Metabolic profiling of shoot apices infested by the peach-potato aphid in susceptible and resistant peach cultivars

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Metabolomics, Vol. 2, No. 4, December 2006 (© 2007)
DOI: 10.1007/s11306-006-0035-1

Proceedings from the 4th International Plant Metabolomics Conference

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OVERVIEW

The 4th International Plant Metabolomics Conference (http://www.pmet06.org) was held between April 7 and 10 2006 at Wokefield Park in Reading, England. The meeting followed a series of three previous meetings held at Wageningen (2002, organised by Raoul Bino and Robert Hall), Potsdam (2003, organised Oliver Fiehn) and Iowa State University (2004, organised by Basil Nikolau and Eve Wurtele). The Reading conference was attended by 180 delegates. The scientific programme was put together by Prof. Mike Beale with assistance from the International Advisory Board and consisted of two full days and two half days of scientific talks. In addition to this, there were 87 poster presentations submitted by attendees. The plenary lecture was given by Prof. Robert Last of Michigan State University whose talk entitled ''The Measure of a Molecule – Confessions of a Biochemical Geneticist'' proved to be an excellent opening talk for the meeting. The rest of the meeting was organised into seven sessions, comprising 13 invited lectures and 24 talks which were selected from abstracts. Session 1 entitled ''Technology'' was opened by Thomas Moritz, who gave a comprehensive overview on GC–MS and its applications in plant metabolomics. The second invited speaker in this session was Jane Ward who described the utility of NMR in plant metabolomics, highlighting recent research in the generation of large datasets from dynamic growth experiments using Arabidopsis. The session was complemented with four talks selected from abstract submissions. This session was followed with a short program on ''Flux Measurements'' where Uwe Sauer gave an excellent talk on intracellular carbon traffic and was complemented by two shorter talks selected from abstract submissions. Session 3, ''Data Mining and Databasing'' was opened by Eve Wurtele, who described the current status of the MetNet platform at Iowa State University. Age Smilde also provided a comprehensive guide to the analysis of large complex datasets in this session. The ''Integration of Omics Datasets'' is now required by many research groups and this topic provided the subject matter for Session 4 where Kazuki Saito described his laboratory's omics work in this area using *Arabidopsis* anthocyanin biosynthesis mutants. The organisers of the meeting were very pleased to also have Oliver Thimm speak in this session. Dr. Thimm described his work on the pathway visualisation tool, MapMan. The largest session in the meeting covered ''Pathway Analysis'' and was broadly split into two sections with Steve Smith opening The Primary Metabolite section with an excellent talk on the TCA cycle. In the Secondary Metabolite section of this session, Lloyd Sumner described the advances in his work on *Medicago truncatula*. Session 6 dealt with ''Interactions'' and again was broadly split into two areas. Dieter Strack described his lab's work on plant–fungal interactions and Nicole van Dam covered the plant-insect interactions area. Finally, Session 7 dealt with ''Applications''. The organising committee were delighted to have Peter Beyer speak in this session and he gave an excellent account of his experiences in developing Golden Rice as a new nutritional product. The last talk of the meeting was given by Robert Hall who not only described his group's work on quality trait analysis in tomato, but also provided a summary on the current status of Plant Metabolomics.

The scientific sessions were complemented with a dedicated workshop, ''Standards in Metabolomics''. This consisted of an open discussion led by a panel consisting of Oliver Fiehn, Basil Nicolau, Nigel Hardy and Lloyd Sumner. Discussions in this area continue under the auspices of The Metabolomics Society.

In summary, the meeting was well attended and provided a good coverage of work currently being carried out in the field of Plant Metabolomics. In this meeting, it was evident that science has moved forward from simply method development studies to utilising the technique to answer actual biological questions. Advances in data analysis and databasing capabilities are now allowing researchers to integrate their ''omics'' datasets allowing a fuller systems biology approach to plant biological problems. Finally, the plant metabolo-

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mics community are looking forward to the 5th International Plant Metabolomics Conference which is to be hosted by Kazuki Saito and is due to take place in Yokohama, Japan in 2008.

ACKNOWLEDGMENTS

We are indebted to Tracy Goodenough for her help in organising the conference. In addition, many thanks go to Kim Rodford for her assistance in formatting the final manuscript. For the help with the smooth running of the event itself, thanks go to staff at Wokefield Park. Finally thanks go to all our sponsors (Leco, Bruker Biospin, Syngenta, Dionex, MeT-RO, Advion, Waters, BlueGnome and Springer).

PLENARY LECTURE

The measure of a molecule – confessions of a biochemical geneticist

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Genetics-based plant biochemical pathway analysis has benefited greatly from fast and accurate methods for metabolite measurement. Such 'brute force' mutant screening has traditionally relied on various compound-class analytical methods such as thin layer chromatography, gas chromatography and liquid chromatography. The advent of more accessible mass spectrometry-based methods has allowed genetic dissection of a wider variety of pathways. Development of more 'omic' scale technologies allow us to consider the feasibility of looking at mutation-induced changes in hundreds or thousands of molecules at a time. In parallel, model organism plant functional genomics resources such as large sequence-indexed insertion mutant collections are blurring the distinction between 'forward' and 'reverse' genetic screens.

I will discuss some recent examples of the application of analytical chemistry to functional genomics. A large collaborative project is being carried out at Michigan State University to discover functions for several thousand nuclear genes encoding plastid targeted proteins. Aspects of this project will be used to highlight the opportunities and challenges we face in creating large phenotypic datasets, including metabolite measurements, which will be of utility to the broad community of plant biologists.

INVITED LECTURES

IL1 – profiling metabolites by mass spectrometry: not just detecting peaks

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Metabolomics has become an important tool the last year in different areas of plant biology. Although there is no general analytical platform that can be used for the metabolomics definition to identify and quantify all metabolites in a biological sample, mass spectrometry (MS) has become the standard instrumentation for metabolomics analysis. The reason for this is its low detection limits, general robustness and in many cases user friendliness. GC/MS is now widely used for profiling metabolites from plant extracts that are volatile or can be made volatile after derivatisation. However, the importance of also including LC/MS analysis to cover more of the metabolome is obvious in order to come close to the definition of metabolomics analysis.

Both GC/MS and LC/MS generate large amounts of data, which sometimes is informative, e.g., the identity of the peaks is known. However, as generated peaks not always represent known compounds or even relevant information it is of importance to develop strategies for both identification of compound and extract relevant information from GC/MS or LC/MS analysis. We will present the principle of GC/MS and LC/MS generated metabolomics data, and discuss quality control of analysis and identification of compounds. A lot of focus will be on the instrumentation (GC/TOFMS and LC/TOFMS; TOF = time-of-flight) and its advantages and disadvantages, and how to move from GC/MS or LC/MS peaks towards information about peak identity.

IL2: NMR fingerprinting and beyond

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Using examples drawn from projects aimed at systematic gene function analysis in *Arabidopsis thaliana* as well as from field experiments studying the growth of wheat under different nutritional regimes, we will describe how the [^{1}H]-NMR–PCA screening methodology, developed in the clinical arena, has been adapted and extended for application to plant metabolomics.

The model plant *Arabidopsis thaliana* is small and has a rapid growth cycle and the availability of collections of ecotypes, knockout mutants, and transgenic lines and a fully sequenced genome makes this an ideal system for large-scale metabolomic analysis in the context of gene function analysis. The ability to produce large numbers of genetically identical subjects in controlled environments leads to a distinct advantage in experimental statistical design for plant metabolomic experiments. Experimental protocols for the uniform growth and harvesting of Arabidopsis have been extensively researched and developed into a high-throughput screening operation on a 600 MHz instrument. Results from the analysis of mutants as well basic studies on the diurnal variation in metabolites over light–dark growth cycles will be drawn on to describe how [^{1}H]-NMR–PCA provides a comprehensive fingerprint that reports rapidly on the status of the more abundant metabolites in the plant. The resultant fingerprint database can be used to classify plant lines by PCA, HCA and PLS-DA cluster analysis. Automated comparison of loadings and contribution plots from these analyses indicate metabolites and pathways that can be targeted for selective analytical regimes.

Using field-grown wheat as an example, we will also demonstrate how coupling of NMR analysis with ESI–MS can increase the fingerprinting information content of the screening regime. We will also demonstrate how introduction of SPE separation into the system can further refine the analysis to target particular classes of metabolites.

IL3 – metabolic networks in motion: analysis of intracellular carbon traffic

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The two experimentally accessible variables of metabolic networks are protein and metabolite concentrations. Direction and rate of fluxes within the network additionally depend on reaction thermodynamics and kinetic properties of the participating enzyme(s), which might be further modulated through various regulatory mechanisms that include allosteric regulation and protein modification. Intracellular fluxes are thus the functional output of integrated biochemical and genetic interactions within complex metabolic networks that are pivotal for understanding of network operation (Hellerstein, 2003). In contrast to the directly measurable concentrations of metabolites and proteins, fluxes are per se non-measurable and must be inferred from other quantities. For this reason, quantification of intracellular fluxes has long lagged behind our capability to track global metabolite, mRNA or protein concentration changes. The key advance were recent developments in ^{13}C-labeling experiments (Sauer, 2004) that paved the road for large-scale experimental analysis of intracellular fluxes (Fischer *et al.*, 2004). After a brief introduction to the principles of flux analysis, I will discuss design principles of carbon traffic that include network rigidity and robustness as well as the relevance of genetic regulation. The focus is on metabolism in the model microbes *Bacillus subtilis*, *E. coli* and yeast, for which large-scale data sets are available (Fischer *et al.*, 2005; Blank *et al.*, 2005).

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IL4: the Metnet platform elucidation of metabolic and regulatory networks

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A major challenge in the post-genome era is to understand how interactions among the molecules in a cell determine its form and function. MetNet is an emerging open-source software platform for exploration of disparate experimental data types and regulatory and metabolic networks in the context of Arabidopsis systems biology. The MetNet platform features graph visualization, interactive displays, graph theoretic computations for determining biological distances, a unique multivariate display and statistical analysis tool, graph modeling using the open source statistical analysis language, *R*, and versatile text mining.

The use of the MetNet tools is illustrated with transcriptomics and metabolomics data. Our studies reveal transcriptional modularity of core metabolic and biological processes. These modules incorporate transcriptional control of a wider set of transporters, enzymes, cofactor and substrate producing proteins and regulatory molecules that may represent a common task.

IL5: analysing complex metabolomics data

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Systems biology is the study of biology as an integrated system of genetic-, protein-, metabolite-, cellular- and pathway events that in flux and independent. Due to the availability of advanced instrumentation it is possible to generate very complex data sets and a systems biology approach becomes a possibility.

A part of systems biology is metabolomics. The amount of data generated in metabolomics studies is huge and the type of data can be very complicated. such data can have a multiway-, multiset- or multilevel structure or combinations thereof. These data structures ask for special models to summarize and visualize the data in useful information. Moreover, there is an increasing awareness that time and spatially resolved metabolomics is crucial for systems biology. This also calls for new data analysis and bioinformatics tools.

