each component detected across all the samples.

PCA analysis of the two parent species showed clear separation of both aqueous and non-polar fractions from fruit. Differences were observed in the amounts of several metabolites, including those with masses corresponding to organic acids, sugars and amino acids.

Profiling of samples from the introgression lines is underway and data arising from this study will be discussed.

*Eshed & Zamir (1995) Genetics 141, 1147-1162

ABSTRACT # 18

HIERARCHICAL METABOLOMICS AND MACHINE LEARNING FOR PHENOTYPING AND FUNCTIONAL GENOMICS

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Genome sequencing and systematic mutagenesis programmes provide the opportunity to examine altered phenotypes in organisms with single gene knock outs in order to determine function. For metabolomics to be effective for predictive gene classification/phenotyping it is essential to develop a strategy that will cope both with the high dimensionality of metabolomics data and its inherent variance (both biological and instrument) when produced over long periods of time.
A hierarchical approach to this problem is described using high-throughput metabolite fingerprinting by flow injection ESI-MS and supervised data analysis approaches to initially classify plants into phenotype clades. This process is followed by more targeted metabolite profiling (GC-MS and LC-MS) coupled with machine learning data analysis to determine a subset of metabolites with discriminatory/explanatory value for classification of individual genotypes.

Although unsupervised clustering analysis can be used to separate metabolome data representative of 10-15 different genotypes with a fairly small number of replicate samples (provided they were all grown and analysed in the same batch) it remains a major challenge to make predictive phenotype class assignments with much larger mutant populations using data produced over a long period of time.

We have been evaluating a range of supervised data modelling approaches for robustness and predictive accuracy when handling metabolomics data sets with limited numbers of replicates. Progress with Decision Tree methods and Discriminant analysis, coupled with variable selection using Genetic Algorithms (GAs) to reduce data dimensionality, will be described.

ABSTRACT # 19

PROFILING OF DIURNAL PATTERNS OF METABOLITE AND TRANSCRIPT ABUNDANCE IN POTATO LEAVES REVEALS SPECIFIC SET OF METABOLIC PATHWAYS ARE TRANSCRIPTIONALLY REGULATED BUT SUGGESTS THAT THE MAJORITY OF THE METABOLIC NETWORK IS UNDER POST-TRANSCRIPTIONAL CONTROL.

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Diurnal changes in carbohydrates and a broad range of primary metabolites were analyzed through a diurnal period in potato leaves (Solanum tuberosum cv. Desiree) using a recently established gas chromatography-mass spectrometry based metabolic profiling protocol alongside conventional spectrophotometric technologies. In tandem we profiled transcript levels using both a custom array containing approximately 2500 cDNA clones predominantly representing transcripts involved in primary metabolism and commercially available arrays containing approximately 12000 cDNA clones that gave coverage of transcript levels over a broader functional range. The levels of many metabolites and transcripts varied during the diurnal period with 56 significant differences observed in the metabolite contents and 966 significant differences recorded in transcript levels. Whilst a large number of the differences would be expected from what has been known previously, several novel changes were observed in these experiments. Notably, qualitative comparison of the combined data sets obtained from the parallel analysis of transcripts and metabolites suggests relatively few changes in gene expression strongly correlate with changes in metabolite levels during a diurnal cycle. Furthermore, these changes appear to be contained to the central metabolic pathways. However, principal component analysis of the metabolic profiles obtained here revealed that metabolite patterns change progressively through a diurnal period. These results suggest that although leaf metabolism is clearly tightly regulated throughout the diurnal period the majority of the metabolic network is regulated at the post-transcriptional level.

ABSTRACT # 20

METABOLOMICS OF HOST-PATHOGEN INTERACTIONS IN BRACHYPODIUM DISTACHYON

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Rice blast caused by the fungus Magnaporthe grisea can inflict disease in many species of the grass (poacea) family Brachypodium distachyon has been described recently as a good model plant for host-pathogen interactions in grasses and is a compatible host for rice blast.

Using metabolomics to study dynamic systems presents a considerable challenge. Bearing in mind the spatial and temporal complexity of the fungal/plant interaction we have validated a sampling strategy to allow generation of fingerprints/profiles that can be compared meaningfully. This has included the development of procedures to sample leaf tissue with similar numbers of infection sites at the same stage of development that contain similar total metabolite contents, bearing in mind water loss caused by necrosis.

Further investigation aimed to validate that data produced from different sets of infected plants of the same host/pathogen interaction can be combined for data mining. This has included the development of appropriate test, training and validation data sets for supervised data analysis, which can cope for much of the machine drift that can occur over time, particularly in relation to fingerprinting techniques such as flow injection ESI-MS

The current focus is the development of a metabolite matrix for GC-tof-MS (peak list) for this novel biological system. It is important to be able to unambiguously annotate metabolites in profiles to be used for data mining. Thus we require a robust methodology to develop automatically peak lists representative of both Brachypodium and rice blast for GC-tof-MS raw data.

ABSTRACT # 21

A ceramics supported soil-less cultivation system for Arabidopsis thaliana and its application for metabolomics.

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Arabidopsis thaliana is obviously one of the most important model plants for plant metabolomics. However, it is difficult to observe a real metabolome of Arabidopsis correctly due to its variable tendency caused by environmental factors such as light, temperature, drought, nutritional and the other biotic or abiotic stresses. An exact conditioned cultivation system should be required for reduction of metabolic variances. Several trials by means of soil-less culture systems using rock wool, vermiculite, agar etc have not afforded a solution satisfied. Among several factors that should be normalized, a water control is one of the most important and is difficult to be controlled. We present a new cultivation system without soil based on the ceramics. The ceramics tubes have superior durability and uniformity. In the ceramics cultivation system, water would be pulled up and held by capillary action of micro porous body of support. Consequently, the immediate modulation of c
ultivation condition can be achieved by changing culture medium. We succeeded in repeatable cultivation of Arabidopsis with the reproducibility in growth rate and morphology. The 'GC-MS based metabolomics' of ceramics system and of soil system were performed to reveal that the metabolome variation of ceramics-grown Arabidopsis was obviously smaller than the one of soil-grown. In addition, the ceramics system can reduce a space to be around half of soil system. And the new system without soil can cut down the cleaning up procedure after experiment. In near future we will run an outsourcing business to provide ceramics Arabidopsis for metabolomics study.

ABSTRACT # 22

THE EFFECT OF GROWING SYSTEMS ON METABOLITE VARIATIONS OF ARABIDOPSIS THALIANA

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Arabidopsis thaliana, a model organism in plant biology, is widely used for studies of the cellular and molecular biology. According to its sensitiveness, it has evolved some responses to environmental factors including day-length, temperature, drought, and nutritional supply. Growth-stage variations and morphological changes as a result of altered environmental conditions could be observed and identified readily. In many cases, however, results may be occurred without major morphological changes. The objective of this study is to investigate the effect of growing systems on Arabidopsis primary metabolites. The comparison of metabolites in Arabidopsis grown in a conventional method (soil-based) and those in a ceramic supported propagation system (ceramic-based) was done. In addition, metabolites of Arabidopsis suspension-cultured cells (cell line T87) were investigated. For soil-based and ceramic-based plants, the primary metabolites were extracted from rosette leaves. While,
the 7-days T87 cultured cells were collected, washed, and filtrated before extraction. The polar phased was derivatized and analyzed by GC/MS. Pre-processing of chromatographic data were done before principal component analysis. The PCA data reveal that metabolites of ceramic-based Arabidopsis show smaller variations than those of soil-based plants. It also implies the uniformity of a liquid fertilizer used in the ceramic-based system comparing to a soil. T87 cultured cells show the similar primary metabolites to the soil-based and ceramic-based plants but differences in the metabolite levels. Concerning to their uniformities with high abundance, T87 cultured cells could be applicable for preliminary study in plant metabolomics.

ABSTRACT # 23

IN VIVO 15N-ENRICHMENT OF METABOLITES IN ARABIDOPSIS CULTURED CELL T87 AND ITS APPLICATION FOR METABOLOMICS

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A mass spectrometer is one of the best analytical tools for that. However, its quantitative accuracy might be lacked due to ion suppression caused by insufficient separation on the chromatography. Though a complete separation of all metabolites by ultra high resolution chromatography would be the best solution to avoid an ion suppression, the present technology couldnt provide an sufficient resolution. Here we present a practical solution for quantitative analysis by means of a stable isotope dilution including in vivo 15N-labeling. We employed Arabidopsis thaliana cultured cell T87 as a model plant cell. 15N-enrichment was readily performed by cultivation with the modified LS-media containing 15N-labeled inorganic nitrogen source, K15NO3 and 15NH415NO3. Five days cultivation was enough to completely replace all nitrogen atoms with 15N isotope. No significant morphological change on T87 cell was observed through the 15N-enrichment. A mixture of the extract of 15N culture d cell and of 14N cultured cell was subjected to capillary-LC/MS/MS analysis. Metabolites including 15N atoms were co-eluted at the same time of the corresponding 14N-metabolites. Sufficient linearity was obtained in the relative quantification system. In addition, a time-course sampling was also performed to reveal an apparent turn over rate of the metabolites including nitrogen atoms. A time course was started from the zero time on which culture media was changed from 14N-media to 15N-media. Interesting variations of nitrogen turn over rate in the several metabolites was observed. The 15N-stable isotope dilution system will be a powerful tool for both metabolomics and flux analysis.

ABSTRACT # 24

Cloning, Expression and Characterization of a Putative Flavonoid Glucosyltransferase from Grapefruit (Citrus paradisi) Leaves

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Flavonoids, plant secondary metabolites, are an integral part of our lives. They are chemically modified by glycosylation, hydroxylation, methylation, etc. During glycosylation the sugar moiety from UDP-sugar is transferred to aglycone flavonoid substrates by specific enzymes and those catalyzing the transfer of glucose are known as glucosyltransferases (GTs). Flavanone-specific 7-O-glucosyltransferase (7GT) catalyzes the first glycosylation reaction leading to production of bitter naringin in grapefruit. Primary structure of 7GT couldnt be determined due to low yield, N-terminal blockage, etc. This research was designed to obtain full-length grapefruit GT cDNA clones, express them and characterize them. Specific primers were designed from the Plant Secondary Product Glucosyltransferase (PSPG) box to amplify grapefruit GTs. RNA was extracted from young grapefruit leaves. SMART RACE RT-PCR was used to obtain 5 clones. Clone specific primers were used to obtain the 3 ends. C ompiled sequences were obtained by matching overlaps of partial clones. Primers were designed from compiled sequences to obtain full-length cDNA clones. To date, one full-length clone was inserted into expression vector pET32A and transformed into the expression host BL21(DE3)RIL. The 34 kDa protein was tested for GT activity using 6 different flavonoid aglycones: naringenin, naringenin chalcone, apigenin, kaempferol, quercetin, and hesperetin using 14C UDP-glucose as glucose donor. Young grapefruit leaf extract was tested with naringenin as control for 7GT assay. Results indicated that the expressed protein was probably not a flavonoid GT. An EST library has been constructed and is being evaluated. To date, there are 3-4 more additional unique clones being analyzed.

ABSTRACT # 25

LARGE SCALE INVESTIGATION ON THE RECOVERY OF METABOLITES ACCESSIBLE BY VARIATIONS OF GC-MS PROFILING PROTOCOLS

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More than 1000 GC-MS metabolite profiles were performed in the course of 3 years in the effort to understand the robustness of the method and to optimise recovery, reproducibility, and range of metabolites that were accessible to metabolome analysis. Experiments were performed on two major plant organs, namely root and leaf of tobacco plants from Nicotiana tabacum cv. SNN. The variations of the initial metabolite profiling protocol (Fiehn et al., 2000; Roessner et al. 2000) encompassed the mode of extraction solvent, liquid partitioning into a polar and lipophilic fraction, and the choice of silylation reagent. In detail, we investigated the use of N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide as compared to N-Trimethylsilyl-N-methyltrifluoroacetamide, omission of liquid partitioning, and permutation of solvent composition, pH and temperature during extraction. All variations were performed in direct comparison to the initial protocol of GC-MS metabolite profiling.

We applied multivariate statistical techniques, such as hierarchical cluster analysis, principal components analysis, and analysis of variation, to unravel similar and highly divergent profiling protocols. In an alternate approach the range of accessible metabolites was investigated. In addition metabolites were classified according to observed recoveries and robustness of recovery. Finally we make suggestions for optimal protocols based on the choice of metabolites that are required to be accessible to GC-MS profiling.

Each laboratory involved in metabolome analyses approaches different biological questions and has a different set of additional analytical technologies available that may already cover certain metabolite classes. Therefore, we are convinced that our investigations do not only improve the GC-MS profiling protocol in general, but will finally allow a fine tuning of GC-MS analysis according to the demands of each research group.

ABSTRACT # 26

CLONING AND CHARACTERIZATION OF CYTOCHROME P450S FROM SORGHUM POTENTIALLY INVOLVED IN THE BIOSYNTHESIS OF THE ALLELOCHEMICAL SORGOLEONE

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Sorgoleone is an allelopathic natural product synthesized by root hair cells of sorghum (Sorghum bicolor) and exuded into the soil where it suppresses the growth of competing species. Recent NMR labeling studies have provided insight into the biosynthetic pathway of sorgoleone1. A 16:3 fatty acid precursor is likely synthesized by fatty acid synthase and a plastid-localized desaturase, exported to ER and converted into 5-pentadecatriene resorcinol by a polyketide synthase. This resorcinol intermediate is subsequently methylated by a SAM-dependent O-methyltransferase and dihydroxylated by a P450 monooxygenase to yield the reduced form of sorgoleone. In an attempt to isolate genes encoding these enzymes, we performed an expressed sequence tag (EST) analysis on approximately 5,500 sequences randomly selected from a cDNA library generated from purified root hair cells. In this study, we identified 15 ESTs from the EST database as putative cytochrome P450 monooxygenases according to BLAST analysis. Among these EST clones, three are specifically expressed in root hairs, as demonstrated by quantitative real-time RT-PCR. Full-length clones corresponding to these root hair-specific ESTs were obtained by RACE PCR. Sequence analysis revealed that they belong to the CYP71 and CYP72 P450 families. Functional characterization of these P450s is currently underway. (1. Dayan, F. et al., J. Biol. Chem., 2003)

ABSTRACT # 27

TRITERPENOID SAPONIN GLYCOSYLTRANSFERASES IN OATS

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The biosynthesis of avenacins, triterpenoid saponins produced in oat roots, involves three predicted glycosyltransferases (GTs) catalyzing the sequential transfer of one alpha-L-arabinose and two beta-D-glucose molecules onto a triterpenoid aglycone derived from beta-amyrin. The avenacins are antimicrobial phytoprotectants and the terminal glucose molecules are critical for their antifungal activity. Avenacin-deficient oat mutants are compromised in disease resistance, indicating a role in plant defence.

Twenty-six candidate GT genes that are expressed in oat roots have been identified using an EST mining approach. Searches utilized representative plant sequences from the Family 1 inverting mechanism GT genes in the CAZy Database. Prediction of function based on sequence information is difficult because of the diversity of this enzyme family and the lack of functionally characterized plant GTs. We are therefore taking a systematic approach to functionally characterize the oat GT candidates via heterologous expression in bacteria.

Oat GT candidates have been prioritised based on phylogenetic relatedness to other plant Family 1 GTs, gene expression patterns and genetic mapping information. Metabolite profiling methods have been used to identify multiple alleles of oat mutants that are defective in avenacin glycosylation and provide another powerful tool to identify the oat avenacin GT genes. In vitro activity assays have so far revealed one purified candidate GT that appears biochemically significant with respect to avenacin glucosylation.

ABSTRACT # 28

Evolution of secondary metabolites in a Mimosoid legume, the genus *Inga*

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Secondary metabolites plays a key role in determining plant defenses, especially for young, expanding leaves that lack toughness. We are investigating the full suite of chemical defenses in a genus of tropical rainforest trees in the context of phylogenetic relationships within the genus, permitting evolutionary inferences to be made. Results to date show wide divergence among species of *Inga*. We have found N-methyl hydroxyproline, tyrosine and cinnamoyl glycosides of catechin in monomeric and polymeric forms in *I. umbellifera*. In contrast, *I. goldmanii* accumulates hydroxypipecolic acids and proanthocyanidins. Neither species has saponins, but we recently have found abundant saponins in other species of *Inga*. Even though *Inga* species show few differences in the genes used for phylogenetic reconstructions and appear to have recently diverged, the large differences in chemistry suggest rapid evolution of genes that determine plant chemistry. The chemical arms race between plants and animals probably has selected for large changes in *Inga* defensive metabolites over a relatively short period of evolutionary time. These observations suggest that a detailed knowledge of *Inga* chemistry will help understand the evolutionary mechanisms that have led to chemical diversity within *Inga*.