An overview will be given of the different kinds of complexities in metabolomics data. Some new methods (e.g., ASCA) will be presented that can deal with some of these complexities. All this will be illustrated with real-life metabolomics data sets from different origin, e.g., from the fields of plant-, mammalian-, and microbial metabolomics.

IL6: integration of omics datasets towards metabolomics-based plant systems biology

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Integrated analysis of omics can provide the clues for identification of gene function and the precise information about gene-to-metabolite and/or metabolite-to-metabolite networks. We are running the project combining transcriptome and metabolome in *Arabidopsis thaliana* aiming plant systems biology. Nutritional stress of nitrogen and sulfur resulted in global change of metabolome that could be correlated with the modulation of gene expression, indicating the presence of gene-to-metabolite networks, in particular, in glucosinolate biosynthesis. The comprehensive gene expression and metabolite profiles of anthocyanin overproducing Arabidopsis lines revealed the function of novel genes that are responsible for modification and storage of anthocyanins. The function of those candidate genes was identified by analysis of the T-DNA insertion lines and recombinant proteins.

By expanding this strategy, we used a transcriptome database publicly available for functional identification of unknown genes and networks. Upon these co-expression analyses, the model of co-expressed genes in flavonoid pathway was constructed, leading to speculation of functions of those genes followed by confirmation with reverse genetics strategy. This strategy of integrated analysis of metabolome and transcriptome will be applicable not only for a model plant *A. thaliana*. Recent these efforts towards metabolomics-based systems biology will be presented.

IL7: functional data integration in plant systems biology

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Systems biology aims to understand and model whole plant systems their underlying physiological processes and regulatory networks. With the recent advent of high-throughput techniques the major bottleneck is no longer the generation of necessary data sets, but their biological interpretation to achieve the desired insight into plant systems. Therefore, researchers have recently focused on the integration of complementary data sets generated by transcriptomics, metabolite profiling, proteomics, enzyme assays and phenotyping approaches. Novel bioinformatics tools have been developed that successfully integrate classical statistical analyses into dedicated graphical user interfaces. In this presentation, we will cover examples of integrative analysis of high-throughput data based on biological information and functional categorisation to learn more about physiological networks and systems regulation in plants. The examples will be drawn from the following areas:

(i) development of a knowledge-driven plant ontology (MapMan – BIN system)
(ii) visual integration of expression and metabolite data sets using the pathway visualisation tool (MapMan)
(iii) development of a text-mining tool for updating and transfer plant ontologies (PlantMiner)
(iv) identification of functional hotspots and functional modules in expression data using functional statistics
(v) identification of trait-related lead genes in industrial-scale metabolite profiling data sets

IL8: primary metabolic pathways and the making of the metabolome

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There is a widely held assumption that the primary metabolic pathways of plants are known and understood, and that the 'textbook' versions can be relied upon to provide the framework for metabolomics research. This assumption is not entirely justified. We continue to find surprises and novelty: new pathways, new functions for 'old' enzymes, correct functions for mis-assigned enzymes, and new examples of metabolic plasticity. Much of my research has focused on the fuel supply and consumption that drives plant growth. The fuels in question are starch, sucrose and triglycerides. We have discovered a pathway for the conversion of transitory leaf starch to sugars, which was not even suspected 5 years ago. The fundamentally important role of maltose as a primary metabolite has been revealed, and a novel cytosolic heteroglycan about which very little is known, is implicated in this pathway. In research on fatty acid metabolism, we have discovered a new and essential role for peroxisomal citrate synthase. Furthermore, when we knock out malate synthase, an 'essential' enzyme of the glyoxylate cycle, an alternative pathway comes into play. We have also shown that peroxisomal malate dehydrogenase is not required by the glyoxylate cycle despite popular misconception. Likewise, when we knock out peroxisomal malate dehydrogenase – an 'essential' enzyme of the photorespiratory pathway – the plants grow happily in ambient air. These are steps at the very centre of primary carbon and energy metabolism in plants, and hence at the centre of the making of the metabolome.

IL9: "metabolomics, a mature discovery science"

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Modern metabolomics is now approximately a decade old and many are questioning the relative performance of this technology. This presentation will provide specific examples that illustrate metabolomics as a mature technology and discovery science. These examples originate from an integrated functional genomics project that is studying the response of *Medicago truncatula* to biotic and abiotic stress and includes the large-scale analyses of mRNA, proteins, and metabolites. *Medicago truncatula* is a rapidly growing model for the study of legume biology, and it is an excellent species for fundamental studies of secondary metabolism.

This presentation will focus on the metabolomics aspects of this project within the overall context of an integrated functional genomics approach. Descriptions and rationale will be provided for the technologies being used and a brief discussion will be provided on the critical bioinformatic components of the project. Then, examples will be provide that illustrate how metabolomics and integrated functional genomics are yielding valuable information relative to gene validation, gene discovery, hypothesis building, and enhanced mechanistic understandings of primary and secondary metabolism.

IL10: metabolic profiling of arbuscular mycorrhizal roots of *Medicago truncatula*

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Roots of most land plants in all ecosystems are colonized by soil-borne fungi in symbiotic interactions to form arbuscular mycorrhizal (AM) roots, characterized by exchange of carbon from the plant versus mineral nutrients, especially phosphate, and water from the fungus. This association requires differentiation and integration of both plant root and fungal metabolism. In an unbiased comprehensive time-dependent metabolite profiling of *Medicago truncatula* roots during colonization by *Glomus intraradices*, primary and secondary metabolites were analyzed and compared to non-colonized control plants using GC/MS, LC/MS and HPLC–DAD. Metabolite profiling at various time points of AM root development gave a first insight into trends of mycorrhiza-specific metabolic changes.

Profiling of polar primary metabolites showed an increase in phosphate and fungal trehalose along with certain amino acids and fatty acids indicating activation of root plastid metabolism. Among the unpolar fatty acids, fatty alcohols and glycerides as well as campesterol and methylene cholesterol, a marked time-dependent increase in palmitic, oleic and the fungus-specific vaccenic and palmitvaccenic was observed.

The constitutive secondary compounds, i.e., isoflavones and saponins, slightly increased at later mycorrhizal stages. However, there is a strong mycorrhiza-specific accumulation of C13 and C14 apocarotenoids parallel to AM root development. Analyses of cell wall-bound phenolics showed an induced accumulation of tyrosol. Correlation analyses indicated stronger correlations between metabolites in mycorrhizal roots compared to non-mycorrhizal ones. Fungal root colonization obviously induces a tighter regulation of root metabolism. The data may be used as a platform in transgenic approaches to unravel the molecular dialogue and specific physiological processes in AM symbioses.

IL11 – ecological metabolomics: the interactive chemistry between plants, their friends and their foes

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Plants are sessile organisms. Therefore, they have evolved an arsenal of chemical defences against herbivores. Secondary metabolites (e.g., glucosinolates) are well-known elements of the plant's weaponry. Chemical defences can either act as 'direct defences', when they directly affect herbivores, or as 'indirect defences', when plants attract natural enemies of herbivores by volatile release or providing food sources. Both defence systems can be induced, i.e., increased upon herbivore attack. The effectiveness of plant defences is co-determined by levels of primary metabolites within the plant. This is because the building blocks (e.g., amino acids) of secondary metabolites originate from primary metabolism. Moreover, secondary metabolites may be more effective if the plant tissue has a low nutritive value.

Plant metabolomics, therefore, is a valuable technique for ecologists studying plant–insect interactions, because both primary and secondary metabolism are analysed simultaneously. Unfortunately, ecologists generally do not have unlimited access to the high-tech equipment and knowledge infrastructure needed for plant metabolomics. Moreover, biologically active secondary compounds usually occur in quantities that are several orders of magnitude smaller than those of primary compounds. Thus, the dynamic range of the employed analyses may be too small to observe the compounds of interest. For ecologists, plant metabolomics analyses will most likely be used to construct a metabolite profile, from which key compounds are selected for targeted analysis. We give some examples of our studies on (induced) defences in wild Crucifers and their effects on the aboveground and belowground multitrophic interactions associated with these plants.

IL12: "Golden Rice on a mission" – on the adventure of developing a GM product in the public sector

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Golden Rice (GR) is the generic name given to genetically modified rice (*Oryza sativa*) that produces b-carotene (provitamin A) in the endosperm. Accumulation of ß-carotene confers a yellow color to the grain which becomes visible after polishing, a procedure that is routinely employed to remove the outer grain layers. Research towards GR was initiated to help cope with vitamin A deficiency (VAD), which represents a major global health problem. Through agriculture and local trade GR is expected to reach the target populations, namely the urban poor and rural populations, especially those living in remote areas. Since its original discovery, the prototype GR has undergone intense research to increase the provitamin A content, establish the scientific basis for its carotenoid complement, and cope with regulatory requirements. Getting GR effectively in the hands of farmers stands nowadays in the foreground, a novel avenue for public sector research. Compared to basic science, product development requires a completely different set of logistics, focus, skills and specific expertise. These competences are well established in R&D based companies but is hardly found in public sector research institutions. However, while GR meets a public demand it is of little commercial interest. Hence, the public sector should take over the responsibility to generate and deliver this product, at no extra cost for the technology used.

Additional research is underway to further increase the nutritional value of GR such as the accumulation of vitamin E, iron and zinc, and the increase in deposition in the grain of high-quality protein or essential amino acids.

IL13: current value and future potential of metabolomics for quality trait analysis

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The quality of both crop plants and their derived products is a direct function of their metabolite content. With the continued development of advanced analytical approaches and new bioinformatics tools, the range of useful applications for metabolomics is growing steadily. In particular, non-targeted metabolomics approaches have great potential to extend our knowledge and understanding of complex quality traits, despite our initial lack of information on the related biochemistry. In this presentation, an overview will be given of how metabolomics is already being used to visualise plant quality traits and how genetic and environmental factors play a role in influencing these. Key examples from the literature and from our own work will be used to illustrate how metabolomics is giving us a clearer and deeper picture of scientifically/commercially important traits such as taste, disease resistance, and nutritional value. Furthermore, with the potential to link metabolomics data to complementary data from other analytical sources, we have never been in a better position to associate specific metabolites or metabolic profiles with, e.g., bioactivity, phenotype, health-promoting capacity, anti-oxidant levels, etc. Related to this, reference will also be made to how metabolomics shall prove a useful tool for identifying valuable biomarkers to assist in process development, production chain management and in targeted breeding strategies. To conclude, the topic of further developments and future ambitions will be broached to assess where we realistically might wish to be in several years time and where the greatest metabolomics challenges still lie. How holistic can we be?

SELECTED LECTURES

SL1: high resolution profiling of plant secondary metabolites capillary liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry

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There is an urgent need for LC–MS based metabolite profiling to improve coverage of metabolomes. This need is even more pressing in plant science. A highly rich and diverse secondary metabolism is a hallmark of plant biology. Lacking the ability to avoid or to retreat from unfavorable conditions or potential foes, plants have evolved an enormous metabolic plasticity, which allows them to dynamically respond to environmental changes through the synthesis and/or degradation of particular compounds. We developed a platform for the highly sensitive profiling of mostly secondary metabolites, employing capillary liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry (CapLC–ESI–QTOF–MS). This approach achieves a very good coverage of Arabidopsis secondary metabolism. A recent compilation listed six biosynthetic classes: nitrogen-containing compounds, phenylpropanoids, benzenoids, polyketides such as flavonoids, terpenes and fatty acid derivatives. Metabolites of five of these classes can clearly be detected by CapLC–ESI–QTOF–MS. Furthermore, in-source fragmentation and targeted tandem MS analysis allow to obtain structural information on unknown compounds. This is of paramount importance given, for instance, the conservatively estimated 5000 metabolites in *Arabidopsis thaliana* of which maybe 500 are annotated today. Databases for LC–MS spectra and for known and ''theoretically'' occurring compounds in the Brassicaceae help in structural elucidation and in cataloguing the Arabidopsis metabolome. A systematic evaluation of matrix effects has shown that the good separation achieved allows reproducible quantification. The platform and examples of application for the elucidation of plant interactions with the biotic and abiotic environment will be presented.

SL2: simultaneous collection of on-line LC–MS/MS and LC fractions with post-column splitting maximizing MS information of complex samples with nanoelectrospray ionization

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Purpose: Development of a system incorporating a post-column splitter to enable simultaneous LC–MS and collection of LC fractions for complex samples.

Introduction: There have been several publications on nanoelectrospray ionization (at flow rates below 25 nL/min) providing less ion suppression compared to higher flow ESI for multi-analyte mixtures (Karas *et al*., 2003). Recent publications with the ESI Chip® have demonstrated this benefit at flow rates up to 500-nL/min (Schultz *et al*., 2004; Hop *et al*., 2005). We believe this is due to the unique formation of the electric field with the chip. Electric field models show the field equivalent to that of a 2-mm diameter pulled capillary. This is due to the field being dropped across the dielectric coating on the nozzle (between the fluid/spray voltage compared to the ground potential of the silicon under the dielectric coating). This work extends this benefit to LC with flow rates up to 1.0 mL/min by use of a post-column splitter coupled to the ESI Chip.

LC–MS/MS continues to be the gold standard for acquiring qualitative and quantitative information for complex mixtures. Applications such as metabolomics, biomarkers and proteomics involve the analysis of known and unknown species with a desire to quantify and characterize each component. Sample complexity limits the amount of time for MS/MS and MSn during an LC run.

This work will describe the development of a system that enables coupling of LC columns with flow rates up to 1.0 mL/min to the ESI Chip with use of a post-column splitter. The system enables collection of LC–MS and LC fractions simultaneously. Software links LC–MS retention times to specific LC fractions collected in 96 or 384-well sample plates. Infusion of LC fractions enables optimization of MS/MS parameters such as collision energy for complex, multi-component fractions demonstrating greater than 10 times improvement in S/N compared to online LC–MS/MS.

Ibuprofen metabolites in human urine were investigated by both online LC/MS and chip-based infusion nanoESI of LC fractions. This work will highlight the improved data obtained by extended analysis of LC fractions for obtaining metabolite MS/MS data on low level metabolites.

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SL3: using Ultra Performance Liquid Chromatography (UPLC)–QTOF–MS for Metabolome analysis of tomato fruit peel

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The peel of fruit acts as a protective barrier and as a medium for the exchange of gases and water. We study the peel of tomato to unravel the network of genes and proteins which determine the unique characteristics of this tissue. Four major metabolic pathways synthesizing cutin, wax, triterpenoids, and aromatic metabolites are active in tomato peel. Our analytical platform includes UPLC coupled to a Q-TOF–MS instrument. The UPLC system detects more peaks with faster run times compared to HPLC. Methanol extracts of tomato flesh and peel tissues derived from four developmental stages were separated with a 7 min gradient and analyzed by MS in ESI positive and negative modes. For data analysis, we used the MarkerLynx program that conducted mass signals alignment across samples and identified differential markers. Gross changes in levels of secondary metabolites were detected through peel development. We also examined a natural tomato mutant termed /"y/" showing a colorless peel phenotype compared to the yellow-colored normal peel. Peel and flesh tissue derived from four developmental stages of the /"y/" mutant were profiled. Data interpretation by means of PCA showed that most changes in the /"y/" mutant occur in the orange/red stages of development. Apart from a decrease in the levels of the yellow flavonoid, naringenin chalcone, levels of naringenin and possibly its derivatives were decreased in the /"y/" mutant. Metabolites with elevated levels in the mutant peel were also detected. We are currently conducting structural elucidation of differential compounds and generating equivalent data at the transcriptome level.

SL4: use and limitations of GC–MS based metabolite profiling methods for the analysis of metabolite content in freeze-dried potato tubers

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Compared with targeted analysis, metabolite profiling methods using GC–MS are not suited to all classes of metabolite, and are necessarily a compromise. We have examined parameters effecting the applicability, optimization and limitations of a method for analysis of potato tuber metabolites. Conversion of reducing sugars to methyloximes is a key step in the protocol, and optimal oximation conditions were 50° for 4 h. Lower temperatures or shorter times resulted in incomplete oximation whereas higher temperatures and longer times resulted in hydrolysis of sucrose. Ascorbate is an important metabolite that is readily measured by targeted approaches but can be a problem in metabolite profiling. Using standards and freeze-dried (FD) potato, ascorbate was degraded during oximation; only in fresh material did some ascorbate survive the oximaton conditions. The method was used to demonstrate the presence of radial and longitudinal metabolite gradients within tubers. These findings impact upon how representative tuber samples are obtained and consequently the use of FD material, which is also a convenient form for storage, was investigated. Metabolite compositions in fresh and FD tubers were generally very similar, although several membrane-derived non-polar metabolites were elevated in fresh material. Using FD material the overall protocol was highly linear, and providing acceptable linearity and repeatability, use of FD or fresh material are equally valid. The short- and long-term repeatability of the method was studied, and the use of reference materials to monitor and to improve the quality of the data is discussed.

SL5: molecular structure analysis and its metabolic flow measurement at the atomic level by a hetero-nuclear NMR

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Although ordinal NMR-based metabolomics approach has been performed by conventional proton-NMR, we are trying to establish a hetero-nuclear NMR-based methodologies. This approach has several advantages, (1) able to obtain the information of functional groups of each metabolite that allows to identify metabolites easily, (2) able to measure metabolites non-invasively, (3) no complicate sample preparation is required, (4) able to detect the flow of metabolites and also the flow of each atoms of metabolite using stable isotopes. In addition, using magic angle spinning NMR measurement, the information of insoluble larger biomolecules can be obtained.

We have conducted the stable isotope labeling of several plants, optimization of the extraction solvent for metabolites, building a chemical shift database, and constructing a semi-automatic signal assignment system, SpinAssign written in Java language. Using these developed technologies, as a case for metabolomic analysis, we studied the metabolic changes of chemical (such as plant hormone) responses to Arabidopsis T87 cultured cell both in vivo and in vitro. From our effort, uniqueness of the hetero-nuclear NMR-based approach are not only structure analysis of metabolites, but also for metabolic flow analysis, characterization of biomacromolecules such as carbohydrates and measurement of metabolite localization using non-invasive experiments.

SL6: metabolic flux analysis for the study of the regulatory role of susy on carbon metabolism

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The development of non-photosynthetic tissues is closely related to sucrose import and metabolism. Sucrose is degraded by sucrose synthase (SuSy) or invertase to provide UDP-Glc or hexoses-P for the biosynthesis of structural or storage compounds and for ATP production. Although often suggested, the regulatory role of SuSy in sugar partitioning remains a matter of debate. Short and steady state labeling experiments (Dieuaide-Noubhani *et al.*, 1995, Rontein *et al.*, 2002, Alonso *et al.*, 2005) were used to analyze glucose metabolism in maize root tips of an inbred line and a mutant in two sucrose synthase genes derived from this line. Unidirectional rates of synthesis for storage compounds were determined by short labeling experiments using [U-^{14}C] glucose, and compared with net synthesis fluxes: the difference gives the unidirectional rate of degradation of these storage compounds, which also is the rate of glucose production from these compounds. Data obtained after steady state labeling with [1-^{13}C] glucose, [2-^{13}C]glucose and [U-^{13}C] glucose, were interpreted using the software C13-FLUX (Wiechert et al., 2001). About 30 fluxes in intermediary metabolism were quantified. The reduction of SuSy activity leads to a 52% increase in the wall synthesis flux. Most of the fluxes, in particular the substrate cycles, were not significantly modified.

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SL7: what is new in metacyc and aracyc metabolic pathway databases for plant research and their applications

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MetaCyc (http://metacyc.org/) is a literature-based curated universal metabolic pathway database of many organisms. MetaCyc (version 9.6) has 692 pathways with 9566 literature citations. The database contains many curator comments for pathways and enzymes and contains a wealth of enzyme property information. The growth of data content in MetaCyc has been tremendous in recent years. The number of plant secondary metabolic pathways has grown from only a handful to over eighty. Among other applications, the rich and diverse information on metabolic pathway variants across species can assist comparative studies of metabolism. AraCyc (http://arabidopsis.org/tools/aracyc/) is a species-specific database computationally derived from MetaCyc. It contains only enzymes and pathways found in the model plant *Arabidopsis thaliana*.

Over the last year, AraCyc has undergone intensive curation to enhance data quality and to increase the breadth of coverage. AraCyc (version 2.6) has 228 pathways, of which, 88% have been manually verified with literature supports. An evidence code system was recently deployed in both databases so that each pathway and enzymatic reaction contains an evidence providing the assertion for the existence of the pathway or the enzyme activity. Different types of data (compound, reaction, pathway, enzyme and gene) are highly integrated and can be browsed or queried in a number of ways. In addition to querying and browsing, the OmicsViewer, a data visualization and analysis tool, has been enhanced to allow many different types of large-scale omics data resulting from microarray, metabolite profiling, and proteomic profiling experiments to be overlaid onto the full metabolic pathway map of Arabidopsis.