ABSTRACT # 29

Reverse Genetic Analysis of Wax Synthases in Arabidopsis

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Neutral esters of fatty acids occur widely in the biosphere, the most common being triacylglycerol esters that constitute the fats and oils that organisms use to store carbon and energy. Although more rare, most organisms also accumulate fatty acids esterified to long-chain alcohols (wax esters). These molecules are abundant in whale blubber, beeswax and in the oil that accumulates in the seeds of the dessert bush, jojoba (*Simmondsia chinensis*). In addition, wax esters are a major component of the cuticle of many plants and insects. The cuticle is the lipid component that plants (and insects) secret and deposit on their aerial surface, and it act as the water-barrier separating the plant from its environment. Wax synthase is the enzyme that is assumed to catalyze the formation of wax ester from fatty acyl-CoA and fatty alcohol. Using the jojoba wax synthase cDNA sequence as an entry point into sequence databases, twelve *Arabidopsis* genes were identified to putatively encode wax synthase genes. This genetic complexity of wax synthase gene in *Arabidopsis* may indicate that the plant has the capacity to produce a diverse range of wax ester. Namely, each of these twelve genes may encode for isozymes that are involved in the biosynthesis different wax esters from different precursors, i.e. different fatty acyl-CoAs and different alcohols. We are using T-DNA insertion mutant *Arabidopsis* together with analysis of subcellular and cellular localization of the mRNA and protein products to test this hypothesis. The gene coding one putative isozyme was expressed in *E. coli* and the corresponding antibody was produced. A few alleles with T-DNA inserted in the putative wax synthase genes or promoter regions have been identified and the change of capability to produce wax ester will be studied. In addition, we have analyzed the wax esters that accumulate in the cuticle that coats different organs of maize and have determined the effect of 30 *glossy* mutations on the accumulation of these molecules.

ABSTRACT # 30

Molecular and genetic characterization of the two paralogous genes coding for the BCCP subunit of the heteromeric acetyl-CoA carboxylase of Arabidopsis

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The heteromeric acetyl-CoA carboxylase (htACCase) catalyzes the first and committing reaction of *de novo* fatty acid synthesis in plastids. This enzyme is composed of four subunits, biotin carboxyl-carrier protein (BCCP), biotin carboxylase (BC), alpha-carboxyltransferase (alpha-CT) and beta-carboxyltransferase (beta-CT). Genes coding three of these subunits are nuclear, but a plastome gene encodes the beta-CT subunit. In Arabidopsis, single-copy genes encode all these subunits, with the exception of the BCCP subunit. The two BCCP-coding genes (At5g16390 codes for BCCP-1 and At5g15530 codes for BCCP-2) are located within 0.32Mb of each other on chromosome 5. There are four potential explanations for this apparent redundancy in BCCP genes: 1) One of the BCCP-coding genes is non-functional and is thus a pseudogene; 2) the two BCCP-coding genes provide the identical function in the same tissue, i.e., the genes are redundant; 3) the two BCCP-coding genes provide different functions in the same tissues; or 4) the two BCCP-coding genes provide the same function in different tissues. The first of these hypotheses can probably be discounted based upon initial observations, which indicate that both genes are expressed [1]. To distinguish between the latter three hypotheses we have characterized the spatial and temporal pattern of expression of each BCCP gene using gene-specific antibodies and by *in situ* hybridization to each mRNA. In addition, we are characterizing T-DNA-tagged mutations in the two BCCP-coding genes. These characterizations indicate that BCCP-1-function is essential and cannot be substituted by BCCP-2. But, BCCP-2-function appears to be redundant to BCCP-1, in that BCCP-1 can substitute for the lack of BCCP-2.

[1] J.J. Thelen, S. Mekhedov, J.B. Ohlrogge, Plant Physiol. 125 (2001) 2016-2028.

ABSTRACT # 31

THE FURTHER DEVELOPMENT OF METABOLOMICS AS A FUNCTIONAL GENOMICS TOOL

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Our goal is to promote plant metabolomics as a true functional genomics tool that provides a comprehensive characterization of the biochemical phenotype of a plant. The realization of this goal will require improved technical abilities in the determination of metabolites in complex plant tissues and the integration and dissemination of metabolomics research data. For this we foresee three important objectives: (I) improvement in the comprehensive coverage of plant metabolomics, (II) facilitation of obtaining comparable results between labs, instruments and experiments, and (III) enhancement in the integration of metabolomics information with other functional genomics approaches. As these challenges are widely recognized and endorsed, this encourages a community-based effort to define common criteria and to initiate a number of concerted actions directed towards the release of standard reference materials, the construction of a consolidated metabolite library and the development of metabolite specific data management systems. The International Committee on Plant Metabolomics of which the authors of this article are members (http://www.metabolomics.nl/) represents a platform to facilitate the proposed actions.

ABSTRACT # 32

METABOLIC PROFILING OF A MUTANT MEDICAGO POPULATION OVER-EXPRESSING ARABIDOPSIS TRANSCRIPTION FACTORS

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Medicago truncatula is the model plant for molecular and genetic studies of legumes, which are important agricultural protein crops. Furthermore, legumes synthesize several unique (pharmaceutical interesting) compounds, such as iso-flavonoids, saponins, triterpenes, alkaloids and phytosterols. In order to study regulation of metabolite biosynthesis pathways, and to generate metabolic biodiversity we produced a mutant population of Medicago. This population was generated by introduction of 500 transcription factors (TFs) of Arabidopsis into Medicago hairy root cultures. Metabolic fingerprints of mutants were obtained using high-resolution accurate mass LC-MS (QTOF Ultima with lock spray source).

The dedicated MetAlign software was used to align all chromatographic mass peaks and to find differences in peak areas in mutants. A small group of mutants has been analyzed in detail for changes in the saponins and flavonoid content by LC-MS (IonTrap), followed by principle component analysis. TF lines showing significant changes in the metabolite profile are re-transformed to verify the authenticity of the TF effect.

We show that untargeted metabolic fingerprinting by LC-MS coupled to MetAlign software is a sensitive and reproducible method to detect metabolic mutants in a large population. The specific effect of TF over-expression in Medicago will be analyzed in more detail by targeted metabolomics.

ABSTRACT # 33

Expression and Characterization of a Phenylalanine Aminomutase Functioning in Taxol Biosynthesis

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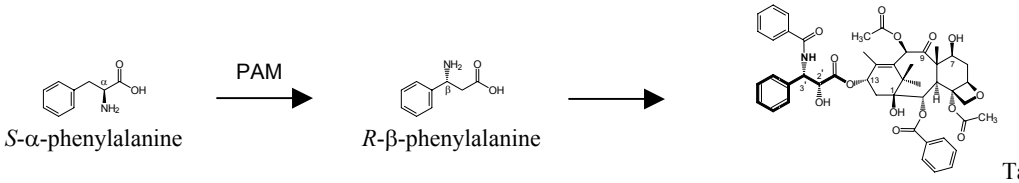
Taxol, an anticancer drug produced by *Taxus* species, is a structurally complex diterpenoid compound comprising, in part, various acyl functional groups, including at C13 the *N*-benzoyl-(2*S*,3*S*)-phenylisoserine sidechain, which is considered to derive from stereospecific conversion of *S*-α-phenylalanine to *R*-β-phenylalanine by phenylalanine aminomutase (PAM) catalysis.



S-α-phenylalanine *R*-β-phenylalanine Taxol

To date, amino acid aminomutases can be categorized mechanistically as 1) radical-based catalyst using either cobalamin or S-adenosylmethionine, PLP and iron-sulfur clusters as cofactors, 2) ATP-dependent, and 3) requiring no cofactors and relying solely on a methylideneimidazol-5-one (MIO) functional group that forms autocatalytically within the active site. A recently isolated tyrosine aminomutase (TAM) clone from *Streptomyces* falls into the latter cofactor-independent category and shows a high sequence homology to a family of ammonia lyases. Previous biochemical analysis to identify PAM in *Taxus* cell free extracts revealed that the *Taxus*-derived mutase also has no cofactor requirements. Therefore, an approach to cloning the corresponding gene was based on the assumption that PAM would resemble the well-known plant phenylalanine ammonia lyase (PAL). The acquisition from a *Taxus cuspidata*, and functional expression in *Escherichia coli*, of PAL-like sequences yielded the cDNA encoding the target aminomutase that encodes a 698 amino acid peptide with a deduced molecular weight of 76.5 kDa.

ABSTRACT # 34

^{1}H NMR METABOLIC FINGERPRINTING FOR GENOTYPE CLASSIFICATION

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Partial least squares discriminant analysis (PLS-DA) has been used together with linear discriminant analysis (LDA) for the classification of different pea lines by ^{1}H NMR metabolic fingerprinting. We show that discrimination not only between the different plant species but also of genotypes within a species is possible. Standard multivariate data analysis methods have been extended for the classification and interpretation of the data. By taking a global view of the PLS models, we have automated a feature selection procedure that provides a subset of points in the NMR spectra with high discriminatory power. We show that using such multivariate methods for dimensionality reduction gives comparable classification results to the original spectra. The aim is to investigate the metabolic differences between genotypes and we show that the method allows easy interpretation of the data. Comparisons are made with commonly used methods such as principal component analysis and hierarchical cluster analysis.

ABSTRACT # 35

VARIATIONS IN THE PHENYLPROPANOID METABOLISM OF ANTHRISCUS SYLVESTRIS; IN-DOOR AND FIELD EXPERIMENTS.

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In a previous study we showed that different populations of Anthriscus sylvestris (L.) Hoffm. (Apiaceae) yielded significantly different phenylpropanoid profiles1. To study a possible genetical factor, we collected the seeds of A. sylvestris from 4 locations, which germinated and grew under the equal laboratory conditions. After 5 months the plants were harvested and profiled using GC-MS. To determine the influences of the development of the plant we collected during one year weekly 5 plants from 2 locations. We profiled roots and aerial parts.
The plants from the seeds of the 4 locations showed significant different phenotypic characteristics (e.g. dry-weight, β-sitosterol content). However the 4 groups did no differ significantly in their phenylpropanoid profile in their roots. Apparently there is no real selection pressure on the lignan concentration in these populations.
Due to the large variation in lignan profiles between each of the individual plants it is difficult to determine if the development of the plant influences the lignan profile. It is clear that the aerial parts of the plant contain equal amounts of lignans as the roots in the early spring and during the season the lignan concentrations in the aerial parts decrease. The lignan profile of the roots does not show such a clear trend. Especially the lignans present in lower amounts (like hinokinin and angeloyl podophyllotoxin) varied highly during the season. The large variations in lignan content of A. sylvestris could be to the advantage of plant and make it more difficult for herbivores to adapt to the toxicity of these compounds.
1 Koulman A, Bos R, Medarde M, Pras N, Quax WJ. Planta Med. 2001;67:858-62.

ABSTRACT # 36

NON-DESTRUCTIVE METABOLIC PROFILING OF THE RHIZOSPHERE USING SOLID-PHASE MICRO EXTRACTION

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Solid Phase Micro Extraction (SPME) is a convenient extraction method for metabolomics. SPME does not demand the destruction of the plant material and therefore allows multiple analysis of a single plant and opens the possibility to analyze the metabolome during the plants development. The application of SPME is already known for headspace analysis of volatile components emitted by the aerial parts of the plant. We developed a methodology that is also applicable for the rhizosphere.
Arabidopsis thaliana plants were germinated and grown on solid agar medium for a period of five weeks at 22 C with a light regime of 16 hours light and 8 hours dark. SPME fibers with different coatings were placed in direct vicinity of the stem into the agar and left there for an hour at room temperature. After this extraction period the fiber was placed in the GC injector port for 45 seconds at 260 C, with a splitless injection mode. The resulting chromatogram showed 12 clear quantifiable peaks (over 10 times the background noise) and 20 detectable peaks (over 3 times the background noise).
The retention indexes were determined for the different peaks and compared to literature data on Arabidopsis exudates1. The main peaks were aliphatic alcohols (e.g. decanol) and phenylpropanoids (e.g. syringilaldehyde) for which the identity was confirmed on basis of reference compounds. The identification of the other compounds will take place using GCMS analysis. The developed method will be applied to study the interaction between Arabidopsis roots and the soil bacterium Bacillus subtilis.
1Walker TS, Bais HP, Halligan KM, Stermitz FR, Vivanco JM. J. Agric. Food Chem. 2003;5:2548-54.

ABSTRACT # 37

MASS SPECTRAL LIBRARIES FOR METABOLITE IDENTIFICATION AND CHARACTERIZATION IN COMPLEX GC-EI-MS PROFILES

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One of the most severe limitations, that each laboratory involved in GC-MS based metabolite profiling meets, is the identification and characterization of hundreds of metabolite derivatives. Occurrence of multiple isomers in biological preparations impedes unambiguous identification by mass spectral match alone. The crucial step of metabolite identification is currently solved by large-scale series of time consuming standard addition experiments. These experiments map commercially available or purified metabolites to specific mass spectra and the sequence of chromatographic retention. Currently no platform exists that facilitates exchange of metabolite identifications and of information on yet non-identified metabolic components. We present a world wide web based collection of mass spectral libraries, which were compiled from pure reference compounds and a range of plant as well as non-plant samples (MSRI; http://csbdb.mpimp-golm.mpg.de/databases/CSBDB_MetaDBs.html). We demonstrate three general applications of these libraries. (1) The composition of still non-characterized biological samples, for example blood plasma or microbial extracts, is screened for identified constituents, and tentative best matching compounds. (2) The occurrence of identified metabolites is analysed in a large range of biological samples, such as different plant organs or species. For this purpose we provide libraries comprising samples of from tomato, related wild type Lycopersicon species, and other Solanaceae, collections of different organs of Lotus japonicus, Arabidopsis thaliana, and preparations from microbial species. (3) The subsequent analysis of samples on two different GC-MS systems facilitates transfer of identifications made on the first system to the second and vice versa. We present data on identifications, which were made in-parallel on quadrupole GC-MS and GC-TOF-MS systems. We are convinced that our effort will not only be highly useful for those laboratories, which enter the field of metabolomics, but will also lead to an effective exchange of information between expert laboratories.

ABSTRACT # 38

SCREENING TOMATO FRUIT FOR FLAVOUR AND HEALTH USING INTEGRATED METABOLOMICS APRROACHES

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For the consumer, the most important tomato fruit quality traits are their flavour and their positive health impact. Breeding for these traits is nowadays a major goal of tomato seed companies. By screening ripe fruits from 96 different tomato cultivars, using both targeted and non-targeted LC- and GC-based metabolic profiling techniques, we aim to find correlations between genes (molecular markers) and known or unknown metabolites and metabolic pathways specifically related to these key quality traits.
Targeted screening of tomatoes for health-related compounds was directed towards carotenoids, tocopherols and flavonoids, using HPLC with PDA and fluorescence detectors, while screening for flavour compounds was directed towards sugars, organic acids and amino acids, using GC-TOF/MS with derivatized extracts.
Non-targeted metabolic fingerprinting of all cultivars was achieved by using high-resolution accurate mass LC-QTOF/MS as well as GC-TOF/MS. The dedicated MetAlignTM software was used to perform mass-based chromatographic alignments and to create mass peaks lists for unsupervised analyses of both LC- and GC-derived data sets. Subsequent multivariate analyses, including clustering and PCA, so far revealed many (new) correlations between mass peaks and cultivars.
These targeted and non-targeted screening approaches revealed clear genotype-associated variation in metabolites correlating with tomato fruit quality.

ABSTRACT # 39

ELUCIDATION OF ANTHOCYANIN ACCUMULATION MECHANISM BY OMICS APPROACH USING <I>PAP1</I> OVER-EXPRESSING MUTANTS (2) -FUNCTIONAL ANALYSIS OF CANDIDATE GENES-

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The <I>pap1-D</I>, activation tagging mutant over-expressing Myb-like transcription factor PAP1, specifically over-accumulates anthocyanins. Genes up-regulated in <I>pap1-D</I> were expected to be involved in biosynthesis and sequestration of anthocyanins. Microarray analysis revealed that genes up-regulated in <I>pap1-D</I> included those encoding enzymes involved in anthocyanin biosynthetic pathway, transcription factors such as Myc and WRKY, and glycosyltransferases (GTs), acyltransferases (ATs) and glutathione S-transferases (GSTs) whose function were remained undetermined. Those GTs, ATs and GSTs were expected to be involved in modification of aglycons and sequestration of anthocyanin.

Out of those candidate genes, genes encoding GTs were cloned, and recombinant proteins were expressed in <I>E. coli</I>. Cyanidin 3GT activity was detected with crude extract of <I>E. coli<I> expressing one of candidate GTs. Recombinant cyanidin 3GT accepted cyanidin, pelargonidin, delphinidin, kaempferol, quercetin and myricetin as substrate.
Metabolomic analysis of <I>A. thaliana</I> mutants that defective in each gene was also performed. In a mutant defective in the gene presumably encoding anthocyanidin 3GT, total amount of anthocyanins was reduced, and 4 flavonol glycosides with glucose attached at 3-position were reduced. In other mutant lacking transcripts of the gene possibly encoding anthocyanin 5GT, composition of accumulated anthocyanins was different from that of WT. The anthocyanins lacking modification at 5-position were accumulated more in the mutant than inWT. It was suggested that those genes encode authentic cyanidin 3GT and anthocyanin 5GT, respectively, <I>in planta</I>.

ABSTRACT # 40

BIOLOGICAL SYNTHESIS OF ALKENES: A CASE STUDY OF THE APPLICATION OF METABOLOMICS IN PATHWAY DISCOVERY

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Long-chain hydrocarbons containing carbon-carbon double bonds (e.g., alkenes dienes) are rare biological molecules. Hence the biosynthetic origins of these molecules are obscure. Detailed metabolomic analyses of the cuticular waxes that accumulate on the surfaces of maize aerial organs have lead to the identification of a system that is ideally suited for experimentation for uncovering the biosynthesis of alkenes. Namely, we have discovered that alkenes and dienes constitute a large proportion (50%) of the cuticular waxes that coat the surfaces of pollen and silks of maize. These alkenes and dienes are part of four homologous series of alkenes and dienes of 19 to 31 carbon-chain lengths, with the double bond situated in different positions of the alkyl chains. Moreover, these metabolomic analyses have provided insights into the structure of the biosynthetic pathway that gives rise to these cuticular wax components. These analyses indicate that alkenes and dienes are probably biosynthesized by a combination of fatty acid elongation and desaturation reactions, which are followed by the sequential reduction and decarbonylation of the resulting unsaturated fatty acids. This hypothesized pathway for alkene biosynthesis is being further dissected and confirmed by the metabolomic analysis of the cuticular waxes of the silk and pollen of a collection of *glossy* mutants that affect the normal accumulation of cuticular waxes.

ABSTRACT # 41

METABOLOMIC CLASSIFICATION OF TOBACCO PLANT ENGINEERED WITH POTATO VIRUS Y CP DNA BY NEAR INFRARED SPECTROSCOPY

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Near infrared (NIR) spectroscopy as a fingerprinting method, providing a total chemical profile of a sample was employed for metabolomic classification of transformed and non-transformed tobacco plants with coat protein (CP) of potato virus Y (PVY). The examined tobacco plants were grown up in cultivation room under controlled light, temperature, humidity and soil conditions. The trasflectance NIR spectra were read from fresh, ground whit silica gel and lyophilised leave samples. The raw NIR spectra of transformed and wild genotype tobacco leaves for all of the used sample preparations did not show marked differences. The NIR spectra of fresh tobacco leaves were very similar to that of water. The spectral features were with enhanced differentiation by utilizing second-derivative algorithm. The regression model was employed for wavelength selection and the classification rule was developed by carrying out of discrimination analysis. A proper classification of studied genotypes was achieved only with NIR transflectance spectra of fresh tobacco leaves. The trasngene and wild genotype was correctly classified whit 93 % accuracy (estimated by crossvalidation). The miss-classification of ground with silica gel and lyophilised tobacco leave samples was greater than 30 %. Because the sample preparations affect only hydrogen-bonding environment and content of water respectively and did not exchange the chemical composition of the sample, the correct metabolomic classification of the gene-modified plants appear to be due to detected structure of water continuum in fresh tobacco leaf. Moreover in transgenic plants CP specific RNA transcript were produced, but accumulation of viral CP was not detected (by double antibody sandwich ELISA and Western blot analysis of total immunoprecipitated total leaf protein), which mean that the variation of biological information on the RNA sequence level is also represented at the phenotypic level read by the NIR transflecvtance spectroscopy. The NIR spectra of genomic DNA extracts are under investigation for their properties for discrimination of transformed and wild genotypes.

ABSTRACT # 42

Elucidation of anthocyanin accumulation mechanism by "omics" approach using <I>PAP1</I> over-expressing mutants (1) -Integration of transcriptomics and metabolomics-

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Integration of transcriptome and metabolome can provide the information of whole gene-to-metabolite networks and hence identify the function of unknown genes. The <I>PAP1</I> gene, which encodes an MYB transcriptional factor, up-regulates the flavonoid biosynthetic gene expression. In this study, we integrated metabolomics and transcriptomics of wild-type Arabidopsis, <I>pap1-D</I> mutant and <I>PAP1</I>-overexpressed transgenic plant, to elucidate a detailed anthocyanin accumulation mechanism.
By metabolome analysis using HPLC/PDA/ESI-MS (21 flavonoids) and FT-MS (~1,800 peaks), it was confirmed that <I>PAP1</I>-overexpressing lines highly accumulated cyanidin derivatives in the leaves and roots whereas global metabolic profile excluding anthocyanin did not change remarkably.
Transcriptome analysis using DNA microarray revealed 38 up-regulated genes in <I>PAP1</I>-overexpressing lines. Some of them were known flavonoid biosynthetic genes, whereas others were genes encoding putative glycosyltransferase, acyltransferase, glutathione S-transferase, transporter and transcriptional factor, of which functions are not confirmed.
These results showed that the <I>PAP1</I> gene is a transcriptional factor which regulates specifically the anthocyanin accumulation. Therefore, the induced genes are expected to be involved in production of these anthocyanins. Our approach by integration of transcriptomics and metabolomics provides an innovative way for comprehensive identification of genes involved in plant metabolism.

ABSTRACT # 43

Metabolomics and QTL Mapping What are the genes controlling metabolic variation?.

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A major goal of numerous crop improvement programs is to alter the nutritional status of the various crops. Invariably, this requires that the metabolic content of the crop be altered. Due to the developing consumer intransigence to transgenic foodstuffs, the most alternative method is to utilize inter- and intra-specific genetic variation (aka Natural Variation) in combination with standard breeding to modify the metabolic content. However, very little is known about the genes that control metabolic variation. We are utilizing high-throughput metabolomics to map and clone genes involved in controlling natural metabolic variation in Arabidopsis. This typically requires the use of Quantitative Trait Locus (QTL) mapping. We will present data that addresses the following questions. How pleiotropic are the QTLs? Are the underlying genes regulatory or biosynthetic? Is it possible to utilize QTL information from one pathway to make generalizations about the QTLs for another pathway
? What is the best statistic to identify THE QTLs for a metabolic pathway?

To address these questions, we are focusing on three major secondary metabolite pathways, glucosinolates, phenylpropanoids and camalexin. These three pathways differ in their inducibility with camalexin having a 1000 X or greater inducibility while glucosinolates are by in large 1.5 to 3 X inducible. We have developed high-throughput metabolite profiling technologies to analyze the 70 detectable compounds in these pathways from a single extraction. This has allowed us to identify QTL controlling the production of each of the 70 compounds as well as compounds controlling their induction. This has identified > 100 QTL. We will present data on the identity of the genes controlling these QTLs and what this information tells about the ability to generalize about metabolic QTLs. We will also present data about the average level of transgressive segregation and epistasis in controlling plant metabolism. Further, we have been able to utilize the QTL information in conjunction with c
omparative mapping of biological QTL to identify functions for each of these compounds.

ABSTRACT # 44

FUNCTIONAL GENOMICS OF SOYBEAN COMPOSITION

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Due to the complex genetic and environmental determinants of seed composition, this trait has eluded molecular understanding. We have developed near-isogenic soybean isolines that differ markedly in their seed composition. These lines are ideally suited for dissecting the molecular determinants of soybean composition. These isolines were developed from two independent recombinant inbred populations that were established between three parent lines, Evans, the high-protein line PI 153.296, and the low protein line PI 438.472. The resulting isolines are being used to profile differences in gene expression at the level of mRNAs, proteins and metabolites, in order to identify biochemical differences associated with different seed composition. To conduct RNA profiling experiments we have constructed an expressed sequence tag-based microarray containing about 9,000 unique cDNAs. We have also developed 2-D-PAGE-based proteomic capabilities to monitor changes in protein accumulation
patterns. And finally, we have developed GC-MS-based metabolomic capabilities to monitor changes in metabolite accumulation patterns. These profiling technologies have first been applied to monitor the mRNA, protein and metabolite profiles during the development of soybean seeds in the parental line Evans. This time-course study included 5 time points, from 25 to 50 days after flowering. These data provide the basis for comparing the molecular differences between Evans and the isogenic lines that express different seed composition traits. This understanding will provide valuable insights into the processes that regulate soybean seed composition.

ABSTRACT # 45

MetaCyc and AraCyc: Metabolic Pathway Databases for Plant Research and Application

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MetaCyc (http://metacyc.org/) is a literature-based metabolic pathway database. It currently has ca. 500 pathways (including pathway variants) from over 150 organisms. The goal is to include all experimentally identified pathways and provide a reference metabolism database. A recent enhancement to the database was to include primary metabolic pathways specific to plants. Pathways are associated with species information, brief descriptions, and classification hierarchy; reactions are annotated with EC numbers; compounds are associated with chemical structures; and enzymes are curated with physical and catalytic properties as well as their coding genes. MetaCyc can be used as a reference for predicting the metabolic pathway content of a given set of annotated genes (e.g. a newly sequenced and annotated genome, a set of annotated ESTs). This has been applied to the Arabidopsis genome to generate the Arabidopsis metabolic pathway database AraCyc (http://arabidopsis.org/tools/aracyc/). Manual curation of AraCyc after its initial build is an on-going effort at the Arabidopsis Information Resource (TAIR). Plant secondary metabolism is our next curation focus. We are currently applying an evidence code system to AraCyc, which provides an assertion for the existence of a pathway or an enzyme activity. The bulk data exchange mechanisms between MetaCyc and all of its derived databases, such as AraCyc, facilitate easy data sharing. The expression overview feature in AraCyc allows overlaying of a list of genes and numeric values onto the pathways, ideal for examination of large-scale Arabidopsis gene expression data or a gene family data.

ABSTRACT # 46

AN UNSUPERVISED APPROACH FOR THE DIFFERENTIAL ANALYSIS OF MULTIPLE LC/MS-DERIVED METABOLIC PROFILES

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The success of non-targetted metabolic fingerprinting of large numbers of plant samples, as will be required for population screening, biodiversity studies etc, is dependant both on the accuracy and reliability of the analyses performed and on our capacity to extract the relevant differences from the data obtained for the different lines. Considering the complexity of e.g. full-scan GC-MS or LC-MS fingerprints and the often large numbers of samples concerned, only an unsupervised approach shall prove feasible. To this aim we are applying the dedicated software package metAlign for data manipulation and differential comparison of peak patterns to detect subtle changes in generally unchanging metabolic fingerprints. MetAlign enables the rapid and unsupervised simultaneous analysis of 100’s of either GC-MS or LC-MS datasets and combines background elimination, chromatographic peak alignment, extraction of mass peak information and statistics.
Unsupervised data extraction was performed on the metabolic profiles derived from a progeny of a cross between two genetically distinct potato lines (CxE). A selection of these progeny, previously described only in phenotypic / physiological terms, has been analysed in a non-targetted manner using high resolution LCTOF/MS of aqueous methanol extracts. Applying MetAlign followed by PCA enabled biological and genotypic differences to be assessed. Clusters of mass peaks could be recognised which coincided with known physiological differences. PCA also enabled us to recognise those mass peaks most likely to be responsible for the cluster divergence. Subsequently MS/MS analysis was applied to identify putatively the metabolites concerned. Results indicate that this approach is a reliable screening method for the large–scale analysis of complex metabolomics datasets.

ABSTRACT # 47

METABOLIC CHANGES IN LEAVES DURING PHOTOPERIODICALLY INDUCED GROWTH CESSATION

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In many woody species subjected to large seasonal variations of temperature, the initiation of cold acclimation and dormancy are synchronised with the end of the growth season and the onset of low temperatures in the autumn. Long days (LD) sustain shoot elongation, whereas short days (SD) induce growth cessation and formation of terminal buds as a prerequisite for further cold acclimation and bud set. The site of daylength perception is considered to be in the leaves, and the response to occur in the shoot apical meristem.
To identify putative photoperiodically regulated signal compounds in the model tree hybrid aspen (Populus tremula x P. tremuloides) we have used a metabolomics concept. Hybrid aspen were grown in long days (LD; 18h (12 h + daylength extension)) and short days (SD; 12h). Leaf samples (1-20) were sampled in LD and SD (2 and 6 days).
Samples were extracted and analysed by GC/TOF-MS, and thereafter the non-processed GC/MS files were subjected to multivariate analysis according to a recent published method (Jonsson et al. 2004). To identify more compounds, a fractionation method based on SPE-extraction was used for the analysis of metabolites from extracts of leaf 10. Together with the SPE-analysis and the traditional metabolomics analysis, more than 20 identified compounds were shown to be consistently photoperiodically regulated in leaves. The metabolites were both primary and secondary metabolites. Potential important photoperidocally regulated signal compounds will be presented, and the role of these compounds in short-day induced growth cessation will be discussed.

ABSTRACT # 48

METABOLIC PROFILING OF THE SHORT TERM *Arabidopsis thaliana* RESPONSE TO ENVIRONMENTAL PERTUBATIONS

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Abstract:

Metabolic Profiling has emerged as a high throughput technique for the measurement of the cellular metabolic state. In this study, metabolic profiling was used to probe the short-term response of the *A. thaliana* to perturbations. Specifically, the experiment involved growing a set of liquid culture plants for 12 days in Gamborg media under constant light and 23°C. On the 13th day, the control set of plant liquid cultures were fed air of ambient composition. The perturbed set of plant liquid cultures were fed air with composition a) 1% CO_2, 21% O_2 & 78% N_2 in case of elevated CO_2 experiment and b) 0.03% CO_2, 3% O_2, 97% N_2 in case of Hypoxia experiment. The plants were harvested at different time intervals during the course of 13th Day.

Gas Chromatography – Mass Spectrometry was used to measure the average (over the entire plant) metabolic plant profiles, using protocol described by Roessner et. al. [1]. We thus obtained time series profile for 150 polar metabolites out of which 53 had known structure. The time-series data was analyzed using multivariate statistical algorithms incorporated in the multi experiment viewer of TIGR TM4 software [2]. This analysis allowed for the identification of the group of metabolites whose concentration changed drastically in response to each environmental stresses. Specifically in response to elevated CO_2 stress, the concentration of metabolites related to cell wall production increased, and of metabolites related to photorespiration decreased. The concentrations of the nitrogen storage metabolites in plant decreased, indicating reduced nitrogen assimilation in the presence of elevated CO_2, similar to what has been reported from long-term response studies.

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ABSTRACT # 49

METABOLIC PROFILING: PLATFORM TECHNOLOGIES TO DETECT UNINTENDED EFFECTS FOLLOWING GENETIC MODIFICATION OF POTATO (SOLANUM TUBEROSUM).

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Current concerns regarding the acceptance of genetically modified crops are based, at least in part, on whether unexpected or unintended adverse changes have taken place following the genetic modification. These unintended alterations may arise from insertion mutagenesis or as a result of metabolic effects of the novel gene product(s). All encompassing procedures to detect and assess the safety of any unintended effects are currently unavailable due to paucity of information on the nature of genes associated with the synthesis of, for example, natural toxic compounds and in addition genes (pathways) that are normally silenced in specific tissues/organs will not generally be assessed for unintended changes and therefore may go unnoticed. To address this we have exploited combined state-of-the-art LC-MS, GC-TOF-MS and 1H NMR approaches to study metabolite changes in (field grown) potato tubers following several distinct transformations: sense and antisense potato fructokinas
e (FK), maltase (Mal1), S-adenosyl methionine decarboxylase (SAMDC), granule bound starch synthase (GBSS) and the appropriate vector-alone and tissue culture controls. In addition, field grown potato cultivars and landraces were analysed to determine whether the unintended effects were within the boundaries of natural variation.