SL8 – KNApSAcK: search tool for relationship between metabolites and species

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Many metabolites have been identified from plants, microbes and other organisms. Secondary metabolites are highly species specific and play a role for the survival of the producing organism within its natural habitat. The number of metabolites present in the plant kingdom is estimated to exceed 100,000 – an enormous number indicating a large scale of structured diversity of compounds. Several databases have been made by collecting metabolite information of various organisms, and provide some chemical information and biological pathways on metabolites, however, they don't provide the relationships between metabolites and their biological origins. To systematically and comprehensively understand species-specific diversity of metabolites, we have designed a database system called KNApSAcK. This system is useful for obtaining information on metabolites and their corresponding species, chemical structure and biological activity. In addition, the database has a tool that can be used for analyzing datasets acquired using Fourier transform ion cyclotron mass spectrometry. We collected information on 24,604 metabolite-species pairs encompassing 10,181 metabolites and 7362 species from published references (October 11th, 2005). These data have been stored on a server which is located in Nara Institute of Science and Technology. When the KNApSAcK system is started, these data are automatically downloaded from the server. This database system and online manual are freely available at http://kanaya.naist.jp/KNApSAcK/. This database system is available in Web- and Download-version. To use the Web-version, get into http://kanaya.naist.jp/KNApSAcK/KNApSAcK.php. If you want to use the Download-version, at the beginning, you have to install KNApSAcK and Java 1.4.2 on your local computer.

SL9: integration of metabolomics, proteomics and multivariate data analysis reveals improved pattern recognition and novel insights into systemic responses

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GC–TOF–MS is well suited for the fast and comprehensive analysis of ultracomplex metabolite samples (Weckwerth *et al*., 2004). For the integration of metabolite profiles with quantitative protein profiles a method is required with comparable throughput. Here, we have implemented the shotgun proteomics approach using LC-Iontrap-MS and ion count for identification and quantification of tryptic peptides from unique proteins in a complex protein digest.

Multivariate statistics are applied to examine pattern recognition and biomarker identification. In particular, protein dynamics and metabolite dynamics alone were compared with a combined data matrix. The integration of the data revealed different patterns of samples and novel multiple biomarkers giving evidence for an increase of information in such holistic approaches (Morgenthal *et al*., 2005).

The approach is exemplified for a set of cold-adapted Arabidopsis thaliana plants. With the proteomics approach alone typical protein markers for stress response were identified. The combined data give rise to novel biomarkers for cold-adapted plants.

In the same experiments dynamic changes of metabolite correlation networks (Weckwerth, 2003) in temperature stress response were correlated to posttranslational modification of sucrose-phosphate synthase (Wolschin *et al*., 2005), a key enzyme in sucrose metabolism.

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SL10: integrated time-series metabolomic and transcriptional profiling analyses of *Arabidopsis thaliana* response to elevated CO_2 and osmotic stress

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Integrated time-series metabolomic and transcriptomic analyses of a systematically perturbed biological system can provide clues about gene and metabolic regulation, correlations between parallel occurring phenomena, the reconstruction of bioreaction networks and even the function of unknown genes. Even further, comparing the system's response to individual perturbations with their combined effect might reveal information about the robustness of the regulatory networks, missed when studying individual perturbations only.

In this context, we subjected 12-day old *Arabidopsis thaliana* liquid cultures, grown at constant light and temperature, to elevated CO_2 levels (1%) and osmotic stress (50 mM NaCl), both individually and simultaneously, continuously for 30 hrs. Two liquid cultures were harvested at each of eight time points throughout the duration of the experiments. The metabolomic and transcriptomic profiles were acquired by Gas Chromatography–Mass Spectrometry (GC–MS) and full genome cDNA microarrays, respectively. The GC–MS metabolomic data were corrected using a new normalization and validation strategy (Kanani *et al*., 2006). Both transcriptomic and metabolomic data were further analyzed using multivariate statistical analysis and a new rigorous technique (Dutta *et al*., 2006) for the analysis of time-series ''omics'' data. The results revealed a unique, identifiable response of *A. thaliana* to the individual and the combined stresses and also indicated the extent to which the plant's response to a particular stress is affected by the presence of the other. This information is deemed extremely valuable for understanding the plant's regulatory network, because it provides clues about which parts of the network are robust for a particular stress and which become active under special circumstances.

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SL11: integration, visualization and comprehensive analysis of diverse metabolite datasets

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The applications of DNA array technology in trasnscriptome analyses and chromatographic separation techniques coupled with mass spectrometry for metabolome analyses have resulted in the generation of qualitative and quantitative data crucial for understanding plant metabolism. However, the large quantity of information generated by modern techniques requires more efficient tools to process and visualize the diverse data. We are currently developing a legume specific tool aimed at the integrated analysis of transcript and metabolite data as well as visualization of individual transcripts and/or metabolites within the context of metabolic pathway maps. All metabolites currently detected using multiple XC-MS techniques (unidentified and identified) were tabulated, and putative biological functions were assigned. In parallel, all transcript elements contained on the recently released Medicago Affymetrix chip were annotated and grouped into functional categories using MIPS terminology.

Furthermore, genomic annotations were utilized to predict the legume specific metabolic pathways using the MetaCyc (see URL http://MetaCyc.org), which comprises a collection of metabolic pathways and enzymes from a wide variety of organisms including microorganisms and plants. The automated alignment was manually curated and Medicago-specific pathways such as isoflavonoid biosynthesis were added. The resulting pathways are being imported into a commercial software package GeneSpring for a statistical analysis and automated visualization of integrated transcript and metabolomics data. The presentation of data in this manner provides improved visualization and interpretation of the integrated data. Data acquired by microarray and XC-MS metabolite profiling while studying the response of *Medicago truncatula*cell cultures to different elicitors will be presented.

SL12: using metabolomics to decipher functions of Arabidopsis genes in the context of metabolic and regulatory networks

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A multi-institutional consortium of labs is developing metabolomics as a new functional genomics tool for elucidating the functions of Arabidopsis genes that are currently annotated as having an ''unknown function''. Approximately one third of the Arabidopsis genes are so annotated. The consortium utilizes five distinct analytical platforms that couple different separations methods (GC, LC, and CE) to mass-spectroscopic detection systems. These analytical platforms are used in both ''non-targeted'' and targeted metabolomics analyses, which in combination detect approximately 2000 metabolites, of which 700 are chemically defined. The consortium is applying these platforms to reveal changes in the metabolome associated with knockout mutations in genes of unknown function and comparing these to similar mutants in genes of known functions. We will discuss initial data generated from a small set of exemplar mutants. These data indicate that metabolomics can reveal metabolic changes in mutants that are otherwise ''silent'' in phenotype. These data are being interpreted via two strategies: (1) as a ''fingerprint'' of the metabolic consequence of each mutation, which can be used to functionally cluster genes; and (2) by mapping metabolite changes on metabolic and regulatory network maps, such as AraCyc and MetNetDB, to identify specific functions that are affected by each mutation. Thus, metabolomics, in combination with other ''-omics'' technologies promises to be a new resource for determining the function of Arabidopsis genes.

SL13: metabolite profiling of ascorbate-deficient Vtc mutants of *Arabidopsis thaliana*

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Ascorbate (vitamin C) is an abundant antioxidant and is also a cofactor for 2-oxoglutarate-dependent dioxygenases in plants. A range of ascorbate deficient *Arabidopsis thaliana* mutants have been isolated (Conklin *et al*., 2000; *Genetics* 154, 847), which have proved useful in investigating the pathway of ascorbate biosynthesis and the functions of ascorbate in plants. For example, some of the mutants are hypersensitive to ozone and high light stress. Three of these mutants (vtc1, 2 and 4) are affected in enzymes of the D-Man/L-Gal ascorbate biosynthesis pathway, while vtc3 is not yet identified. Metabolite profiling or fingerprinting offers the potential of identifying specific metabolic consequences of each mutation, as well as the metabolic consequences of ascorbate deficiency itself (which should to some extent be common to all the mutants). An extensive ^{1}H NMR analysis of the vtc mutants was carried out. Principal components analysis clustered the mutants into distinct groups from the wild type plants. vtc2 and 4, which are affected in neighbouring enzymes of the D-Man/L-Gal biosynthetic pathway, cluster together while vtc1, which is affected in an enzyme earlier in the pathway clusters separately. vtc3, whose function is so far unidentified, is similar to vtc1. Further analysis of this dataset, along with LC analysis of aromatic compounds will be presented.

SL14: acyl-CoA, oxylipin and triacylglycerol profiling as tools to investigate gene function in Arabidopsis

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We have developed targeted metabolite profiling methods to help determine the biochemical phenotype of Arabidopsis mutants disrupted in genes expected to be involved in various aspects of lipid, fatty acid and branched chain amino acid metabolism in *Arabidopsis*. We have used acyl-CoA and oxylipin profiling to characterize mutants in the peroxisomal ABC transporter, acyl-CoA oxidase, multifunctional-protein and thiolase genes of peroxisomal b-oxidation. This work has allowed us to demonstrate in-vivo acyl-CoA substrate specificity of the corresponding enzymes and the involvement of the peroxisomal ABC transporter and a specific acyl-CoA oxidase in jasmonic acid biosynthesis. Acyl-CoA profiling of mutants disrupted in the mitochondrial electron-transfer flavoprotein:ubiquinone oxidoreductase have allowed us to demonstrate that this protein is involved not only in branched chain amino acid catabolism but also, most remarkably, in the breakdown of phytanic acid derived from the phytol chain of chlorophyll. We have also developed a profiling method for triacylglycerols that we are now using as a diagnostic tool for screening plant oils in fast-track breeding and metabolic engineering projects. We are also employing this method to determine the function of genes involved in regulating the composition of the 80 plus species of triacylglycerol in *Arabidopsis*.

SL15: structural kinetic modeling of plant metabolic networks

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Construction and evaluation of detailed mathematical models of cellular metabolism are primary challenges in current systems biology. However, explicit kinetic modelling of metabolic pathways using differential equations is often severely hampered by inadequate knowledge of the enzyme-kinetic rate laws and their associated parameter values. Recently, we proposed a method that aims to give a detailed and quantitative account of the dynamical capabilities of metabolic systems, without requiring any explicit information about the particular functional form of the rate equations.

Our approach is based on constructing a local linear model at each point in parameter space, such that each element of the model is either directly experimentally accessible, or amenable to a straightforward biochemical interpretation. This ensemble of local linear models, encompassing all possible explicit kinetic models, allows for a systematic exploration of the comprehensive parameter space. It opens the way to identify important regulatory steps and allows assessment of the robustness of the system in a quantitative way. The method is exemplified with paradigmatic examples of plant metabolic pathways, such as the photosynthetic Calvin cycle and adjacent pathways.

SL16: metabolome adjustements induced by salt treatment in the extremophile *Thellungiella halophila*, an Arabidopsis relative species

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Plants submitted to osmotic stress associated to water deficit, high soil salinity or freezing, develop responses that involve overall physiological organisation, from morphogenetic to molecular adjustments. However, adaptative efficiency of these responses highly differs between tolerant and sensitive species, especially at the cell scale. At that level, osmo-induced regulations occurring in metabolism, such as osmolyte accumulations and metabolite fluxes, may play a key role in tolerance. Therefore, the comparative analysis of metabolome adjustments induced by stress in sensitive and tolerant plants should be usefull to question tolerance strategies.

The extremophile *Thellungiella halophila*, that shows more than 90% genome homology with its relative *Arabidopsis*, is well designated for such a purpose. We present here a pilot study where metabolic adjustments of *Thellungiella* following salt treatment are investigated, through GC-Q and GC-TOF analysis of methoxyme-trimethylsilylated metabolites and ICP quantification of cationic mineral contents.