ABSTRACT # 50

High throughput metabolite profiling of the suspension-cultured Arabidposis thaliana T87 cells by combination of various mass spectrometric technologies

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Our laboratory has initiated a functional genomics program to study the suspension-cultured Arabidposis thaliana T87 cells at the transcriptome and metabolome levels. Homogeneous cultured cell in sterile medium are advantageous of controlling experimental conditions over the whole plant even grown under environment-controlled conditions. We analyzed preliminary metabolite profiling data of non-targeted metabolite extracted from Arabidposis T87 cells under the different growth times after subculturing.
Metabolic profiling of Arabidposis T87 cells was performed using multidimensional analysis technique based on the sensitivity and selectivity of mass spectrometry (MS). These analytical systems include reverse-phase HPLC coupled to UV photodiode array detection (PAD) and electrospray ion-trap mass spectrometer, capillary electrophoresis (CE) coupled to electrospray ionization mass spectrometer, and gas chromatography (GC) coupled to time-of-flight (TOF) mass spectrometry. Profiling of the phenolic compounds in Arabidposis T87 cells using HPLC/PAD/MS detected kaempferol-type flavonoid metabolites accumulating in leaf of wild-type A. thaliana. However, quercetin-type flavonoid metabolites could not be detected in Arabidposis T87 cells. Cationic compounds were detected by CE/MS as more than 700 metabolite peaks using selected ion monitoring (SIM) technique.
Principal component analysis (PCA) was applied to the data of metabolite profiling to find the relationships between the samples. PCA of Arabidposis T87 cells in normal growth conditions and leaves of A. thaliana indicated the different group between cell culture and leaves using sets of GC/TOF/MS data. From PCA result, Arabidposis T87 cells were able to easy control of biological variation for metabolome analysis.

ABSTRACT # 51

CHEMOMETRIC ANALYSIS FOR UNDERSTANDING COMPLEX BIOLOGICAL SYSTEMS

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Significant progress has been made in developing analytical techniques to quantify the biochemical processes in living systems. For example, microarray techniques measure the relative gene expression levels, 2D gel-electrophoresis coupled with mass spectroscopy (MS) quantifies the production of cellular proteins, and nuclear magnetic resonance spectroscopy (NMR) or mass spectroscopy both provide the metabolic status (fingerprint) of an organism. All these techniques are biologically complementary and central tools for the study of complex biological systems. They share similarities in the complexity and in the substantial amount of data produced. It is clear that traditional statistical tools that assume independent variables are not well suited for these kinds of data. Chemometrics is an established field in data analysis and provides tools to make good use of measured complex data, and to quantitatively model and produce visual representations of the extracted information. In addition, it also provides means of collecting relevant information through statistical experimental design.

Principal Component Analysis (PCA) and Partial Least Squares (PLS) [Ref. 1] have been successfully applied to ongoing research projects at Umeå Plant Science Centre (http://www.upsc.nu). The focus is often on the interpretation of the multivariate models, e.g. what are the metabolic differences between wildtype and transgenic poplar. Lately, some pitfalls on interpreting PLS models have been identified. These pitfalls perturb the interpretation and are related to the complexity of the biological system. The recently published O-PLS method [Ref. 2] (a modification of the PLS method) has shown to better handle these pitfalls and simplify the model interpretation. The motivation for O-PLS and its advantages compared to PLS, and similar methods, will be discussed and demonstrated in examples.

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ABSTRACT # 52

THE UK NATIONAL CENTRE FOR PLANT AND MICROBIAL METABOLOMICS

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The recently funded UK National Plant and Microbial Metabolomics Centre aims to build and operate a high-throughput primary and secondary metabolite fingerprinting service for the plant and microbial community. The role of the Centre will be to provide both a service, based on ongoing research, and a training facility. It is based at Rothamsted Research where a purpose-built analytical chemistry facility will operate an integrated process of metabolite fingerprinting and high-resolution structural annotation, which will serve the needs of both large scale screens and targeted analysis. The core of the facility will be a hyphenated 600MHz NMR spectrometer configured to collect fingerprint data from crude extracts and to provide structural data on purified samples. Further targeted analysis by MS techniques will then be carried out on selected samples with signals of potential interest. Centre facilities in bioinformatics are located at Aberystwyth University and will provide
the expertise for the development of data models and the construction and refinement of a metabolomics database. Together with the UMIST team, advanced data analysis methods will be applied to further mine metabolomic data, including that from the FT-MS research instrument at UMIST.

Staff at the Centre will carry out both large and small-scale metabolomic analyses of any plant and microbial material. Multi-level access to the facility will allow users to submit, plant material, extracts, or data for analysis and/or visit the Centre for training. The Centre will also seek to develop metabolomic projects with groups having interests in systems biology, metabolic engineering and bioinformatics.

ABSTRACT # 53

CONSISTENCY IN PLANT GROWTH: THE KEY TO SUCCESSFUL METABOLITE PROFILING

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In order to make accurate conclusions about differences in metabolite profiles between plants from different backgrounds and mutants altered in activity of different genes it is necessary to be able to constantly produce plant material of a consistent nature.

Using Arabidopsis thaliana a number of experiments have been carried out to investigate the reproducibility of the plant growth protocols employed in metabolomics projects. Using a simple extraction method and analysis by 1H NMR spectroscopy, Principle Component Analysis has been used to assess the differences between sets of plants grown under identical controlled environment conditions at different times.
Further comparisons have been made between plant material grown, harvested and analysed following a standard operating procedure at two different sites. In addition data will be presented on the assessment of biological variability using extracts from replicated single plants compared to those made from batched material.

It is evident from the data collected that for meaningful metabolomics, considerable attention needs to be given to the consistency of plant growth conditions and experimental protocols.

ABSTRACT # 54

GENE EXPRESSION AND METABOLITE PROFILING OF TOMATO LEAF TISSUE TREATED WITH THE PHYTOTOXIN CORONATINE

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Coronatine (COR), a phytotoxin produced by several pathovars of *Pseudomonas syringae,* often functions as a virulence factor in host-pathogen interactions. In addition to chlorosis, COR induces an amazing array of effects in plants including anthocyanin production, alkaloid accumulation, ethylene emission, inhibition of root elongation, and induction of proteinase inhibitors. COR consists of the polyketide coronafacic acid (CFA), a structural and functional analogue of jasmonic acid (JA), and coronamic acid (CMA), an analogue of 1-aminocyclopropane-1-carboxylic acid. In our biological assays, COR inhibited root growth, induced anthocyanin accumulation, and elicited chlorosis. In gene expression profiling studies, approximately 485 unique genes showed a significant change in expression, and the results suggested that COR impacts signaling in tomato via multiple phytohormone pathways. Genes involved in JA biosynthesis and/or JA-wound responsive genes were further analyzed using real-time quantitative PCR analysis showed 4-5 ($\log_2$) fold induction upon treatment with COR. Preliminary results of metabolic profiling using GC-MS indicate an increase in endogenous levels of JA, related octadecanoids, and other volatile metabolites following COR treatment of tomato leaves. Root growth of the *jai1* mutant, a tomato line insensitive to JA, was not inhibited by COR; furthermore, COR did not induce chlorosis or anthocyanin accumulation in *jai1* plants. In summary, our results using biological assays and gene expression and metabolic profiling confirm that COR targets the JA pathway and induces a wide array of biological activities.

ABSTRACT # 55

Metabolite profiling during leaf development in transgenic and wild type Arabidopsis

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Metabolic profiling provides a powerful tool to understand the ultimate consequence of altering gene expression. In this study, changes in the metabolite profiles during leaf development between transgenic and wild type Arabidopsis are compared.
ATP citrate lyase (ACL; EC 4.1.3.8) is a heterodimer that generates acetyl-CoA and OAA from citrate and CoA utilizing ATP. Each ACL subunit (ACL-A and ACL-B) was targeted to plastids and transformed into Arabidopsis plants. Transgenic lines identified as overexpressing ACLA and ACLB were intercrossed. Progeny from this cross were identified that overaccumulated both ACL subunits. Young and mature leaves of these transgenic Arabidopsis and sibling wild type plants were collected for metabolite profiling. Plants were sampled before bolting (stage3, according to Boyes et al., 2001) and after flowering (stage 6). Metabolites were extracted with methanol and then divided into hydrophilic and lipophilic phases. GC/MS and HPLC were used to detect metabolites in both phases. GeneGobi and FC modeler, emerging software for analysis of gene expression data were used to delineate changes in metabolites during leaf development of wild type Arabidopsis and ACL overexpressing plants.

ABSTRACT # 56

CHANGES IN THE METABOLOME OF Arabidopsis IN RESPONSE TO LOW TEMPERATURE: ENVIRONMENTAL STRESS VERSUS COLD ACCLIMATION?

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Metabolism is the fundamental process dictating material and energy fluxes through organisms. Our goal was to examine the effects of cold acclimation on metabolism from a global perspective; incorporating changes from all metabolic pathways using an unbiased, non-targeted approach afforded us by Fourier transform ion cyclotron mass spectrometry (FTMS) technology. Multivariate data analyses indicates in Arabidopsis a global reprogramming of metabolism occurs as a result of cold acclimation. We have been able to separate transient environmental stress responses from true acclimation responses at the level of the metabolome. Whereas leaves shifted to low temperature present metabolite profiles that are constantly changing, leaves developed at low temperature demonstrate a stable metabolite complement. By measuring an entire spectrum of metabolites versus an individual or group of metabolite(s), a global unbiased assessment of metabolic processes relative to cold acclimation was determined. It appears that some metabolic networks are dynamically able to channel with the environment (post-development), but most require significant hard-wiring that can only be achieved through development under low temperature conditions. Understanding how metabolism as a whole is regulated allows the integration of cellular, physiological and ecological attributes in a biological system, a necessity if complex traits are to be modified by breeding or genetic manipulation.

ABSTRACT # 57

Targeting of ATP-Citrate Lyase to the Plastids of Arabidopsis thaliana

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Acetyl-CoA is necessary for the production of many important plant chemicals, and since organelle membranes are impermeable to this large molecule, it must be synthesized in the compartment in which it will be utilized. The enzyme ATP-citrate lyase (ACL) produces a cytosolic pool of acetyl-CoA in the plant Arabidopsis thaliana. ACL catalyzes the ATP-dependent conversion of citrate and CoA to OAA and acetyl-CoA. Plastids are the site of fatty acid synthesis from acetyl-CoA; the expression of ACL in that organelle might, alter the rate of acetyl-CoA formation, and hence affect synthesis of fatty acids and other processes that require plastidic acetyl-CoA. The functional ACL enzyme consists of two subunits A and B (ACL-A and ACL-B respectively) individually encoded by genes named ACL-A1 and ACL-B2. ACL-A1 and ACL-B2 have been targeted to the plastids of A. thaliana using a transit peptide from the small subunit of Rubisco. Several lines have been obtained that are over-exp ressing the RNA for both ACL-A1 and ACL-B2. We are evaluating the protein levels and activity of the ACL enzyme in these lines, in concert with global expression studies, to understand how expression of the ACL gene in plastids may affect metabolite flux through acetyl-CoA-requiring metabolism in that organelle.

ABSTRACT # 58

Metabolomic Profiling of Hypericum Species

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Hypericum perforatum, commonly known as St. Johns Wort, is a popular herbal supplement today in the United States taken for its reported anti-depressant and anti-viral properties. This species contains a wide range of phytochemicals including naphthadianthrones, phloroglucinols, flavonoids, biflavones, phenylpropanes, and proanthocyanidins. However, the full complement of bioactive compound(s) has not yet been elucidated. Furthermore, little has been done to standardize the quantity of putatively bioactive compounds in commercial preparations of this herb.

There are over 300 additional species in the Hypericum genus world-wide, growing mostly in the temperate climates of the northern hemisphere, most of which have been little characterized biochemically. We are using metabolomic profiling to determine metabolic variation among different accessions and species of Hypericum. Using HPLC-PDA and HPLS-MS-MS we are comparing retention times, mass spectra, and electromagnetic radiation absorbance spectra of compounds of extracts from different species of Hypericum. This enables an initial identification of new compounds, and the quantification and comparison of known compounds. We are currently focusing on the variety of flavonoid components of different Hypericum species.

ABSTRACT # 59

PLANT CHARACTERISTICS AND ARTEMISININ PRODUCTION IN TRANSGENIC *ARTEMISIA ANNUA* WITH OVEREXPRESSION OF THE FARNESYL PYROPHOSPHATE SYNTHASE (*FPS*) GENE

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The production level of the artemisinin, an antimalarial substance derived from *Artemisia annua* was aimed to improve by control the plant metabolic pathway. The *Agrobacterium tumefaciens*-mediated gene transformation for the overexpression of the Farnesyl pyrophosphate synthase (*FPS*) gene, the proposed key enzyme in artemisinin biosynthesis was investigated. The artemisinin content in leaflets of transgenic plants detected by HPLC was compared to the control and other induced mutation-derived plants. Transgenic with overexpression of the *FPS* gene significantly performed high artemisinin levels, which was 2-3 times higher than other mutative clones and control. The relative mRNA expression of *FPS* was performed to characterize the relation between the expression level of this gene and artemisinin accumulation. The tendentious correlation of them was observed in transgenic *FPS* clones, whereas the differential expressions among clones were detected in mutative lines. This result suggested that the overexpression of *FPS* gene could able to convey the metabolic flux to artemisinin. Nevertheless, alteration of plant morphological characteristics in some transgenic lines revealed that the expression of *FPS* gene may affect to alter other metabolic pathway such as cytokinin and gibberellins biosyntheses, which regulate in plant hormonal system and need to be further investigated for better understanding in the metabolic flux

ABSTRACT # 60

METABOLOMIC PROFILING OF LAMB BLOOD PLASMA BY GC/TOF-MS

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Gas Chromatography/ Time of Flight Mass Spectroscopy (GC/TOF-MS) has been used to profile blood plasma samples that were taken from lambs grazing on improved upland pasture from July to October 2003. GC/TOF-MS is a non-scanning mass spectral technology that has been applied with great success to the analysis of metabolites in plant extracts. It combines excellent chromatographic resolution and reproducibility with high rates of spectral data acquisition.

In this study we have determined that GC/TOF-MS is well suited to the acquisition of metabolomic profile data from blood plasma samples. We found that a non-supervised comparison of deconvoluted data from the plasma samples against a mass-spectral/ retention time index database compiled from GC/TOF-MS profiles of plant extracts was able to identify many common metabolites. In addition, other metabolites, not present in the plant library, were detected in the plasma samples.

The impact of plant chemical composition on the grazing animal is poorly understood and the availability of a comprehensive GC/TOF-MS library that contains metabolites from both animals and plants will be an invaluable tool for its characterisation. We propose to participate in the construction of such a library and use it to investigate the transfer of primary and secondary metabolites from plants to the blood metabolome of animals grazing at pasture on diets of known composition.

ABSTRACT # 61

FORMATION OF ARSENIC-PHYTOCHELATIN COMPLEXES BY PLANTS IN RESPONSE TO ARSENIC STRESS

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Plants form phytochelatins (PCs) of different chain length as response to exposure to heavy metals, like copper (Cu), arsenic (As) and cadmium (Cd). Until recently the complex formation was derived more from indirect measurements. We have developed a method for direct determination of As-PC complexes, which combines high-resolution reversed-phase chromatography with an element-specific detector (ICP-MS) for the detection of As and an organic mass-spectrometer (ES-MS) for the detection of the complexes by their molecular ions. Using this new technique we successfully separated in vitro formed As-PC complexes using a mix of GSH (reduced gluthatione), PC_2, PC_3 and arsenite as starting material for the first time. Arsenic forms preferential $As^{(III)}$-PC_3, followed by GS-$As^{(III)}$-PC and $As^{(III)}$-$(PC_2)_2$ complexes.

Separation of extracts of the vegetative parts of the arsenic hyperaccumulator Pteris vittata and an arsenic tolerant strain of Holcus lanatus showed that the GS-As-PC_2 complex, which was somewhat a surprise in the in vitro experiment, is formed as well in vivo in plants during arsenic exposure. In Pteris vitatta GS-$As^{(III)}$-PC complex was found, whereas Holcus lanatus contained $As^{(III)}$-PC_3 and GS-$As^{(III)}$-PC. Non-tolerant Helianthus annuus (cultivar Giant Yellow) formed after exposure a whole range of As-PC compounds, especially in the roots, among others $As^{(III)}$-PC_3, GS-$As^{(III)}$-PC, $As^{(III)}$-$(PC_2)_2$. $As^{(III)}$-PC_3 seems to be the preferred complex formed by plants in response to arsenic exposure. The main reason for this is the increased stability of this complex compared to other possible complexes, like GS-$As^{(III)}$-PC_2 and $As^{(III)}$-$(PC_2)_2$. Studying xylem sap in H. annuus showed that As is transported in non-bound inorganic form as As(III) and As(V) and not complexed to PCs.

ABSTRACT # 62

IDENTIFICATION OF METABOLITES ASSOCIATED WITH FREEZING TOLERANCE IN CONSTITUTIVELY FREEZING TOLERANT MUTANTS OF ARABIDOPSIS

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Many plants develop a better freezing tolerance through a process of cold acclimation (CA), which also results in many changes in metabolite levels. To identify and distinguish metabolites that contribute to the increased freezing tolerance from those that are merely responsive to low temperature stress, we investigated the changes of metabolite profile in several constitutively freezing tolerant (cft) mutants and Col wild type(Col-WT) plants of Arabidopsis on HPLC system equipped with ELSD, UV and Fluorescence detectors. Metabolites associated with constitutive freezing tolerance and/or associated with induced freezing tolerance in response to cold acclimation were identified by comparing the metabolite profiles 1) in those mutants with non-acclimated and cold acclimated Col-WT Arabidopsis, 2) with the ability of CA to enhance the freezing tolerance in Col-WT and cft mutants, and 3) with decreased freezing tolerance in Col-WT and cft mutants during de-acclimation process af
ter 48 hours of CA treatment. Preliminary results indicate that, in addition to accumulating metabolites commonly known associated with temperature stress, such as proline, raffinose and maltose, non-acclimated cft mutants also accumulated several other unique compounds in significant amount. Some of these compounds were also identified in cold acclimated WT plants. The changes of these compounds during cold acclimation and de-acclimation in WT plants were correlated with the development of freezing tolerance. This integrative approach, which combines genetic, metabolic profiling, and physiological analysis, provides a powerful tool to identify metabolites that play a key role in temperature stress tolerance in higher plants.

ABSTRACT # 63

Metabolomics Approach to Analysis of Bioactive Constituents of Echinacea

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Historically, Echinacea was used by Native Americans as an herbal medicine to treat colds, coughs and snake bites. Currently, Echinacea extracts are a popular herbal-remedy used for general enhancement of the immune system. However, the specific compounds responsible for immunostimulatory properties of Echinacea extracts are still unknown and the levels of phytochemicals in commercial Echinacea extracts are not standardized. As part of a larger NIH-funded study on the efficacy and mechanism of action of Echinacea, we are investigating the accumulation of potentially bioactive compounds in the context of developmental, environmental and genetic diversity. Our long-term goal is to explore the metabolic network of alkamides (a group of putatively bioactive compounds) in Echinacea. Using HPLC and GC-MS, we have characterized developmental and genetic factors affecting the accumulation of the alkamides. Analysis of five stages of flowers of E. purpurea indicates that two tetraene alkamides predominate: N-isobutyl-(2E,4Z,8Z,10E/Z)-dodecatetraenamide; alkamide concentrations are up to 3-fold higher in mature flowers than in flower buds. An analysis of 120 individual plants from 40 accessions from nine Echinacea genotypes indicates that at least six species (E. angustifolia, E. sanguinea, E. simulata, E. tennesseensis, E. atrorubens and E. laevigata) accumulate three 3 diacetylenic isobutylamides (E-N-isobutyl undeca-2-ene-8,10-diynamide, Z-N-isobutyl undeca-2-ene-8,10-diynamide and E-N-isobutyl dodeca-2-ene-8, 10-diynamide). Interestingly, the qualitative distribution of alkamides among different accessions of E. pallida, one of the major medicinally used species, varies greatly. These studies will facilitate our understanding of how potentially bioactive metabolites change during development and across genotypes.

ABSTRACT # 64

Impact of interaction of salt and water stress in brassica juncea

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Seedling response of two *Brassica juncea* genotypes (CS-52 and RH-30) for interaction of water and salt stress was studied under controlled set of conditions. Iso-osmotic levels of stress were created by using polyethylene glycol for water stress and sodium chloride for salt stress. To study the interactive effect, water stress adapted seedlings were exposed to salt stress for two days and vice versa. Observations were recorded for proline, sodium, potassium and chloride content and SDS profile. It was observed that water stress adapted seedlings exposed to salt stress exhibited higher potassium to sodium ratio for CS-52 rather than RH- 30. Both the varieties showed higher proline and sodium content when salt stress adapted seedlings were exposed to water stress than water stress adapted seedlings exposed to salt stress, whereas reverse pattern was observed for chloride content; however values were on higher side in RH-30 as compared to CS-52. SDS profile of shoots of water stress adapted seedlings of CS-52 variety when exposed to salt stress lead to appearance of 80 Kd polypeptide and disappearance of 24 Kd polypeptide whereas in reverse case there was only slight appearance of 80 Kd and 22 Kd peptide. In case of roots of CS-52, complete disappearance of 80 and 26 Kd peptide was observed when water stress was followed by salt stress. In case of RH-30 shoots, when salt stress followed water stress disappearance of 70 Kd polypeptide whereas it appeared under water stress followed by salt stress.

ABSTRACT # 65

CLASSIFICATION OF ILEX SPECIES BASED ON METABOLOMIC FINGERPRINTING USING 1H NMR AND MULTIVARIATE DATA ANALYSIS

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The metabolomic analysis of eleven Ilex species such as I. argentina, I. brasiliensis, I. brevicuspis, I. dumosa var. dumosa, I. dumosa var. guaranina, I. integerrima, I. microdonta, I. paraguariensis var. paraguariensis, I. pseudobuxus, I. taubertiana, and I. theezans was carried out by NMR spectroscopy and multivariate data analysis. The analysis using principal component analysis (PCA) and classification of the 1H NMR spectra showed a clear discrimination of those samples based on the metabolites present in the organic and aqueous fractions. The major metabolites that contribute to the discrimination are arbutin, caffeine, phenylpropanoids, and theobromine. Among those metabolites, arbutin which has not been reported yet as a constituent of Ilex species was found to be a biomarker for I. argentina, I. brasiliensis, I. brevicuspis, I. integerrima, I. microdonta, I. pseudobuxus, I. taubertiana, and I. theezans. This reliable method based on the determination of a large number of metabolites makes it possible to perform chemotaxonomical analysis of Ilex species.
Key words: Metabolomic analysis. Ilex species. Arbutin, Phenylpropanoids, NMR. Principal component analysis. Classification.

ABSTRACT # 66

THE IMPACT OF SAFENERS IN ARABIDOPSIS USING METABOLIC FINGERPRINTING WITH GC/MS-TOF

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The objective of this research was to examine how herbicide safeners perturbed metabolism in Arabidopsis. Safeners are members of chemically diverse groups including dichloroacetamide derivatives (dichlormid, benoxacor), phenylpyrimidines (fenclorim), quinolinoxycarboxylic acid esters (cloquintocet-mexyl), phenyl pyrazoles (fenclorazole-ethyl). Research has shown that safeners induce the activity of cytochrome P450 mono-oxygenases, glucosyl transferases and glutathione S-transferase to protect grass crops. Safeners also increase cysteine and glutathione, and affect the regulation of gamma-glutamyl-cysteine synthetase.

In this study we analyzed the effect of the safeners benoxacor, dichlormid and fenclorim, and the glutathione biosynthesis inhibitor buthionine sulfoximine (BSO) on the metabolic fingerprint of Arabidopsis. Statistically significant increases in the concentrations of peaks identified as glutaric acid, glutamic acid, asparagine, sucrose and isobutylamine as well as reduced concentrations of tryptophan, maleic acid, valine, and glucose 6-phosphate were observed in extracts from safener treated plants. Buthionine sulfoximine induced statistically significant changes in the profile of metabolites, but without peak identification. Using hierarchical cluster analysis, the metabolic profiles obtained from safener-treated Arabidopsis plants grouped together, with the dichlormid and benoxacor samples overlapping and the fenclorim treatment in the same branch. In contrast, the profile obtained from BSO-treated seedlings was distinct from the cluster of safener samples, and aligned

in the same branch as the control sample. This work provided insight into the metabolic networks affected by safeners and contributed to the development of new methods for metabolic fingerprinting.

ABSTRACT # 67

Reverse Genetic Analysis of the Metabolism of Cytosolic Acetyl-CoA

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Acetyl-CoA is an intermediate of both primary and secondary metabolism. Because acetyl-CoA cannot readily cross membranes, compartmentalized biosynthesis is required. ATP-citrate lyase (ACL) catalyzes the ATP-dependent reaction between citrate and CoA to form oxaloacetate and acetyl-CoA. We have shown that ACL is located in the cytosol, and is not detectable in the plastids, mitochondria, or peroxisomes. To further test the hypothesis that ACL is a source of cytosolic acetyl-CoA, we have generated transgenic Arabidopsis plants that have reduced ACL activity due to the expression of an antisense ACLA RNA. These plants show a complex, miniaturized phenotype with similarity to wild type plants treated with mevinolin (an HMG-CoA reductase inhibitor) and mutants with altered sterol metabolism. To address the hypothesis that plants with reduced ACL may be deficient in the accumulation of sterols, metabolite profiling is being conducted and will be discussed. In addition, T-DNA or D
s insertion mutants for each of the ACLA and ACLB (subunits of ACL) genes have been obtained. Cytosolic acetyl-CoA converted to malonyl-CoA via homomeric acetyl-CoA carboxylase (hmACCase), contributes to the elongation of fatty acids and the biosynthesis of a variety of other phytochemicals, including flavonoids and malonic acid. To identify the role of hmACCase derived from the ACC1 locus (one of two ACC loci) in growth and development, plants with T-DNA tagged acc1 alleles were obtained and analyzed. Results indicate that the ACC1 gene is essential for the formation of viable seeds and may be essential in early embryo development.

ABSTRACT # 68

METABOLOMIC DISCRIMINATION OF CATHARANTHUS ROSEUS LEAVES INFECTED BY PHYTOPLASMA USING 1H-NMR SPECTROSCOPY AND MULTIVARIATE DATA ANALYSIS

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A comprehensive metabolomic profiling of Catharanthus roseus (L.) G. Don infected by 10 types of phytoplasmas was carried out using 1D- and 2D-nuclear magnetic resonance (NMR) spectroscopy followed by principal component analysis (PCA), an unsupervised clustering method requiring no knowledge of the data set and used to reduce the dimensionality of multivariate data while preserving most of the variance within it. With a combination of these techniques, we were able to identify those metabolites which were present in different levels in phytoplasma-infected C. roseus leaves than in healthy ones. The infection by phytoplasma in C. roseus leaves causes an increase of metabolites related to the biosynthetic pathways of phenylpropanoids or terpenoid indole alkaloids: chlorogenic acid, loganic acid, secologanin, and vindoline. Furthermore, higher abundance of glucose, glutamic acid, polyphenols, succinic acid, and sucrose were detected in the phytoplasma-infected leaves. The prin
cipal component analysis of the 1H-NMR signals of healthy and phytoplasma-infected C. roseus leaves shows that these metabolites are major discriminating factors to characterize the phytoplasma-infected C. roseus leaves from healthy ones. Based on the NMR and PCA analysis, it might be suggested that the biosynthetic pathway of terpenoid indole alkaloids, together with that of phenylpropanoids, is stimulated by the infection of phytoplasma.

ABSTRACT # 69

THE SEARCH FOR FLOWERING HORMONES IN PEA

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In the garden pea (Pisum sativum L.), there are several single gene, day-neutral mutants which flower much earlier than the wild type in short days. The flowering of wild-type shoots is markedly promoted by grafting to certain mutant rootstocks, while grafting early-flowering shoots to wild-type rootstocks can delay flowering. These results provide evidence for mobile, hormone-like, flowering signals in pea. The chemical nature of these hormones has been an elusive target of flowering research ever since the florigen concept of the 1930s. We are exploring metabolomics approaches to tackling this fundamental problem, exploiting our extensive collection of mutants and their corresponding wild-type isolines. The flowering hormones may well be present in trace amounts, as indicated by our research with other mutants (some of which also have a graft-transmissible basis). Several of our dwarf mutants, for example, owe their phenotype to a deficiency of gibberellin, but even in th
e wild-type, the level of bioactive gibberellin is only 10 to 20 ng per g fresh weight.

ABSTRACT # 70

Insertion of high flux pathways in Arabidopsis thaliana without impact on plant morphology, metabolome and transcriptome.

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Genetic engineering of plant offers the possibility to design plants that produce increased amounts of new compounds. Here we report the effect on the metabolome, transcriptome, and plant morphology of insertion of the dhurrin biosynthetic pathway from Sorghum bicolor in transgenic Arabidopsis thaliana. As previously shown (1), these plants are able to produce up to 4% (dry weight) of dhurrin, a tyrosine derived cyanogenic glucoside believed to be involved in protection of plants against herbivores (1). The dhurrin pathway is catalyzed by three enzymes: two cytochromes P450 and one UDP glucosyl transferase. Arabidopsis lines transformed with the first gene in the pathway, the two first genes or the complete pathway were obtained and analyzed using metabolite profiling and global transcriptome analysis. We demonstrate that it is possible to engineer plants to express a new high flux biosynthetic pathway and to accumulate large amounts of a new natural product without unexpecte
d significant changes in transcriptome and metabolite profiles. If incomplete pathways are inserted, metabolic cross-talk or detoxification reactions are more likely to induce changes in plant morphology, transcriptome and metabolome.

(1) Tattersall D. et al., Science, Vol 293, Issue 5536, 1826-1828, 7 September 2001

ABSTRACT # 71

A New TLC-Densitometric Technique for Simple Artemisinin Analysis

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A simple TLC-densitometric technique was developed for rapid analysis of artemisinin content in various Artemisia annua crude extracts. This new analytical method is based on the structural conversion of artemisinin on a silica gel plate by NH3 to form a chromophore-containing compound that can be detected by UV-based TLC-densitometric analysis. Structure elucidation of the NH3-treated artemisinin product indicated that compound was 10-azadesoxyartemisinin which has its max at 320 nm. Practically, various crude extract samples of A. annua mutants were subjected to artemisinin separation on a silica gel plate. The TLC plate was then exposed with ammonia vapor before being scanned by TLC-densitometer using the wavelength of 320 nm. The technique appeared to be accurate and sensitive as compared with a complicated HPLC-UV technique.

ABSTRACT # 72

Supervised and Automated Unsupervised GC/MS Metabolomics for the Diagnosis of Agrochemical Targets

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The reliable diagnosis of pesticide targets is of paramount importance to the discovery of novel crop protection products. For herbicides a total of about 20 different modes of action (MoA) have been commercially exploited to date. In most cases, inhibition of enzymes of essential biosynthetic pathways constitute the primary herbicide action. It is conceivable that perturbations of metabolic processes cause changes in the metabolite complement of treated plants that are specific to the MoA of a given herbicide class.

We have developed a metabolomics approach to diagnose herbicide MoA based on GC/MS analysis of plant (*Arabidopsis thaliana*) tissue extracts. The complexity of the GC/MS data requires sophisticated methods for an unbiased data extraction. Two different approaches for GC/MS data processing are presented.

The fingerprinting approach using metAlign is dedicated for a fast comparison of GC/MS data acquired under similar chromatographic conditions. Small changes in retention time can be accounted for.

The other approach taken includes peak deconvolution, compound identification and quantification. The decoupling of compound information from the chromatographic separation facilitates a long-term comparison of GC/MS data that is a prerequisite for a reliable classification of metabolic changes caused by herbicide treatment.
Examples will be shown to emphasize the potential of both data extraction strategies.

ABSTRACT # 73

Gene Discovery via Metabolite Fingerprinting of Arabidopsis Activation Tagging Lines

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Metabolomics depends on the analytical methods, including optical spectroscopy, nuclear magnetic resonance, and mass spectrometry. However, a single approach cannot analyze all the compounds quantitatively and qualitatively. This is due to the chemical complexity of the metabolome, the biological variations inherent in plant organism and the dynamic range limitations of most instrumental approaches. Consequently, choosing a most appropriate technology for a specific research is important in efficient analysis of the target samples. Fourier Transform Infrared (FT-IR) Spectroscopy is relatively quick and provides metabolic fingerprints that can determine similarities or differences of concerned samples via pattern recognition software. We used FT-IR spectroscopy in metabolic fingerprinting of wild type and 500 activation tagging lines of Arabidopsis. The most significant differences occurred in the range of 2000-880 cm-1. Principal Component Analysis (PCA) combined with Discri
minant Function Analysis (DFA) and Hierarchical Cluster Analysis (HCA) in MATLAB software revealed that most of the activation tagging lines at T1 generation were clustered together to form a single mass in PC1 vs PC2 plotting, whereas approximately 10% of the lines show significant differences. To understand molecular nature of the metabolite difference in these lines, T-DNA flanking sequences were obtained through a modified thermal asymmetric interlaced PCR (TAIL-PCR) protocol. Some of the genes were identified to be metabolic enzymes that mediate various biosynthetic pathways. The present approaches based on biochemical phenotypes than morphological alteration prove to be helpful in characterization of the gene functions whose knock-out or knock-in mutants do not display obvious visible phenotypes.