Discrimination of *Thellungiella* and *Arabidopsis* plants grown hydroponically under optimal conditions is clearly established by PCA, on the basis of metabolite profiles. Noticeable differences are found among several analyte levels, including a greater fumarate content in *Arabidopsis* and two compounds only detected in *Thellungiella*, i.e., caffeoylquinic acid and digalactosylglycerol. Furthermore, metabolite and mineral ions profiling in different organs of *Thellungiella* submitted to salt treatment, associated to growth parameters and water status measurements, provide information about short and medium term dynamic of metabolic adaptation in this new model of extremophile.

SL17: metabolomics meets genetics linking metabolite profiles to QTLs

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Though variation in metabolite profiles is a well-known phenomenon in plants, it is still poorly understood to what extent this variation has a genetic basis. Here we present a novel approach integrating genetic and untargeted metabolite analyses in plants, using *Arabidopsis thaliana* as model. Instead of focusing on specific metabolites, we applied an empirical metabolomics approach using high resolution LC-QTOF MS followed by unbiased data processing and alignment. This strategy revealed many qualitative and quantitative differences in metabolite composition between *Arabidopsis* accessions, grown under identical conditions, with only 13.4% of the mass peaks being common between all 14 accessions analyzed. Quantitative Trait Locus (QTL) analysis of more than 2000 relevant mass signals, detected in a Recombinant Inbred Line (RIL) population derived from the two most divergent accessions, enabled the identification of QTLs for about 75% of the mass signals. More than one-third of the signals were not detected in either parent, demonstrating the large potential for modification of metabolic composition through classical breeding. Combining identification of mass signals and QTL profiles allowed us to analyze not only known metabolic pathways, but also to identify and elucidate novel biosynthesis steps.

The results show the power of untargeted metabolomics combined with QTL analyses in the identification of loci controlling metabolic profiles and in elucidating biosynthetic pathways. This can lead to the identification of the underlying genes and the construction of biochemical networks in relation to other phenotypic traits.

SL18: effects of insect herbivory and pseudomonas syringae infection on secondary metabolite profiles in *Arabidopsis thaliana* leaves

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Because plants are not mobile, their success depends on their ability to sense and generate chemical defenses to a wide range of threats including herbivory by insects and pathogen infection. Secondary metabolites play a critical role in plant–insect interactions, but we have limited information about the interactions of perception, signal transduction, and metabolism are coordinated as the basis for a functional phenotype. To address these issues, our recent studies have explored the biochemical phenotype of *Arabidopsis thaliana* via LC/MS analysis of secondary metabolites including glucosinolates, phenolics, and other known and unknown metabolites. Comparisons of metabolite profiles from plants subjected to specialist and generalist herbivores (both chewing and sucking insects) and virulent and avirulent strains of *P. syringae* can often be clearly distinguished. Among the more striking findings are the substantial alteration in methylation patterns of sinapoyl malate and compounds and changes in ratios of thioether versus methylsulfinyl glucosinolates. These findings have steered us toward new functional hypotheses about plant defense responses that are being tested using assorted mutants.

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SL19: mining the pathogen metabolome in infected susceptible plants using a 'three host/one pathogen' system

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Sampling the host–pathogen interface is a challenging prospect in any biotropic or hemi-biotrophic fungal disease of plants; not only is the pathogen tissue intimately entwined with that of the host, but in early phases of the infection process, the host tissue biomass dominates. Thus, in an infected susceptible plant it is difficult to determine whether metabolome changes are specific to the diseased state and equally difficult to attribute these changes to either host or pathogen. Compounding this difficulty, pathogen metabolome profiling *in vitro*, using common laboratory media such as potato dextrose agar, produce only a limited number of detected signals and do not provide an accurate representation of the organism's metabolic status *in planta*. To overcome these difficulties, metabolomic studies involving a three host/single pathogen system was successfully employed from which an *in planta* "infectome" characteristic of *Magnaporthe grisea* was compiled using machine learning data analysis techniques. By using three hosts, species-specific plant defence metabolites (or any derived microbial transformation products) were prevented from entering the short list of potential fungal metabolite signals, thus allowing discrimination of plant versus fungal origin. Infected tissues were extracted at a series of post infection time points and analysed using direct infusion ESI/MS and GC-tof-MS. Data were then subjected to Decision Tree analysis to mine out metabolite signals (m/z) that were discriminatory towards a diseased state and shared between all the infected hosts. Results from this approach offer up novel areas of metabolism not previously reported which may provide future targets for disease control.

SL20: gene discovery in secondary metabolism through a combination of transcriptome and metabolome analysis

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The terpenoids are one of the largest classes of secondary metabolites with over 35,000 structures known that are mainly produced by plants. They are extremely important because of their economic value as flavour and fragrance compounds and pharmaceuticals and because they play an important role in the interaction between plants and other organisms. For example, plants can defend themselves against insects by the insect-induced emission of terpenoids that attract enemies of these insects. We are coupling metabolomics and transcriptomics to obtain insight in the biochemical pathways and signal transduction involved in this induced plant defence.

Here we will discuss the results of such analyses on spider mite induced volatiles and gene expression using GC–MS metabolomics, micro-array transcriptomics and principle component analysis (PCA) for data-analysis. We were able to show that the expression patterns of specific biosynthetic genes cluster with products of the corresponding pathway. For example, Z-hexenyl-3-acetate clusters with a strongly up-regulated lipoxygenase cDNA and the volatile product E,E-b-farnesene with an up-regulated sesquiterpene synthase fragment. This resulted in the cloning and characterisation of the cucumber E,E-b-farnesene synthase. We will present results of more extended analyses on this data-set and discuss why there are only few examples in the literature of successful gene discovery using the integration of metabolomics and transcriptomics.

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SL21: metabolic profiling of shoot apices infested by the peach-potato aphid

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^{1}H-NMR and targeted HPLCs were used to analyse the response of peach (*Prunus persica* L. Batsch) to infestation by the peach-potato aphid (*Myzus persicae* Sulzer, PPA). The response was studied in ''Rubira'', a red-leaf cultivar resistant to PPA (Pascal *et al*., 2002) that shows strong level of induced resistance two days after infestation (Sauge *et al*., 2002) and ''GF305'', a susceptible cultivar. Growing shoot apices, that are the preferred settling and feeding site of PPA, were sampled 48 h after infestation and analysed for their ^{1}H NMR profiles and their levels of main primary and secondary metabolites. Whereas no obvious differences were detected between metabolic profiles of infested and non-infested plants in the ''GF305'' susceptible cultivar, clear-cut changes in the ^{1}H-NMR and HPLC profiles were observed in Rubira following infestation. Carbohydrates and most organic acids showed a marked decrease. Several amino acids, including lysine and branched-chain and aromatic amino acids, showed a large accumulation whereas levels of glutamine, proline and threonine were greatly reduced. Infestation of Rubira by PPA also triggered accumulation of secondary metabolites, including phenolic and cyanogenic compounds. This first metabolomic approach of plant responses to infestation by an aphid species provides new insights into the metabolic pathways affected by insect feeding. Hypotheses on the mechanisms involved in induced resistance are discussed.

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SL22: genetics of metabolite content in fruits of interspecific introgressions of tomato

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Tomato represents an important source of fibre and nutrients in the human diet and a central model for the study of fruit biology. To identify components of fruit metabolic composition we phenotyped tomato introgression lines (IL) containing chromosome segments of a wild species in the genetic background of a cultivated variety. Using this high diversity population we identified 889 quantitative fruit metabolic loci and 326 loci that modified yield associated traits. The mapping analysis indicated that at least 50% of the metabolic loci were associated with quantitative trait loci (QTL) that modified whole plant yield associated traits. Based on correlation analysis we generated a cartographic network that revealed whole plant phenotype-associated and independent metabolic associations including links with metabolites of nutritional and organoleptic importance. The results of this genomic survey illustrate the power of genome-wide metabolite profiling and detailed morphological analysis for uncovering novel traits with potential for crop breeding.

SL23: metabolic modeling of cytosolic sucrose and trehalose metabolism in sugarcane

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The regulation of sucrose accumulation in sugarcane is still poorly understood. Components of trehalose metabolism are involved in the regulation of carbon flux in bacteria, yeast and more recently a similar role has been suggested in plants. The previously described kinetic model of cytosolic sucrose metabolism in sugarcane has been expanded to include the trehalose pathway (TPS, TPP and trehalase). The aim was to identify potential points of control between sucrose and trehalose metabolism.

The majority of control of flux over the trehalose pathway resided in the TPS step, with flux control coefficients of 0.75–0.89. Incorporation of the trehalose branch into the original sucrose model showed that reactions from the original model significantly affected the steady-state attributes of the trehalose pathway. The trehalose branch had no significant effect on either steady-state cytosolic sucrose concentration or flux of sucrose into the vacuole. This is largely due to the relatively low flux through the trehalose branch of the expanded model, complete recycling of trehalose, and the lack of allosteric regulation by trehalose-6-phosphate or trehalose on any of the reactions from the original sucrose model. The expanded model affords a basis from which to further investigate trehalose metabolism in the context of plant sucrose accumulation.

SL24: molecular breeding of stress tolerant plants by metabolic engineering

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We present here a good example of metabolic engineering of plants through modifying flavonoid and NAD pathway leading to stress resistant plants. The ectopic-expression of a novel DFR in rice promoted the level of NAD(P)(H) through up-regulating enzymes required for NAD bio-synthesis as well as flavonoid level. Such promotion in nicotinamide nucleotide levels and flavonoids may serve as a pool of redox substances needed for quenching ROS effects.

Measurement of organic acids of transgenic plants revealed that the concentration of cis-aconitate, isocitrate, and 2-oxoglutarate were higher in leaves, whereas fructose-1,6-bisphosphate and glyceraldehyde-3-phosphate were lower in roots. The free-amino acids, total sugars and starch contents in seeds were similar in the control and transgenic plants.

Engineered plants showed tolerance to oxidative stress induced by H_2O_2, UV-C and salts and pathogens. The maize ubiquitin (Ubi-1) promoter warrants the site-specific expression in plant tissues which has been exposed to external stresses like sheath blight pathogen (*Rhizoctonia solani*) and also by the infection of brown stripe pathogen in rice leaves. Such stress tolerance was due to prevention of cells from ROS induced cell death (Uchimiya *et al*., 2002, Kawai-Yamada *et al*., 2004)

Comparison of metabolic patterns by FT-MS revealed that compositions of organ-specific metabolites were altered in transgenic plants, providing significance of high throughput metabolite analysis. These results suggested that the regulation of a small proportion of metabolites in transgenic rice contributed to multiple stress tolerances.