ABSTRACT # 74

DEVELOPMENT OF A NOVEL TECHNOLOGY PLATFORM FOR ROOT CULTURE SYSTEMS FOR FUNCTIONAL GENOMICS

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Agrobacterium rhizogenes-mediated transformation is a high-throughput system for obtaining large numbers of transgenic root clones. The rol-gene cluster in the bacterial Ri plasmid is responsible for hairy root induction. We have developed rol-type binary vectors, including hairy-root activation (HR-AT), hairy-root sense overexpression (HR-Ox), and hairy-root RNAi (HR-RNAi). Our root culture system is unique because i) It can be applied to any plant species as required, ii) Genes for factors responsible for various secondary metabolic processes in the root can be isolated.

To evaluate the HR-Ox and HR-RNAi vectors, we have developed hairy root clones for Ox and RNAi of SMT (Sterol methyltransferase) 1, SMT2, and SMT3 in Arabidopsis. Gene expression data suggest that these vectors work in planta; all the Ox root clones expressed each introduced gene strongly, and all the clones of RNAi showed suppressed gene expression. Interestingly, the infiltration transformation method using these HR-vectors was successful in Arabidopsis. The T1 transformants showed dwarf and rooty phenotypes and set seeds. This means that we can maintain transformants using seeds and establish transgenic root cultures on demand. We have started to establish HR-AT transformant lines using the infiltration transformation method.

ABSTRACT # 75

FTIR AND GC-TOF-MS ANALYSIS OF BLOOD OF GRAZING LAMBS WITH DIFFERING GROWTH RATES

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Growth rates of animals grazing upland pastures are often characterised by great variability between individuals that is difficult to explain. Diet selection and differences in feed intake both contribute to variation, but other factors such as animal genotype and plant secondary compounds could also be important.

Deproteinised blood plasma from 146 of a group of 210 lambs that had grazed permanent pastures containing ryegrass and white clover for four months from weaning were analysed by FTIR spectrometry. Lambs with relatively poor growth rates (-6 to 71 g live weight gain (LWG)/d; n=77) were compared with lambs with relatively good growth rates (134 to 189 g LWG/d; n=69). Normalised data were analysed using principal components analysis, cluster analysis, and genetic programming. A selection of samples (n=3) from lambs in each range of growth rates (poor, good, and also mean: about 103 g LWG/d) were further analysed by GC-TOF-MS with compound identification.

No clear separation of FTIR spectra was achieved, although preliminary analysis of GC-TOF-MS data showed that plasma concentrations of certain metabolites correlated with growth rate. Thus, FTIR fingerprinting seems not to provide sufficient information to correlate with lamb growth rates, while metabolite profiling did, which through identification of plant secondary compounds in plasma may also provide information on diet selection.

ABSTRACT # 76

PUSHING THE LIMITS OF METABOLOMICS: DEVELOPMENT OF MS PROCEDURES FOR SCREENING OF NOVEL LOW ABUNDANCE BIOACTIVE SIGNALS

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We are attempting to identify novel signals that regulate shoot branching, using metabolomic analysis of xylem sap. Because of predicted low abundance, a sensitive method is required to generate unique identifiers for each sap component. However, most metabolomic methods (GC-MS, flow-injection MS, data-dependent acquisition LC-MS) are unsuited to discovery of low abundance unknowns. Data-dependent acquisition LC-MS generates unique MS2 or MSn spectra from selected precursors, but high background noise of electrospray ionisation leaves many signals undetected. High sensitivity LC-MS-MRM is only suitable for known compounds.

However, hybrid quadrupolelinear ion trap instruments such as Q-Trap (Applied Biosystems) reportedly allow enhanced product ion scans at similar sensitivity to MRM1. We have evaluated a method which scans precursor masses at unit resolution, with a low precursor signal intensity cut-off. MS2 spectra are generated from each trapped precursor, then the system moves to the next unit mass window. Each MS2 ion with its parent is treated as one possible unique component for further data analysis.

A complete unit-by-unit scan of the mass range requires several minutes, which precludes use of normal LC separations. Instead, samples are fractionated off-line and injected at nanoflow rates into the Q-Trap. We have validated the method by analysing known plant hormones present at nanomolar levels in xylem sap (cytokinins, jasmonic acid). We will present comparative analysis of pea xylem sap from a range of genotypes differing in branching phenotype and in predicted levels of signal, and will contrast results with analysis of parallel samples by standard capillary GC-MS.

1Xia Y-O et al. (2003) Use of a quadrupole linear ion trap mass spectrometer in metabolite identification and bioanalysis. Rapid Communications in Mass Spectrometry 17, 1137-1145

ABSTRACT # 77

MONITORING OF PLANT DEVELOPMENT USING HR/MAS NMR AND MULTIVARIATE DATA ANALYSIS

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High resolution magic angle spinning proton nuclear magnetic resonance spectroscopy, 1H HR/MAS NMR, and multivariate data analysis using batch processing, were applied for the study of two different genotypes of hybrid aspen (Populus tremula L. x tremuloides sMichx.), transgenic trees, containing an antisense construct of PttMYB76 and control (wild type). A gene encoding a MYB transcription factor, with unknown function, pttMYB76, was selected from a cambial EST library of hybrid aspen (populus tremula L. x tremolides Michx.) for metabonomic characterisation. pttMYB76 is believed to affect different paths of the phenyl propanoid synthetic pathway. This pathway leads to the formation of S- and G-lignin, flavonoids and sinapate esters.
Ground poplar samples collected by the internodes of the tree were analysed using 1H HR/MAS NMR spectroscopy. The application of multivariate statistical batch processing allowed elucidation of a growth related pattern in the plant internode direction as well as highlighted the discrimination between the trees with down regulated PttMYB76 expression and wild type populations. The presented results could be of great relevance to the uncovering and understanding of the different lignification processes occurring within different types of trees. The present findings are also emphasizing the importance of applying robust and organised multivariate statistical approaches in order to facilitate modelling and interpretation of complex biological data sets as generated by high-throughput bioanalytical techniques.

ABSTRACT # 78

LC/ESI-MS ANALYSIS FOR PLANT METABOLOMICS: METHOD DEVELOPMENT AND DATA MINING.

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LC/MS method development and obtained data mining were performed for metabolome analysis of the crude extracts of model plant Arabidopsis thaliana. MetAlignTM software (RIKILT) has been used for data mining.

Samples were prepared by cold methanol or acetonitrile extraction of the frozen grounded plant organs. Liquid chromatography was coupled with the electrospray ionization / ion trap mass spectrometry. Modified sheatless ESI source was used. Separation efficiency, reproducibility and robustness, using different commercial and commercially unavailable monolithic capillary columns, were compared for reversed-phase chromatography.

Utilization of high flow micro LC/ESI-MS with the use of monolithic capillary reversed-phase columns and modified sheatless ESI source for non-targeted metabolome analysis of crude extracts of Arabidopsis thaliana plants enabled detection of hundreds of metabolites.
Quality of HPLC separation and an appropriate ESI parameters were found a key points for
recording valuable data. The use of the MetAlignTM software significantly accelerated data mining in terms of finding differences among sets of the biological experiments. Identification of unknowns still remains a question of on-line or off-line accurate mass measurement and off-line NMR experiments.

ABSTRACT # 79

ANALYSIS OF THE METABOLITE PROFILE OF ARABIDOPSIS THALIANA DURING DEVELOPMENTAL AND DIURNAL CYCLES

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Metabolite profiling has become a key component in the analysis of genetically and environmentally stressed plants. To study the metabolite profile, it is important to harvest the plants in a consistent manner to minimize experimental variation. The aim of this study is to gain insight into the variation of metabolite levels of wild-type Arabidopsis during its developmental and diurnal cycles. Plants grown under normal conditions were harvested every two days after bolting and every two hours from 0800 to 1800 hours on day 10 for analysis of the developmental and diurnal cycles, respectively. Lyophilized plant tissues were extracted using a methanol and dichloromethane protocol and extracts were derivatized and analyzed using GC/MS. The metabolite levels for amino acids, organic acids, sugars, sugar alcohols, fatty acids and sterols were determined for each time-point.

For the diurnal cycle, the data showed that amino acid content peaked at 1200 hours, while organic acids, sugars and sugar alcohols steadily increased throughout the daylight period while fatty acids and sterols decreased. For the growth cycle, similar data will be reported.

ABSTRACT # 80

SPECTRUM-BASED CHROMATOGRAM ALIGNMENT

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One key challenge in high throughput GC-MS and LC-MS-based methods for metabolomics is that retention times of individual components change from run to run, either on the same instrument over time, or on different instruments, resulting in complications in the comparison of datasets for differential display analyses.

Chromatogram alignment is a bioinformatic technique to compensate for such retention time differences. By aligning chromatograms before further processing, one is able to pass more and cleaner information as input to downstream analysis.

We have developed a technique based on spectral similarity to align chromatograms. This dynamic-programming algorithm shifts retention times to maximize the spectral similarity of the aligned chromatograms.

Various methods for calculating spectral similarity have been suggested in the literature. To validate our algorithm and to compare these methods, we developed a benchmark of five GC-MS chromatograms, taken from samples of wild type *Arabadopsis thaliana* grown over four months and analyzed over that time period on two different instruments. The benchmark is scored by counting how many peaks match, based on the algorithm, with the number of true matches, based on human analysis. Our spectrum-based alignment algorithm increases the rate of correct matching of peaks on this benchmark from under 3% (without alignment) to over 90%.

ABSTRACT # 81

APPLICATION OF METABOLITE AND FLAVOUR VOLATILE PROFILING TO STUDIES OF BIODIVERSITY IN SOLANUM SPECIES

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Globally, potato is an important foodstuff and source of nutrition. Traditionally potatoes have been developed for agronomic traits such as yield and disease resistance. To meet changes in consumer demands, considerable effort is being put into improving additional characteristics such as nutritional value and organoleptic properties. We are using a wide range of potato germplasm to explore phytochemical diversity. Several genotypes of the potato species Solanum phureja, with favourable organoleptic properties, have been developed and are the subject of studies concerning the relationships between tuber metabolites, volatile flavour compounds and taste. Comparisons have been made between S. phureja and established varieties of the more familiar S. tuberosum, of which the former were assessed as having a more appealing flavour by specialist taste panels. The effect of low temperature storage and cooking of tubers has been studied. Volatile compounds entrained from boiled tuber
s of both species were analysed by automated thermal desorption gas chromatography mass spectrometry (ATD-GC-MS). Tuber metabolites from both raw and cooked tubers were analysed by time of flight (TOF) GC-MS. Data analysis clearly differentiates between species and also between different durations of storage. These differences were related to the abundance of specific compounds.

ABSTRACT # 82

Profiling of Metabolites and Volatile Flavour Compounds from Solanum Species using Gas Chromatography-Mass Spectrometry

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We are using high throughput profiling techniques linked to automated data processing to study substantial equivalence and unintended effects of genetic modification in Solanum species. In addition, metabolite variation within Solanum germplasm collections is being measured with the objective of exploring phytochemical diversity. One specific example of this is the investigation of the role of tuber metabolites and volatile compounds in relation to the organoleptic properties of potato as characterised by specialist taste panels. We have developed a metabolite profiling technique based on the use of GC-Time-Of-Flight (TOF) MS for analysis of tuber metabolite composition. Separate polar and non-polar extracts are prepared from freeze-dried or fresh potato tubers in the presence of internal standards, and are analysed alongside retention index markers following appropriate derivatization. Volatile compounds generated on the cooking of potato are entrained on porous polymer
s and are subsequently analysed using a mass spectrometer coupled to an Automated Thermal Desorption (ATD) autosampler. Appropriate data reduction techniques are used for subsequent data analysis.

ABSTRACT # 83

MetNetDB: a database for Arabidopsis systems biology

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MetNetDB is a public database used to create and store information about metabolic and regulatory networks. The purpose of MetNetDB is to facilitate our understanding of genetic functions in biological networks during development and across genotypes. MetNetDB is designed to capture the special features of plants, with a specific focus on Arabidopsis; it can be easily adapted for use with other eukaryotic organisms. It is assembled from a combination of metabolic and regulatory pathway information from experts in specific areas of molecular biology, on-line genomic and metabolic pathway information (Aracyc, KEGG, Brenda and PubMed), and sequence-based information on protein complexes. This information comprises a network composed of metabolites, RNA, genes, polypeptides, protein complexes, together with the interactions between each of these entities. Information provided for each entity includes: subcellular location(s), pathways, supporting data, references. The network contains more than 30 predefined types of interactions (catalysis, translation, assembly, positive regulation of unknown mechanism, etc.). Users can add literature references and comments to enhance the data quality. The relations between polypeptides in protein complexes can be edited via an intuitive graphical user interface. The pathway topology together with gene relation information can be exported as an XML file and combined with gene expression profiles (e.g., metabolomic, proteomic, microarray). The graph and profile data can be subjected to statistical analysis and visualization tools such as GeneGobi, and can be visualized and modeled in 2D by FCModeler, or in the C6, an immersive virtual reality environment by MetNetVR.

ABSTRACT # 84
Reverse genetic analysis of novel biotin proteins in plants

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Arabidopsis contains novel biotin proteins whose physiological functions are poorly characterized. These are: 3-methylcrotonyl-CoA carboxylase (MCCase), one of the four molecularly characterized biotin enzymes expressed by Arabidopsis (encoded by genes At1g03090 and At4g34030); a seed biotin protein (SBP), encoded by At2g42560; and three genes (At1g52670, At3g15690 and At3g56130) that encode proteins (BCCP-like proteins) that show significant sequence similarity to the Cyanobacteria biotin carboxyl-carrier protein subunit of acetyl-CoA carboxylase. MCCase is a nuclear-encoded, mitochondrial-localized enzyme that is required for leucine catabolism in mitochondria, one of two parallel leucine catabolic pathways expressed in plants. Moreover, MCCase may play important roles in the catabolism of isoprenoids via the mevalonate shunt. SBP accumulates late during seed development and is thought to act as a store of biotin. The biochemical and physiological functions of the BCCP-like proteins are completely undefined. To test these hypotheses, and decipher the functions of these proteins, plants carrying mutations in genes encoding the above proteins were identified. GC-MS and HPLC approaches are being used to characterize the metabolomes of each mutant. The physiological functions of these proteins will be further analyzed.

ABSTRACT # 85

STARCH GRANULE METABOLISM AND ACETYL-COA UTILIZATION

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Starch accumulates and degrades in leaf chloroplasts during the light and dark phases of the diurnal cycle respectively, indicating formation of granules is highly regulated throughout the diurnal cycle. We are exploring the starch metabolic network in the context of other metabolic processes by investigating mutants with single-gene changes that perturb starch metabolism. The central metabolite, acetyl-CoA, is required for many biochemical pathways critical to plant growth and development. ATP citrate lyase (ACL) produces acetyl-CoA from citrate in the cytosol. Transgenic Arabidopsis with decreased levels of ACL activity contain abnormally enlarged starch granules. To study the underlying molecular events involved in utilization of acetyl-CoA during starch metabolism in wild-type (WT) and antisense-ACLA1 (acla1) plants, leaves of seedlings grown under short day (SD) photoperiod were analyzed by microarray, and starch was characterized. Zymogram analyses of starch metabolizing enzymes were used to compare enzyme activity to transcript accumulation profiles. Data analysis included use of the GeneGobi and FCModeler software. Differences between WT and acla1mutants indicate additional aspects of the starch metabolic network.

ABSTRACT # 86

METABOLITE PROFILING OF THE MUTANT LINES OVEREXPRESSING AtIAMT1 AND AtJAMT1, TWO MEMBERS OF THE SABATH FAMILY METHYLTRANSFERASES IN Arabidopsis thaliana

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The Arabidopsis thaliana genome contains 24 related genes encoding enzymes that belong to a SABATH family of methyltransferases (MTs). Preliminary experiments suggest that SABATH MTs convert several important hormones and other plant constituents into their methyl esters, thereby exerting important effects on the biological activity of these molecules and consequently on myriad important physiological processes. Two of the SABATH enzymes have been recently characterized as jasmonic acid methyltransferase (AtJAMT1) and indole-3-acetic acid methyltransferase (AtIAMT1). The aim of our project is to identify the function of all the methyltransferases belonging to this family (i.e., identify biologically relevant substrate for each enzyme) by a combination of methods that involve molecular genetics, enzymology, protein structure analysis, and metabolite profiling. The results are expected to provide a better understanding of plant responses to environmental conditions, thus helping improve crop yield and nutritional value. In addition, by developing methodologies for determining which Arabidopsis genes are involved in the synthesis of the plant's diverse repertoire of small molecules, the project will contribute to the elucidation of the function of other Arabidopsis genes involved in hitherto unknown biochemical pathways. Preliminary metabolite data obtained from a suite of techniques and methodologies will be presented.

ABSTRACT # 87

"PACLITAXEL STABILITY AND CONVERSION THROUGH METABOLITE ANALYSIS IN TAXUS CELL SUSPENSION CULTURES"

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Effective plant-derived anticancer drugs such as paclitaxel are subject to research in chemical engineering because the supply is limited through conventional means (i.e., harvestation). Plant cell tissue culture has received significant attention, and is a very promising technique for paclitaxel supply. However, an understanding of cellular metabolism (biosynthesis, degradation and transport) is critical in the design of effective strategies to enhance productivity so that plant cell tissue culture processes can become economically feasible.
The main goal of this research is the study of paclitaxel stability in cell suspensions, including gaining mechanistic information on paclitaxel degradation and solubility. Paclitaxel is a member of the taxane family of structurally-similar compounds, which can broadly be divided into side-chain (i.e., containing a side-chain derived from phenylalanine) and nonside-chain taxanes. Cell cultures produce a variety of taxanes that can be analyzed and quantitated via HPLC-MS. Taxane feeding experiments were performed in conjunction with HPLC-MS analysis to determine the levels and types of taxanes present in Taxus cell suspension cultures under a variety of conditions (e.g., elicitation with methyl jasmonate). Radiolabeled paclitaxel was also utilized through addition to the extracellular medium and analysis via HPLC equipped with both a PDA and b-RAM detector, providing additional mechanistic insight into paclitaxel stability. Studies demonstrated significant interconversion between nonside-chain taxanes, and conversion from side-chain taxanes to nonside-chain taxanes and other degradative metabolites. This information, combined with complimentary information on paclitaxel biosynthesis provides a more complete model of paclitaxel metabolism.

ABSTRACT # 88

CORRELATION OF METABOLITE PROFILING AND GENE EXPRESSION IN PACLITAXEL-ACCUMULATING TAXUS CELL SUSPENSION CULTURES

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Plant cell cultures represent a potential method for the large-scale production of important secondary metabolites. One important example is the anti-cancer agent paclitaxel (Taxol - Bristol-Myers-Squibb), which is a secondary metabolite produced by the Taxus genus of plants. Paclitaxel is one of a family of related compounds, referred to as taxanes. In Taxus suspension cultures, paclitaxel is often less than 10% of the total taxanes present. This low selectivity represents an excellent opportunity to increase the overall accumulation of paclitaxel in culture.

The addition of the enzyme elicitor methyl jasmonate has been shown to increase secondary metabolism in a wide variety of plants, including the Taxus system. Specifically, methyl jasmonate increases total taxane accumulation as well as paclitaxel levels. In addition, elicitation with methyl jasmonate can be used as a tool to elucidate biosynthetic pathway regulation. We have examined the distribution of various taxanes produced in Taxus suspension cultures with and without methyl jasmonate elicitation.

We have also used Northern blot analysis of several known genes in the taxane pathway to determine the relationship between gene expression and the accumulation of taxane metabolites. This is evidence for regulation of taxane biosynthesis occurring at the mRNA level. Work presented here provides the basis for ongoing efforts to understand the regulation of the taxane pathway. In the future, the identification of pathway bottlenecks may lead to the development of directed strategies, such as genetic transformation, to increase paclitaxel accumulation.

ABSTRACT # 89

MARKER METABOLITES CAPTURING THE METABOLITE VARIANCE PRESENT IN A PLANT DEVELOPMENTAL PERIOD

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Variation in crop development due to genotype and environment greatly impacts yield. Many samples are needed to characterize quantitative aspects of developmental variation, for which a metabolomics approach could be limited by analytical resources. Correlations among metabolites during a key developmental period, tillering, of the rice (Oryza sativa L.) plant were observed in a metabolomics study, indicating that representatives from clusters of metabolites could capture much of the metabolite variance of the developmental period. The objective was to construct a set of marker metabolites to capture this variance while being amenable to medium-throughput assay technologies. The metabolome profiles from the rice developmental study were analyzed. Principal components (PCs) in standardized centered metabolite space were determined. Graphic patterns in the PC scores over development suggested that the first several PCs were interpretable as responses to developmental and enviro nmental variables. Patterns in metabolite loadings on PC1 suggested some metabolite variation could be interpreted via well-known metabolism. Individual metabolites were selected to provide a spread of variation in loadings on the first three PCs, while being easy to assay. Metabolite selection involved K-means clustering of the ranked loadings on the three top PCs, plus hand-picking of promising metabolites. The resultant set of marker metabolites captures much of the metabolite variance in development as identified by metabolome profiling, and could be used in comparative screening of metabolite patterns of plant developmental periods, of plant response to specific environmental factors, or of genotypes in set conditions.

ABSTRACT # 90

PMETABOLIC PROFILING OF POTATO CULTIVARS VARYING IN LEAF RESISTANCE TO LATE BLIGHT, PHYTOPHTHORA INFESTANS.

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Potato cultivars Caesar (moderately resistant) and AC Novachip (susceptible) to late blight were grown in greenhouse, inoculated with Phytophthora infestans (US-8 genotype), 12 leaf discs of 15 mm diameter were sampled at 24 h, extracted with methanol, derivatized and metabolites were analyzed using gas chromatograph/mass spectrometer. The disease severities on 6th day after inoculation were 64 and 174 mm2, in Caesar and AC Novachip, respectively. 120 metabolites were detected, of which, 51 occurred in all the four experiments conducted. These metabolites belonged to different chemical groups, such as alkaloids, nitrogens, phenols, sulfurs, etc. Out of 51, only two compounds were unique to, pathogen inoculated, Caesar: 1H,10H-Furo[3',4':4a,5]napht and Heptadecanoic acid,16-methy as compared to 18 to AC Novachip: some for example: 1,3-Dicyano-2-phenyl-3-amino., 1,3,3-Trimethyl-6-hydroxy-2-., 10-Dicyanomethylene-benz(a)a., 2-Methyl-2-(4-methoxyphenyl), etc. While 2 and 14 metabolites were unique to water inoculated Caesar and AC Novachip, respectively. Five compounds, including Acetic acid, [(trimethylsilyl and Myo-Inositol,1,2,3,4,5,6-he were detected in both cvs., but the abundance of the latter was higher in Caesar. These results indicate the specificity of certain metabolites to cultivars and inoculations, with water or pathogen. Such differences in metabolic profiles among cultivars and inoculations can be exploited as markers to discriminate the level of resistance, and used in breeding to produce potato cultivars with improved levels of resistance.

ABSTRACT # 91

METABOLIC FLUX ANALYSIS OF SUCROSE METABOLISM INTO PROTEIN, OIL AND STARCH IN SOYBEAN SEEDS

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Soybeans are used as a major source of raw material for animal feeds as well as other food products. An increase in nutritional value of the seed would render them commercially more attractive. To develop a desired seed product, it therefore becomes necessary that the carbon flow through the metabolic pathways in soybean be clearly understood.

Metabolic flux analysis (MFA) using carbon bond labeling experiments (BLE) is an effective tool for quantifying intracellular metabolite fluxes. It helps in quantification of fluxes in various pathways, e.g. glycolysis, tricarboxylic acid cycle, pentose phospate pathway, anaplerotic pathways, glyoxylate shunt and GABA shunt simultaneously. To the extent of our knowledge, our group is the first to systemically apply this technique on a plant system.

The main purpose of this study is to use MFA to assess the temperature effects on metabolism in developing soybean cotyledons cultivated *in vitro* under different temperature conditions. The ^{13}C labeling pattern, which is reflected in the proteinogenic amino acids and starch, can be analyzed using Nuclear Magnetic Resonance (NMR) spectroscopy. 2-dimensional (2D) HSQC (^{1}H, ^{13}C Heteronuclear single quantum correlated spectroscopy) and TOCSY analyses were performed to evaluate the extent of carbon-carbon bond coupling and fractional carbon enrichments. Extracellular measurements coupled with NMR analyses help quantify the fluxes in the pathways, using computer software that we have developed for this purpose. We will present metabolic maps for soybean with quantitative values of the intracellular fluxes under different temperature conditions. A comparison of the same will give an understanding of the effects of temperature on metabolism.

ABSTRACT # 92

ASP-295 IS IMPORTANT FOR PHOSPHORYLATION IN THE ACTIVE-SITE LOOP OF PYRUVATE DEHYDROGENASE E1alpha BY PYRUVATE DEHYDROGENASE KINASE

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The pyruvate dehydrogenase complex (PDC) is a large multi-component structure involved in pyruvate metabolism that generates CO2, acetyl-CoA and NADH. The decarboxylation of pyruvate is catalyzed by pyruvate dehydrogenase (E1), an alpha2beta2 heterotetramer. The catalytic activity of eukaryotic PDC is modulated by phosphorylation of specific Ser residues on E1alpha subunit by an E1-specific kinase (PDK) that results in an inactivation of the complex. A highly conserved region surrounding phosphorylation site 1 forms the active-site loop that is involved in catalysis of pyruvate and phosphorylation by PDK. Site-directed mutagenesis of the conserved Asp-295 and Gly-297 residues was used to study their mechanistic roles in the active-site loop of pyruvate dehydrogenase. Asp-295 was substituted with Ala, Asn, or Leu, while Gly-297 was substituted with Ser or Asp. The E1alpha and E1beta were cloned into pT7-7 and pET28 vectors, respectively, and coexpressed in E.coli BL21(DE3). The Asp-295 mutant proteins were much less efficiently phosphorylated by PDK. The D295L mutant had ~3% residual phosphorylation, while phosphorylation in the D295A and D295N mutants was 8 and 17%, respectively. The G297S and G297D mutants had 87 and 91% phosphorylation. The results from two-hybrid analysis suggested that neither the D295A nor D295N mutations affect binding of PDK to the active-site loop. Data on the catalytic activity, measured as oxidative decarboxylation of pyruvate, will be presented. Based on these observations, we propose a role for Asp-295 in stabilizing the active-site loop, which facilitates transfer of the alpha-phosphoryl group of ATP to E1alpha Ser-292.

ABSTRACT # 93

CLASSIFICATION OF UNKNOWN METABOLITES USING LC/MS: HOW MUCH CAN WE LEARN USING NOMINAL MASS ANALYSIS?

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Identification of unknown or unanticipated plant metabolites remains a substantial challenge owing to the diversity of plant metabolomes. While accurate mass measurements are possible with high resolution mass spectrometers and MS/MS scans can provide structural information, the costs and technical expertise needed to perform metabolite analysis can be substantial. For many laboratories, such resources are either out of reach or they limit the number of experiments that can be performed. Our recent efforts have aimed to identify and quantify plant using LC/MS with single quadrupole mass analysis, with a focus on metabolites with potential to mediate plant-insect and plant-insect-pathogen interactions. Metabolites have been extracted from leaves of Arabidopsis, cotton, and oak as well as from wood and bark from genera Acer and Pyrus.
The number of carbon atoms in a metabolite can be calculated from the ratio of abundances of its monoisotopic molecular ion to the "A+1" peak which contains one heavy isotope (mainly 13C). While the number of carbon atoms is not particularly diagnostic for a group of compounds, the percentage of molecular mass that is carbon can be readily calculated from the number of carbon atoms and the molecular mass. Metabolites generated through similar biosynthetic pathways cluster together based on %C values. In-source fragmentation can be generated using alternating cone voltage programming. Structural information about unknowns can be obtained from fragment masses and %C of individual fragments. These approaches are accessible using virtually all LC/MS instruments.

ABSTRACT # 94

REFERENCING IN METABOLITE SPACE THROUGH LARGE-SCALE GENERATION OF PREDICTIVE FUNCTIONS BASED ON RATIOS OF METABOLITE CONCENTRATIONS

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The analysis of patterns of metabolites in plant tissues is useful to characterize biochemical phenotypes. Multivariate statistics identifies broad-scale changes in patterns. Supplemental approaches are desirable to discern more local (i.e, small groups of metabolites) variation. The objective was to evaluate the use of ratios of metabolite concentrations for prediction of other metabolite concentrations. This approach has some loose parallels to triangulation in geo-referencing, and could serve to provide a non-metabolism based structure for referencing portions of metabolome profiles. Metabolome profiles collected during a key developmental event, tillering, in the rice (Oryza sativa L.) plant were analysed. In preparation, a set of marker metabolites was selected that represented much of the metabolite variance present during development. All possible ratios among these marker-metabolite concentrations were generated. Each ratio was used, via univariate linear regressions of the developmental series data, as a predictor of each metabolite concentration. The calibration and cross-validation sets each consisted of a randomly selected half of the developmental series samples. All predictive functions (regressions) were carried into cross-validation. The functions were quantified for ability to predict concentration of a particular metabolite based on fits and slopes of the regression equations of predicted vs. known values. Response surfaces for predictive ability in metabolite space could then be constructed. If valid, then observed local stabilities in metabolite structure will aid in comparison of metabolome profiles and have potential to shed new light on plant metabolite networks.

ABSTRACT # 95

METABOLOMICS AND INTEGRATED FUNCTIONAL GENOMICS OF MEDICAGO TRUNCATULA RESPONSES TO BIOTIC AND ABIOTIC STRESS

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An integrated functional genomics approach to study the relationships between gene expression, protein levels and metabolites in the model legume Medicago truncatula, following biotic and abiotic elicitation will be described. Particular emphasis will be placed on metabolic profiling of natural products. M. truncatula suspension cell cultures were separately treated with methyl jasmonate, yeast cell wall extract, or UV light. Samples were collected at 21 timepoints following each elicitation and analyzed at the metabolite, protein, and mRNA levels. Significant changes were observed in both primary and secondary metabolism. Several amino acids and two organic acids increased; whereas sucrose levels decrease. These specific changes were observed for all three elicitations and suggest a generalized stress response. Additional changes in beta-alanine were also observed and this implication will be discussed. Changes were also observed in secondary metabolism. Specifically, methyl jasmonate elicitation resulted in increased levels of beta-amyrin as well as a significant number of triterpene saponins. Changes in the metabolome will be discussed in relation to corresponding changes in transcriptome and proteome.

ABSTRACT # 96

ELUCIDATION OF A WHOLE ADAPTIVE PROCESS TO NUTRIENT DEFICIENCY BY INTEGRATION OF METABOLOMICS AND TRANSCRIPTOMICS

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Plant has mechanisms to adapt to nutrient deficiency and optimize metabolic balance according to nutritional status given to plants. To elucidate a whole adaptive response to sulfur deficiency (S), we analyzed metabolome and transcriptome of Arabidopsis under S and related stresses. To elucidate long-term responses, plants were grown under continuous S, nitrogen (N) or SN conditions for 3 weeks. To clarify early responses and adapting process to S, plants grown under +S were shifted to S and harvested at several time points after transfer. In both experiments, plants were grown apparently normal. Non-targeted metabolome were analyzed by Fourier-transform ion cyclotron MS. Targeted metabolic profiling was also conducted using HPLC and capillary electrophoresis. These data was integrated with transcriptome data obtained by using DNA array.

Global changes in metabolome and transcriptome were shown by principal component analysis. Metabolome and transcriptome depended mainly on plant organ type and method of culture, and secondly on nutritional condition. Self-organizing map (SOM) analyses were performed to classify metabolites and genes according to their accumulation and expression patterns, respectively. Specific responses to S and general responses to nutritional stresses were suggested. Glucosinolates, S-containing defense compounds, were considered playing a role as S storage source. SOM analyses on metabolome and trasncriptome indicated that glucosinolate metabolism is coordinately regulated at mRNA accumulation level. By assumption that genes involved in the same metabolic pathway will cluster together in SOM, we found an array of candidate genes responsible for glucosinolate biosynthesis.