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METABOLOMICS STANDARDS INITIATIVE

Workshop led by Oliver Fiehn, Basil Nikolau, Nigel Hardy and Lloyd W. Sumner

In fall 2005, an initiative has been formed by the Metabolomics Society (www.metabolomicssociety.org) to draft a document on best practice, reporting and data exchange for metabolomic data. So far, standards are lacking that guide researchers to report in detail how data were actually acquired, which experimental designs were used, and how data were processed and structured to eventually reason scientific conclusions. Furthermore, the flexibility of metabolism and the need for validation of biochemical events across different genotypes or environmental perturbations requires comparison of different metabolomic datasets between laboratories and beyond specific techniques. However, without establishment of a common ontology and structure how metabolomic data should be reported, comparisons will be disabled despite the willingness of most researchers to report details on experimental procedures and other metadata.

The scope of the 'Metabolomics Standards Initiative' will be to identify, develop and disseminate best practice in all aspects of metabolomics. The aim will not be to *prescribe* how to do metabolomics experiments but to formulate a minimum of reporting standards that *describe* the experiments. Consequently, there will be no attempt to restrict or dictate specific practices but to develop better descriptors to support the dissemination and re-use of metabolomic data. Such reporting standards will specify the data identified as necessary for complete and comprehensive reporting in a range of identified contexts, such as submission to academic journals. Data exchange standards will be developed to provide a technical vehicle which meets or exceeds the requirements of reporting standards.

Five working groups have been formed, and preliminary draft documents will be available as PDF files and as hardcopies on the conference:

A. Biological sample context
B. Chemical analysis
C. Data analysis
D. Ontology
E. Data Exchange The workshop will give a short introduction into documents and the roadmap for each of these working groups. Specific emphasis will be given to discussing the minimal information requirements for defining a plant biology experiment in the context of metabolomic data, such as genotype, organ and tissue type, and environmental and physiological conditions such as daylight period, temperature, humidity or watering regime. The outcome of this workshop will be reported to the participants of the annual conference of the Metabolomics Society (June 2006, Boston) when the document drafts will be refined and disseminated thereafter. The eventual aim of such documents is to implement standards that will be required for publishing data, or reporting results and data to funding bodies and governmental agencies.

POSTER ABSTRACTS

P1: basic tools for complex tasks: creative use of principal component analysis in ecological metabolomics

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Metabolomics is focused on constructing profiles that characterize the metabolism based on the relationships between the metabolites present in an organism. These profiles are obtained by using multivariate data analysis techniques. Principal Component Analysis (PCA) is the most basic and the most widely used of the many available multivariate data analysis techniques. A considerable drawback of PCA is that the amount of information that can be extracted from the data is limited, because it treats all variation as equal. The applicability of PCA can be significantly increased when it is used as a more flexible data analysis tool. The amount of information that can be extracted from a metabolomics dataset increases by focusing the PCA model on specific experimental questions. This can be accomplished in different ways.

First, it is possible to select subsets of samples from the experiment that are sufficient to answer one of the experimental questions. Second, PCA can be extended to focus on other types of data analysis questions. For example, Principal Component Regression can be used to examine relationships between different datasets and Principal Component Discriminant Analysis can be used to focus on differences between treatment groups in the experimental design. By doing so, (extended) PCA answers specific experimental questions in a way that is understandable to a broad audience.

Here I demonstrate the advantages of a flexible use of PCA for extracting specific information from the data using an ecological metabolomics study of the induced defenses in Brassica species.

P2: ^{1}H-NMR metabolic profiling reveals tissue specificity in tomato fruit

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Tomato fruit is a complex organ composed of heterogeneous tissues that develop from ovary tissues after ovule fertilization. Up to now, most of the studies were devoted to the whole fruit or the entire pericarp. In order to investigate the role of each fruit tissue, we initiated a metabolome study based on proton NMR analyses along fruit development. Fruits of tomato (*Solanum lycopersicum* L.) cv. Ailsa Craig were harvested at six stages of fruit development from division phase to red ripe fruit. The different fruit tissues (exocarp, pericarp, placenta + columella, locular tissue and seeds) were separated and metabolites were extracted and analysed using ^{1}H-NMR (Moing *et al*., 2004). About 30 metabolites were detected and quantified in all tissues and stages of development, comprising five sugars, 12 amino acids, three organic acids, one phenolic compound, two quaternary amines and four unknown compounds. Starch was quantified using enzymatic analysis. Multivariate analyses such as Principal Component Analyses and Artificial Neural Network analyses with Kohonen's Self Organizing Maps (De Boishebert *et al*., 2005) were used to describe the global metabolite variability between tissues and stages of development and to point to marker metabolites of individual tissues. The NMR metabolome data will be completed with targeted analyses of isoprenoids, and interpreted in relation with transcriptome and cytology data of selected tissues.

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P3: aliphatic carbon flux during induced suberization

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Suberin is a cell specific, wall-associated biopolymer formed during normal plant growth and development as well as in response to biotic and abiotic stresses. Suberized tissue is characterized by the deposition of both a poly(phenolic) domain (SPPD) in the cell wall and a poly(aliphatic) domain (SPAD) thought to be deposited between the cell wall and plasma membrane. The monomeric components that comprise the SPPD and SPAD have been well characterized by analyzing the compounds released after chemical degradation of suberized tissue. However, the biosynthesis of SPPD and SPAD components and their deposition during suberization is poorly understood. Using wound healing potato tubers as a model system, and targeted metabolite analysis, we have tracked the flux of carbon into the aliphatic monomers of the SPAD in a time course fashion, monitoring both soluble and insoluble components. From these analyses, we demonstrate that newly formed fatty acids undergo one of two main metabolic fates during wound-induced suberization: (1) desaturation followed by oxidation to form the 18:1 omega-hydroxy and dioic acids characteristic of potato suberin, and (2) elongation to very long chain fatty acids (C20–C28), associated with reduction to 1-alkanols, decarboxylation to *n*-alkanes and minor amounts of hydroxylation. The partitioning of carbon between these two metabolic fates illustrates a high degree of metabolic regulation during wound healing, and provides insight into the organization of fatty acid metabolism.

P4: data correction strategy for enhanced accuracy of metabolomics analysis

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Metabolomic profiling using Gas Chromatography–Mass Spectrometry (GC–MS) requires the derivatization of the extracted metabolite mixture. Therefore, what is indeed measured is the profile of the mixture of the metabolite derivatives. Hence, quantitative GC–MS metabolomics would only be possible, if the derivative was in one-to-one proportional relationship with the original concentration profile, the proportionality remaining constant among samples. Two types of biases, however, might alter these conditions. The first change only the proportionality size between the original and the derivative profiles among the various samples; they are corrected by the use of an internal standard. The second type, however, might distort the one-to-one relationship and change the proportionality between the original and the derivative profiles among the various samples to a different fold-extent for each metabolite. It is imperative that the metabolomic profile is corrected for these biases, because of the high risk of assigning biological significance to changes due only to chemical kinetics. Moreover, a class of molecules that have been traditionally used as bio-markers, the amino acids in particular and the compounds with amine groups in general, are significantly affected by this type of biases. For the first time ever, a streamlined data correction and validation strategy for this type of errors not jeopardizing the high-throughput nature of the metabolomics analysis is presented. In the context of this study, it also became possible for 11 amino acid derivative peaks that had to-date either not been reported or considered unknown in public databases to be finally ''annotated''.

The work presented here is part of:

US Provisional Patent #US60/657,605 and Kanani H. and Klapa M.I. (2006). ''Data correction strategy for metabolomics analysis using gas chromatography-mass spectrometry (under review)

P5: integrated time-series metabolomic and transcriptional profiling analyses of *Arabidopsis thaliana* response to elevated CO_2 and osmotic stress

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Integrated time-series metabolomic and transcriptomic analyses of a systematically perturbed biological system can provide clues about gene and metabolic regulation, correlations between parallel occurring phenomena, the reconstruction of bioreaction networks and even the function of unknown genes. Even further, comparing the system's response to individual perturbations with their combined effect might reveal information about the robustness of the regulatory networks, missed when studying individual perturbations only.

In this context, we subjected 12-day old *Arabidopsis thaliana* liquid cultures, grown at constant light and temperature, to elevated CO_2 levels (1%) and osmotic stress (50 mM NaCl), both individually and simultaneously, continuously for 30 h. Two liquid cultures were harvested at each of eight time points throughout the duration of the experiments. The metabolomic and transcriptomic profiles were acquired by Gas Chromatography–Mass Spectrometry (GC–MS) and full genome cDNA microarrays, respectively. The GC–MS metabolomic data were corrected using a new normalization and validation strategy (Kanani *et al*., 2006). Both transcriptomic and metabolomic data were further analyzed using multivariate statistical analysis and a new rigorous technique (Dutta *et al*., 2006) for the analysis of time-series ''omics'' data. The results revealed a unique, identifiable response of *A. thaliana* to the individual and the combined stresses and also indicated the extent to which the plant's response to a particular stress is affected by the presence of the other. This information is deemed extremely valuable for understanding the plant's regulatory network, because it provides clues about which parts of the network are robust for a particular stress and which become active under special circumstances.

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P6: plant metabolomics addressing scientific concerns and uncertainties with genetic modification

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The genetic modification of plants has been shown repeated to be successful at altering target (bio)chemical components. However, concerns over the specificity of these modifications, especially when undertaken in food crops, have fuelled the public GM debate. Subsequently the more general question has arisen of whether the existing, generally targeted, safety assessments applied to traditionally bred crops are sufficient or are new strategies required. Targeted analyses will, by definition, miss unexpected or unintended compositional changes and the application of 'catch all' analytical technologies such as LC–MS^n, GC-ToF-MS^n and NMR, in to metabolomics technologies, have been proposed as the potential next wave of safety assessment approaches.

Potato, the fourth largest global food crop, has been the subject of many specific genetic modifications and served here as an excellent model to assess the relative efficiencies of metabolomic technologies in determining unintended effects, or deviations from substantial equivalence. Several transgenic lines and their tissue culture and vector-only controls were subject to metabolomics. In addition, and of equal importance, a broad range of potato varieties and landraces (lines which have not been subjected to controlled introgression of traits from a variety of wild species) were subject to metabolomics. The data from this allowed changes accompanying genetic modification, both intended and unintended, to be reported upon within a broader scale of germplasm biodiversity.

P7: molecular breeding of stress tolerant plants by metabolic engineering

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We present here a good example of metabolic engineering of plants through modifying flavonoid and NAD pathway leading to stress resistant plants. The ectopic-expression of a novel DFR in rice promoted the level of NAD(P)(H) through up-regulating enzymes required for NAD bio-synthesis as well as flavonoid level. Such promotion in nicotinamide nucleotide levels and flavonoids may serve as a pool of redox substances needed for quenching ROS effects. Measurement of organic acids of transgenic plants revealed that the concentration of cis-aconitate, isocitrate, and 2-oxoglutarate were higher in leaves, whereas fructose-1,6-bisphosphate and glyceraldehyde-3-phosphate were lower in roots. The free-amino acids, total sugars and starch contents in seeds were similar in the control and transgenic plants.