ABSTRACT # 97

NECTARIN-4 IS A HOMOLOGUE OF XYLOGLUCANSE INHIBITORY PROTEIN

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Higher plants like other living organisms have evolved their own strategies for successful fertilization and propagation. Energy-rich nectar is one of the most important factors in this strategy. Until 1999 nectar was not reported to have any protein. Then our laboratory, for the first time demonstrated the presence of at least five proteins in the nectar of ornamental tobacco. Four proteins have been previously identified and reported. In this study we report on the characterization of the last of the nectar proteins. Nectar proteins were analyzed in 20% SDS-PAGE followed by excision of a band corresponding to 60 kD. Tryptic peptides from this gel slice were analyzed by Mass-spec sequencing. Three unique peptides were found to be similar to the xyloglucan-specific fungal endoglucanase inhibitor protein precursor in tomato, and its homolog in potato. A pair of primers was designed based on these sequences and used to clone a 1018 bp internal piece of Nec-4 cDNA from a cDNA library. The remaining 3'end has been captured by 3'RACEwhich has revealed presence of at least two different genes for this protein. Nectar protein extract has however, failed to show any activity again *Aspergillus niger* and *Trichoderma brachiatum* xyloglucanases. Further studies are under way to elucidate potential role of this protein in nectar.

ABSTRACT # 98

Biologic diversity of ecosystems forest and consistent of the dforestation by remote sensing in a zone semi arid

zegrar ahmed

Zone steppique presents a diversity floristique and faunique, and seen the unfavorable climatic conditions in the zone, one notes a deterioration of the surroundings physics notably the natural forest regression. This deterioration of forests known under the no dforestation provokes an unbalance of the lively environment of the serious consequences and misleading to the desertification. The use of pictures of remote sensing of the LANDSAT satellite of the stage 195-36 of the month of May 1989 and the one of the month of April 2001 (ETM+) and that have sudden different treatments have permit to follow and to value the phenomenon of dforestation in a region steppique of floor bioclimatique semi arid. A thematic analysis of middle steppique informed us on the physical parameters of the middle (forest populations, vegetation steppique and soil) on the one hand and of the human activity in these surroundings (reclamation of earths, cut of wood and fire) on the other hand. A classification of the different components of the middle has been elaborated forests and their level of deterioration that have permitted notably of valued the phenomenon of dforestation in middle steppique in order to permit an analysis of the process of the desertification phenomenon.

ABSTRACT # 99

OVEREXPRESSION OF ATP CITRATE LYASE IN ARABIDOPSIS

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Cytosolic acetyl-CoA is formed by the action of ATP citrate lyase (ACL), a heteromeric protein encoded by two separate sets of genes: *ACLA* and *ACLB*. I am testing 2 hypotheses in Arabidopsis: 1) introduction of both *ACLA* and *ACLB* transgenes, but not either one individually, will increase ACL activity in the plant; and 2) an increase in ACL activity will lead to increased levels of specific cytosolic acetyl-CoA derived metabolites under defined conditions. Arabidopsis lines have been developed to overexpress ACLA or ACLB by introducing an *ACLA* or *ACLB* transgene. Independent transgenic lines have large increases in the level of the appropriate *ACL* mRNA, but only small increases in the level of the corresponding protein. Neither ACLA overexpression nor ACLB overexpression alone increases ACL activity relative to the wild-type level, indicating that neither subunit alone is limiting for enzyme activity. Lines that overexpress ACLA have wild-type levels of ACLB; conversely, overexpression of ACLB does not affect ACLA accumulation. Thus, expression of neither transgene influences the expression of the other enzyme subunit. ACLA- and ACLB-overexpressing lines have been crossed to obtain plants with elevated levels of both ACL subunits. These ACLA + ACLB plants show no phenotypic difference from wild-type plants under normal growth conditions; however, when grown at 10°C they have increased accumulation of anthocyanins relative to wild-type plants. Under all experimental growth regimes tested, ACLA + ACLB lines show high levels of accumulation of RNA from both *ACL* transgenes and a slight increase in ACL protein concentration. Microarray experiments delineate specific genes affected by increasing ACL expression *in planta*.

ABSTRACT # 100
EPICHLOE AMARILLANS IN ASSOCIATION WITH AGROSTIS HYEMALIS PRODUCES ONLY N-ACETYL NORLOLINE IN PLANTA

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Abstract: Loline alkaloids (1-aminopyrrolizidines) have been shown to have insecticidal properties and may also increase drought tolerance in plants. Neotyphodium uncinatum and related sexual teleomorphs (Epichloe species) are fungal endophytes that have been shown to produce loline alkaloids in association with certain grass species. Previous studies have shown that loline, methylloline, N-acetylnorloline, N-formylloline, and N-acetylloline were produced by N. uncinatum in culture. Agrostis hyemalis (winter bentgrass) is a Poaceae species that is common throughout the United States. In previous reports Agrostis hyemalis in association with an isolate of Epichloe amarillans did not contain any loline alkaloids. Preliminary observations in our greenhouse indicated that Agrostis hyemalis seemed to be resistant to aphids during outbreaks. This observation along with the presence of the loline genes in this fungal isolate led us to look for the alkaloids in low concentrati
ons. Using GCMS we were able to determine that E. amarillans produces only N-acetylnorloline (NANL) in vivo with Agrostis hyemalis. We calculate that this association produces 48 grams N-acetylnorloline /gram of dry weight. The formation of one loline could have uses in future studies for elucidating the loline biosynthetic pathway.

ABSTRACT # 101

GCTOF METABOLITE PROFILING OF ARABIDOPSIS MUTANTS AND ECOTYPE ACCESSIONS UNDER HIGH LIGHT, COLD, AND FERTILIZER STRESS: IMPACT ON THE CITRAMALATE PATHWAY

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Metabolite profiling analyses describe physiological processes as responses to developmental, genetic or environmental changes. In addition to genomics and transcriptomics, metabolomics is a useful method to characterize the biological function of novel metabolites. GCTOF-analysis of Arabidopsis thaliana ecotypes as well as mutant lines under high light, cold and fertilizer stress leads to the suggestion of a coregulation of TCA-cycle intermediates and citramalate. This connection has previously been described only for bacteria, in which citramalate is formed by the condensation of acetate and pyruvate, and is subsequently degraded to succinate and malate, as an anaplerotic re-filling reaction. Citramalate has been discovered for the first time in plants in Arabidopsis thaliana leaf extracts, and to date, its metabolic pathway in plants is unknown. The level of citramalate increases under high light and decreases under cold stress conditions. Malate, succinate and fumarate be
have in the same way as citramalate, but all other TCA intermediates show no significant changes. On the other hand fertilizer stress (organic nitrogen)has no effect on the citramalate pathway. Further experiments are necessary in order to prove the hypothesis of the citramalate pathway as an anaplerotic re-filling of the TCA intermediates succinate and malate, comparable to microorganisms such as Rhodospirillum rubrum.

ABSTRACT # 102

Systems Biology Maps: tools and approaches for the integration and analysis of genome-scale data

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Post-genomic era research is focussing on studies to attribute functions to genes, their encoded proteins, and to describe the regulatory networks controlling biochemical, protein synthesis and signal transduction pathways. To facilitate the analysis of experiments using post-genomic technologies, new concepts for linking the vast amount of raw data to a biological context have to be developed. Visual representations of pathways help biologists to understand the complex relationships between components of metabolic networks and provide an invaluable resource for the knowledge-based integration of transcriptomics, proteomics and metabolomics data sets. Here we describe a visual interface, termed Systems Biology Maps, for an annotation database covering metabolic and cell biological pathways in the model plant Arabidopsis thaliana, that allows the knowledge-based integration and analysis of genome-scale datasets (oligonucleotide microarrays, proteomics, metabolomics, flux co
ntrol measurements, phenotyping).

ABSTRACT # 103

METABOLITE PROFILING OF MYCORRHIZAL *MEDICAGO TRUNCATULA*

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Fungi from the order Glomales form symbiotic associations (arbuscular mycorrhizas) with many plant roots that improve plant growth by enhanced supply with phosphate and increase stress tolerance. Our aim is the comprehensive analysis of primary and secondary metabolites during the establishment of the symbiosis in the model system *Medicago truncatula/Glomus intraradices* to elucidate the mycorrhiza-specific metabolic alterations.

While *Medicago truncatula* inoculated with the fungus were grown under unsterile conditions, tissues were harvested from progressively aging plants at weekly intervals (2-8 weeks) by freezing in liquid N_2 and lyophilization. Root extracts are analyzed by GC-MS, LC-MS and HPLC for metabolite identification and quantification. During the mycorrhization the occurrence of fungus-specific palmitvaccenic acid as well as an increase in the unsaturated fatty acids was observed. In the polar extracts several trends in primary metabolites (amino and aliphatic acids, sugars) were seen together with the fungus-specific trehalose. Saponins and isoflavonoids, the constitutive secondary metabolites of the roots, showed a time-dependent increase, but no significant differences between mycorrhizal and control plants. In contrast, only mycorrhizal roots accumulate apocarotenoids (cyclohexenone derivatives and the “yellow pigment”) that show levels correlated with the time after inoculation. Metabolites from mycorrhizal extracts showed a higher pairwise correlation than those from controls.

ABSTRACT # 104

GENE CLUSTERS FOR INSECTICIDAL 1-AMINOPYRROLIZIDINE (LOLINE) ALKALOIDS IN THE FUNGAL GRASS ENDOPHYTE NEOTYPHODIUM UNCINATUM

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Some poid grasses are frequently infected by fungal endophytes of the genus Epichlo (anamorph: Neotyphodium), which produce at least four different classes of alkaloids with anti-herbivore activities. Saturated 1-aminopyrrolizidine (loline) alkaloids (LA) produced by some endophyte species have potent anti-insect activities and are of interest as natural chemical plant protectants against insect pathogens. Our work focuses on the biochemistry and genetics of the LA. Two genes, lolA and lolC, have been found to be associated with LA production in Epichlo/Neotyphodium. Both lolA and lolC belong to gene clusters within ~25 kb of the genome of the LA-producer Neotyphodium uncinatum. Seven additional genes, lolF, lolD, lolO, lolU, lolP, lolT, and lolE, are clustered with lolA and lolC. The predicted products of all genes, except lolU, gave significant similarity to known enzymes, including PLP-containing enzymes, FAD and P450 monooxygenases, and oxidoreductases characteristic o
f secondary metabolism pathways. N. uncinatum has two orthologous lol gene clusters, LOL-1 and LOL-2, showing identical gene arrangement and orientations; only the relative position of lolF in LOL-2 remained undetermined. LA production in cultures and in symbio coincided with expression of all LOL cluster genes. Transformation of N. uncinatum with an RNAi-based lolC-silencing construct decreased lolC expression and LA accumulation in culture, further supporting an involvement of lolC in LA biosynthesis. The predicted activities of the LOL-gene cluster products and precursor-feeding studies suggest production of the LA by a novel biosynthetic pathway.

ABSTRACT # 105

Metabolic profiling approach for plant P450 monooxygenase studies using FT-MS, GC-MS, and LC-MS

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Plant metabolomics, in the post genome era, has attracted considerable interest in light of the fundamental importance not only for understanding plant metabolisms but also for unlimited potentials of plant metabolites in both crop quality improvement and industrial applications. In this study, we employed comparative mass spectrometric analyses to investigate plant cytochromes P450 (P450) monooxygenase reactions with the aid of FT-MS together with the combination of GC-MS and LC-MS. In the ESI-FT-MS analysis, we were able to observe ~500 independent ion peaks from crude extracts from different plant tissues including cultured tobacco cells and petunia flowers containing different P450 monooxygenase genes. Processing the observed ESI-FT-MS data by principal component analysis (PCA) was successful to demonstrate distinct metabolomes of petunia flowers from plants of different genetic backgrounds in terms of P450 genes involved in the phenylpropanoid metabolism. A stable isotop ic analysis was performed for metabolic labeling of potential substrates for P450s among plant metabolites. Specifically, the FT-MS analysis was successful to monitor metabolic labeling of secondary metabolites produced through a series of several CYP71 P450 activities in maize. The PCA data and stable isotopic analyses are discussed as a tool to explore novel P450 activities and related metabolic pathways.

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ABSTRACT # 106

Quantification of metabolic fluxes in plants from 2-D NMR data: mathematical framework and flux identifiability

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Metabolic flux quantification is instrumental in the detailed understanding carbon partitioning in plant metabolism. However, it is difficult to perform on a systemic level, because fluxes have to be quantified by back-calculating them from their effect on the distribution of a labeled substrate, and such calculation requires a detailed mathematical model if it has to be accurate. Such models are highly nonlinear and often non-trivial to solve. In this presentation, we report the development of a computer-aided metabolic flux analysis tool that enables the concurrent evaluation of fluxes in several primary metabolic pathways. We implemented this tool on developing soybean (Glycine max) embryos cultured on a mixture of U-13C sucrose, naturally abundant sucrose, and glutamine. NMR spectra of seed storage protein and starch hydrolysates were acquired, and yielded a labeling data set consisting of 156 13C isotopomer abundances. Our flux analysis tool employs recent mathematical d
evelopments toward efficient flux analysis, such as Boolean function mapping and cumomer balancing. It automatically calculates fluxes and reaction reversibilities from raw NMR data, and is capable of working with any user-defined metabolic network model. Also, statistical analyses are performed on the evaluated fluxes to obtain standard deviations for them. We will present a metabolic flux map that depicts carbon trafficking in the soybean embryos, as well as values of all fluxes evaluated from the NMR data. Some of our results are as follows: A high flux was found through the oxidative pentose phosphate pathway (104.2 mol 23.0 mol per 100 mol of sucrose uptake). Separate transketolase and transaldolase fluxes could be distinguished in the plastid and the cytosol, and those in the plastid were found to be at least 6-fold higher. The backflux from triose to hexose phosphate was also found to be substantial in the plastid (113.2 mol 26.0 mol per 100 mol of sucrose uptake)
. Forward and backward directions of anaplerotic fluxes could be distinguished. The glyoxylate shunt flux was found to be negligible, while a moderate flux was detected through the γ-aminobutyric acid (GABA) shunt. Such a generic flux analysis tool can serve as a quantitative tool for metabolic studies and phenotype comparisons, and can be extended to other plant systems.

ABSTRACT # 107

STUDYING PLANT AND FUNGAL GENE EXPRESSION IN SYMPTOMLESS AND CHOKED INFLORESCENCES OF LOLIUM PRATENSE INFECTED WITH EPICHLO FESTUCAE

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Cool season grasses often develop a symbiotic relationship with endophytic fungi of the genus Epichlo (mitotic state = Neotyphodium). These endophytes colonize the intercellular spaces of grass plants, staying invisible for most of their life cycle. Reproductive development of the plant induces the sexual life cycle of the fungus: external hyphae growing out from inflorescence primordia develop into a compact mycelial layer (stroma), enveloping and choking the emerging inflorescence of the host. Embedded in the mature stroma are perithecia, which eject ascospores capable of infecting new host inflorescences (horizontal transmission). In associations with asexual (Neotyphodium) species the endophytes continue intercellular growth in reproductive tillers, infect the developing embryos and efficiently transmit vertically via seeds. Some of these species produce bioprotective alkaloids and improve abiotic stress resistance, traits which are extensively used in forage and turf g
rass production. Epichlo festucae exhibits both growth modes on one host plant. It also produces bioprotective alkaloids and, therefore, represents a suitable model for studying the developmental switch between benign and antagonistic growth associated with mutualistic and parasitic symbioses. This poster represents two strategies for studying the phenomenon: sequencing of ESTs from cDNA libraries of symptomless and choked inflorescences from Lolium pratense infected with E. festucae, and hybridization of cDNA samples from these tissues to cosmid clones of a genomic library from E. festucae.

ABSTRACT # 108

THE ORIGIN OF STRONG CORRELATIONS IN METABOLOMICS

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The quick pace of development of experimental techniques in metabolomics in the recent past has led to a great increase in the amount of data available. A phenomenon recognized early in metabolomics development is that for replicate samples a few metabolite pairs display an unusually strong correlation. Initially it was thought that these reflected the underlying stoichiometric network. However, a recent publication (Steuer et al., 2003, Bioinformatics, 19, 1019) demonstrated that this is not necessarily the case. Here we present a hypothesis based on Metabolic Control Analysis, that the origin of such correlations lies within the concentration control distribution, and that for those highly correlated pairs there is a single enzymatic step with a disproportionate share of control. The interpretation of metabolite scatter plots is then more directly related with metabolic control than with co-response, despite the superficial resemblances. We will present metabolic simulations that illustrate this principle, and accompany them with a theoretical basis. Metabolite pair correlations may be used as a diagnostic of the overall regulation of the system - even if the regulation is essentially genetic rather than metabolic. This makes correlation analysis of replicate metabolomic samples a useful tool in systems biology.