Engineered plants showed tolerance to oxidative stress induced by H_2O_2, UV-C and salts and pathogens. The maize ubiquitin (Ubi-1) promoter warrants the site-specific expression in plant tissues which has been exposed to external stresses like sheath blight pathogen (*Rhizoctonia solani*) and also by the infection of brown stripe pathogen in rice leaves. Such stress tolerance was due to prevention of cells from ROS induced cell death (Uchimiya *et al.*, 2002). Comparison of metabolic patterns by FT-MS revealed that compositions of organ-specific metabolites were altered in transgenic plants, providing significance of high throughput metabolite analysis.

These results suggested that the regulation of a small proportion of metabolites in transgenic rice contributed to multiple stress tolerances.

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P8: metabolite profiling analysis of the impact of NAD status on primary leaf metabolism

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Quantitative metabolite profiling was used to analyze the impact on primary leaf metabolism of elevated NAD in the *Nicotiana sylvestris* mutant CMS, which lacks mitochondrial complex I and respires through alternative respiratory dehydrogenases. Two-fold increases in leaf NAD+ and NADH in CMS were accompanied by marked increases in ammonia and amino acids, particularly nitrogen-rich amino acids such as glutamine, asparagine, and arginine. Leaf organic acid profiles were also modified in CMS, with increases in citrate and malate but decreases in 2-oxoglutarate. While hexoses were similar in CMS and wild-type tobacco, starch was much decreased in the mutant. Flux analysis using ^{15}N labelling showed that increased amino acids in CMS were associated with enhanced leaf nitrate reduction. Analysis of key transcripts and extractable enzyme activities suggests that the principal features of the CMS metabolite signature cannot be explained by changes in gene expression. Taken together, the data point to an important role for NAD+ and NADH concentrations in the coordination of carbon and nitrogen metabolism through modulation of the in vivo activities of enzymes such as nitrate reductase and isocitrate dehydrogenase.

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P9: the strigolactone communication signals that mediate the interaction between host plants, parasitic plants and mycorrhizae are derived from the carotenoid pathway

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Root parasitic plants (*Orobanche* spp and *Striga* spp) are a serious problem in agriculture, causing large crop losses in many parts of the world. The seeds of these parasitic plants will only germinate after induction by a chemical signal exuded from the roots of their host, called germination stimulants. Several germination stimulants have been isolated from a variety of host-plant species and they all belong to one chemical class, the strigolactones (Bouwmeester *et al*., 2003). Until recently, the significance of these signalling compounds for the plant itself has remained elusive. However, an intriguing recent paper in Nature has shown that the strigolactones are used by arbuscular mycorrhizal fungi for their colonisation (Akiyama *et al*., 2005)

Although the strigolactones have been classified by many to be sesquiterpene lactones, the biosynthetic pathway of these molecules is unknown. Here we will present how we have used the high sensitivity of parasitic plant seeds to the germination stimulants in a germination bioassay-guided approach to elucidate this pathway. We have investigated the induction of parasitic plant seed germination by root exudates of host-seedlings treated with isoprenoid-pathway inhibitors and a series of maize (*Zea mays*) mutants (Matusova *et al*., 2005; *Plant Physiol*. **139**, 920–934). Our results demonstrate that the strigolactones are derived from the carotenoid biosynthetic pathway. We will discuss the proposed biosynthetic pathway but also the likely involvement of the strigolactones in the interaction of mycorrhiza and parasitic plants through their host.

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P10: metabolomics tool to discrimination of genetically different or environmentally modified birch trees

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Subject of plant metabolomics is to define changes in the metabolism due to genetic modification or environmental stress. The main aim of our study was to devepol and to test the power of metabolomics tool to woody plant genotype and phenotype discrimination. Samples of leaves (three samples per tree) were taken from two genotypes (GT) of Betula pendula trees: GT2 (tolerant to ozone) and GT5 (sensitive to ozone). Experimental trees grew during of six years on two control plots with ambient air, and two plots with elevated concentration of ozone (about 1.5 × ambient ozone) using an open field exposure system. Birch leaf metabolites were extracted, fractionated into polar and lipophilic compounds, transformed to TMS derivatives and quantified with GC–MS. Polar phenolics were analysed with HPLC-DAD and identified with HPLC-ESI–MS. The metabolomic database of birch trees, that included 331 chemical traits, was analyzed using descriptive statistics, cluster, principle component, and correlation network analyses. We proved that in spite of the big size and the growth of individual trees in highly variable microclimatic conditions, this tool can be applied for comprehensive biochemical phenotyping of genetically different or environmentally modified (ozone) woody plants.

P11: metabolic profiling of shoot apices infested by the peach-potato aphid

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^{1}H-NMR and targeted HPLCs were used to analyse the response of peach (*Prunus persica* L. Batsch) to infestation by the peach-potato aphid (Myzus persicae Sulzer, PPA). The response was studied in ''Rubira'', a red-leaf cultivar resistant to PPA (Pascal *et al*., 2002) that shows strong level of induced resistance two days after infestation (Sauge *et al*., 2002) and ''GF305'', a susceptible cultivar. Growing shoot apices, that are the preferred settling and feeding site of PPA, were sampled 48 h after infestation and analysed for their ^{1}H-NMR profiles and their levels of main primary and secondary metabolites. Whereas no obvious differences were detected between metabolic profiles of infested and non-infested plants in the ''GF305'' susceptible cultivar, clear-cut changes in the ^{1}H-NMR and HPLC profiles were observed in Rubira following infestation. Carbohydrates and most organic acids showed a marked decrease. Several amino acids, including lysine and branched-chain and aromatic amino acids, showed a large accumulation whereas levels of glutamine, proline and threonine were greatly reduced. Infestation of Rubira by PPA also triggered accumulation of secondary metabolites, including phenolic and cyanogenic compounds. This first metabolomic approach of plant responses to infestation by an aphid species provides new insights into the metabolic pathways affected by insect feeding. Hypotheses on the mechanisms involved in induced resistance are discussed.

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P12: a liquid chromatography mass spectrometry based metabolome database for tomato

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For the description of the metabolome of an organism, the development of common metabolite databases is of utmost importance. Here we present the MoTo DB (Metabolome Tomato Database), a metabolite database dedicated to liquid chromatography-mass spectrometry (LC–MS) based metabolomics of tomato fruit (*Lycopersicum esculentum*). A reproducible analytical approach consisting of reversed phase LC coupled to quadrupole time-of-flight (QTOF) MS and photodiode array detection (PDA) was developed for large-scale detection and identification of mainly semi-polar metabolites in plants and for the incorporation of the tomato fruit metabolite data into the MoTo DB. Chromatograms were processed using software tools for mass signal extraction and alignment, and intensity dependent accurate mass calculation. The detected masses were assigned by matching their accurate mass signals with tomato compounds reported in literature and complemented, as much as possible, by PDA and MS/MS information, as well as by using reference compounds. Several novel compounds not previously reported for tomato fruit were identified in this manner and added to the database. The MoTo DB contains all information so far assembled using this LC–PDA-QTOF MS platform, including retention times, calculated accurate masses, PDA spectra, MS/MS fragments and literature references.

P13: MétaboP a French initiative supported by Génoplante for the development of analytical and bioinformatic tools for plant metabolomics

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Of the techniques allowing definition of metabolic profiles, mass spectrometry combined with chromatography separation (GC or LC) as well as NMR (liquid or solid) spectroscopy are two complementary methods of choice. The objective of the 2005–2006 Génoplante project named 'MétaboP' and entitled 'Plant metabolome for genetic resource analysis, mutant and transformant screening and quantitative trait loci determination' is to set up a network of French laboratories which master and share both analytical (from tissue extraction to spectra results) and bioinformatic tools (data bases, data mining) necessary for the high throughput study of plant metabolomics. This goal is being attained by driving and co-ordinating method development, and sharing know-how in the extraction protocols, quality-control standards, high-speed analysis techniques, spectra and data processing. So far, effort has been concentrated on the development of a high-throughput GC–MS method at Orsay, a high throughput semi-quantitative ^{1}H liquid NMR method at Bordeaux, and the construction of a web site (http://cbi.labri.fr/MetaboP/index.html) and database structure following ArMet at Bordeaux (see Poster by Ferry-Dumazet *et al.*). Following the development of these methodologies for liquid and solid-MAS NMR, GC–MS analyses, and associated bioinformatic tools, the know-how generated will be used in functional genomics research programs dedicated to fruit quality in tomato, C/N relations or stress responses for Arabidopsis and maize.

P14 – what is new in MetaCyc and AraCyc: metabolic pathway databases for plant research and their applications

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MetaCyc (http://metacyc.org/) is a literature-based curated universal metabolic pathway database of many organisms. MetaCyc (version 9.6) has 692 pathways with 9566 literature citations. The database contains many curator comments for pathways and enzymes and contains a wealth of enzyme property information. The growth of data content in MetaCyc has been tremendous in recent years. The number of plant secondary metabolic pathways has grown from only a handful to over eighty. Among other applications, the rich and diverse information on metabolic pathway variants across species can assist comparative studies of metabolism. AraCyc (http://arabidopsis.org/tools/aracyc/) is a species-specific database computationally derived from MetaCyc. It contains only enzymes and pathways found in the model plant *Arabidopsis thaliana*. Over the last year, AraCyc has undergone intensive curation to enhance data quality and to increase the breadth of coverage. AraCyc (version 2.6) has 228 pathways, of which, 88% have been manually verified with literature supports. An evidence code system was recently deployed in both databases so that each pathway and enzymatic reaction contains an evidence providing the assertion for the existence of the pathway or the enzyme activity. Different types of data (compound, reaction, pathway, enzyme and gene) are highly integrated and can be browsed or queried in a number of ways. In addition to querying and browsing, the OmicsViewer, a data visualization and analysis tool, has been enhanced to allow many different types of large-scale omics data resulting from microarray, metabolite profiling, and proteomic profiling experiments to be overlaid onto the full metabolic pathway map of *Arabidopsis*.

P15: statistical approach for finding metabolic pathways for environmental stress responses in *Arabidopsis thaliana*

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One of the roles of analyses of global data analyses, such as transcriptome and metabolome data, is selecting key elements that are responsible for specific responses. In this study, we propose an objective criterion for detecting the switching of the interactions of elements (genes and metabolites), concerning the time-dependent biological responses. The criterion is calculated based on a probabilistic model. This method enables us to evaluate the changes quantitatively from a macro viewpoint, and also to focus on the responses without the risk from experimental noises. Therefore, we can select the candidates concerning specific responses effectively. We applied our method to transcriptome and metabolome data of sulfur-deprived *Arabidopsis thaliana*. The results were supported by the reported incidents and those also gave us novel candidate factors and novel metabolic pathway, which are critical for such stress response. We show an example of the finding of novel pathway responding to sulfur depletion. The approach is a basic technology that could be developed for other omics data analyses, for analyzing plasticity of the control systems in living cells.

P16: genetics of metabolite content in fruits of interspecific introgressions of tomato

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Tomato represents an important source of fibre and nutrients in the human diet and a central model for the study of fruit biology. To identify components of fruit metabolic composition we phenotyped tomato introgression lines (IL) containing chromosome segments of a wild species in the genetic background of a cultivated variety. Using this high diversity population we identified 889 quantitative fruit metabolic loci and 326 loci that modified yield associated traits. The mapping analysis indicated that at least 50% of the metabolic loci were associated with quantitative trait loci (QTL) that modified whole plant yield associated traits. Based on correlation analysis we generated a cartographic network that revealed whole plant phenotype-associated and independent metabolic associations including links with metabolites of nutritional and organoleptic importance. The results of this genomic survey illustrate the power of genome-wide metabolite profiling and detailed morphological analysis for uncovering novel traits with potential for crop breeding.

P17: KNApSAcK search tool for relationship between metabolites and species

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Many metabolites have been identified from plants, microbes and other organisms. Secondary metabolites are highly species specific and play a role for the survival of the producing organism within its natural habitat. The number of metabolites present in the plant kingdom is estimated to exceed 100,000 – an enormous number indicating a large scale of structured diversity of compounds. Several databases have been made by collecting metabolite information of various organisms, and provide some chemical information and biological pathways on metabolites, however, they don't provide the relationships between metabolites and their biological origins. To systematically and comprehensively understand species-specific diversity of metabolites, we have designed a database system called KNApSAcK. This system is useful for obtaining information on metabolites and their corresponding species, chemical structure and biological activity. In addition, the database has a tool that can be used for analyzing datasets acquired using Fourier transform ion cyclotron mass spectrometry. We collected information on 24,604 metabolite-species pairs encompassing 10,181 metabolites and 7362 species from published references (October 11th, 2005). These data have been stored on a server which is located in Nara Institute of Science and Technology. When the KNApSAcK system is started, these data are automatically downloaded from the server. This database system and online manual are freely available at http://kanaya.naist.jp/KNApSAcK/. This database system is available in Web- and Download-version. To use the Web-version, get into http://kanaya.naist.jp/KNApSAcK/KNApSAcK.php. If you want to use the Download-version, at the beginning, you have to install KNApSAcK and Java 1.4.2 on your local computer.

P18: LC–MS-based system for the analysis of glycolytic intermediates and sugar phosphates from *Arabidopsis thaliana*

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Glycolytic intermediates and sugar phosphates are central compounds in plant metabolism. They are important intermediates of cellular energy metabolic pathways, such as glycolysis, the pentose phosphate pathway, and starch and sucrose synthesis in plants. However, due to their polarity, structural variety, and their poor UV absorption, these compounds are a challenging subclass of the metabolome to analyse van dam *et al*. (2002).

We have set up and optimised a negative ion mode LC-ESI–MS/MS method for the analysis of glycolytic intermediates and sugar phosphate using a porous graphitized carbon (PGC) stationary phase and an MS compatible mobile phase. This new HPLC method was then applied to selectively detect and quantitate a range of glycolytic intermediates and sugar phosphates from plant tissues using a trichloroacetic acid/ether extraction Weiner *et al*. (1987). Using our optimised method, separation and detection of a mixture of standard compounds was achieved. Differentiation of the isomeric compounds glucose-1-phosphate and glucose-6-phosphate (G1P/G6P) is possible based on their retention times and product ion spectra, which yield very different and characteristic product ions Feurle *et al*. (1998). Calibration curves using our on-line PGC-based separation showed a linear response over the concentration range 0–100 μM.

This poster will describe details of our system and recent data from our application of this method to the analysis of *Arabidopsis thaliana* extracts.

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P19: metabolic profiling of plant–nematode interactions

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Plant parasitic nematodes are responsible for global crop damage estimated at ~$100 billion annually. Of these, the obligate endoparasitic root-knot (Meloidogyne) and cyst (*Globodera* and *Heterodera* spp) nematodes are important causes of yield losses. During the interaction between plant endoparasitic nematodes and their hosts, changes in both localized and systemic gene expression occur leading to either susceptible or resistant interactions. Little is known about how these different interactions affect the host's metabolism and how these relate to changes in gene expression.

Metabolite profiling is being used to monitor changes in tomato and potato hosts in compatible and incompatible interactions with Meloidogyne and Globodera to determine if these 'metabolome' profiles can be used to differentiate these interactions. An extraction protocol has been established for freeze-dried tomato roots and leaves for analysis with GCMS and LC–PDA–MS. Metabolite profiles of infected and uninfected samples from pathogen induced galls, whole roots and leaves showed clear differences 14 day post-infection. Work in progress includes profiling leaves and roots from individual tomato plants from cultivars that are susceptible (Moneymaker) and resistant (Rossol) to *Meloidogyne javanica*. Profiling will be used to follow post-inoculation changes over a 10-day period. These approaches are being extended to potato genotypes which differ in susceptibility to the potato cyst nematode *Globodera pallida*.

Significant changes in known and/or unknown metabolites that are characteristic of either compatible or incompatible interactions will provide insight into the biochemical basis of susceptible and resistant plant responses and provide specific markers to characterise progeny in breeding programs.

P20: network flux analysis of an *Arabidopsis thaliana* cell suspension

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The redistribution of ^{13}C that occurs when a ^{13}C-labeled substrate is introduced into a metabolic network is determined by the structure of the network and the fluxes it supports. If the structure of the network is known, then analyzing the redistribution of the label in a metabolic and isotopic steady state provides a powerful method for determining the fluxes. This steady state approach to network flux analysis relies on extensive characterization of the isotopomeric composition of labeled intermediates and end-products, and typically leads to the definition of a flux map for the pathways of central metabolism. The method is used routinely for the characterization of microbial metabolism, and work is underway in several laboratories to extend the application of the method to the more complex metabolic networks found in plants.

A suspension culture of heterotrophic *Arabidopsis thaliana* cells maintained in a glucose growth medium was sub-cultured into medium containing 100% [1-^{13}C]glucose, 100% [2-^{13}C]glucose or 10% [U-$^{13}C_6$] glucose plus 90% unlabeled glucose. The cells were harvested after 7 days, and the redistribution of the label in a range of labeled carbohydrates and organic acids was determined by ^{13}C NMR analysis of cell extracts. Flux maps for the compartmented pathways of carbohydrate oxidation were determined from isotopomeric data using ^{13}C-Flux® (http://www.simtec.mb.uni-siegen.de/434.0.html). These maps are being used to investigate: (i) the effectiveness of different labeling strategies for defining different parts of the network; and (ii) the extent to which physically separate pathways in a compartmented system are functionally distinct.

P21: time course metabolite profiling of arbuscular mycorrhizal roots of Medicago truncatula/Glomus intraradices

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Within a functional genomic approach metabolite profiling of primary and secondary metabolites of developing mycorrhizal roots (2–8 weeks after inoculation) was performed using GC/MS, LC/ESI–MS and HPLC-DAD to determine relevant changes and clear trends in the metabolite pattern in comparison to nonmycorrhizal controls with different phosphate supply. The degree of mycorrhization determined by staining, MtPT4 and Glomus rRNA expression follows a sigmoidal behaviour. Mycorrhization and high phosphate supply lead to a similar shoot biomass increase, but the metabolite levels of roots differ significantly. However, phosphate supply does not lead to considerable metabolite alterations. Due to its fungal origin trehalose, palmitvaccenic and vaccenic acids as well as certain sterols occur only in mycorrhizal roots. An increase in some amino acids and fatty acids (palmitic, oleic) indicate a general activation of plastid metabolism. Some constitutive secondary metabolites (isoflavonoids and saponins) show higher levels at later mycorrhization stages. C13 and C14 apocarotenoids (cyclohexenone and mycorradicin derivatives including the yellow pigment), occurring solely in mycorrhizal roots, accumulate during the symbiotic interaction. The analyses of cell wall-bound components demonstrate the presence of typical phenolics in all samples in addition to mycorrhiza-induced 2-(4-hydroxyphenyl)-ethanol (tyrosol). As tyrosol is only a trace component in cell wall-bound phenolics of Glomus intraradices its formation and integration into root cell walls is a mycorrhiza-specific phenomenon. Correlation analyses between primary metabolites reveal a stronger metabolic relation in mycorrhizal roots than in nonmycorrhizal ones indicating a tighter control of the intensified mycorrhizal root metabolism.

Acknowledgement

This work was supported by German Research Foundation.

P22: phenoarray analysis of plant–bacteria interactions

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Endophytic plant pathogens such as Pseudomonas syringae colonise the intercellular spaces of plant tissues, drawing nutrients from apoplastic fluid and using toxins and secreted proteins to manipulate and suppress plant defence responses. To understand Pseudomonas–plant interactions we must analyse and interpret how both pathogen and host interact with a common pool of metabolites in the form of apoplastic fluid. A number of techniques can be applied to this problem, including metabolomic analyses of apoplastic fluid, bioinformatic analyses of bacterial genome sequences and transcriptomic analyses of gene expression by bacterium and host. However, to complete the picture we require bridging technologies that can identify which metabolites are used and modified by plant and parasite. One approach that can be used to address this question is phenoarray technology. Microplate-based phenoarrays provide high-throughput profiles of cellular nutrient utilization and stress responses, providing data on metabolic activity in a wide range of defined media. We have used commercial Biolog phenoarrays and custom arrays to profile and compare the metabolic capacity and stress responses of pathogenic and non-pathogenic Pseudomonas. We have also developed a phenoarray-based method that allows us to profile bacterial metabolism in response to specific environmental conditions, and have used this approach to analyse P. syringae metabolism during growth in apoplastic fluid. We discuss how phenoarray data can be integrated with metabolomic data and comparative bioinformatic analyses of genomes, protein domains and domain architecture to provide new insights into the evolution and biology of plant–pathogen interactions.

P23: towards understanding of ryegrass-endophyte symbiosis through data integration of transcriptome and metabolome

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Fungal endophytes (*Neotyphodium lolli*) in perennial ryegrass (*Lolium perenne*) produce a range of bioactive alkaloids which are implicated in toxicity to grazing animals and insect resistance. The understanding of regulatory and biochemical mechanisms of the symbiosis will provide clues on the genetic manipulation of beneficial alkaloid production. About 15,000 ESTs have been generated from six suppressive substractive hybridization (SSH) libraries at three developmental stages of the ryegrass including immature, blade and mature tissues. The metabolome of the symbiosis has also been investigated in these tissues using unbiased direct infusion ion trap mass spectrometry (DIMS) and targeted GCMS and LC–PDA. About 850 ions and 70 targeted measurements of sugars, amino acids and alkaloids were collected as a representative metabolome for joint analysis with the transcriptome. Global gene expression and metabolic profiles of the symbiosis were analyzed using machine learning algorithms. Various feature selection approaches were applied to identify salient genes and metabolites in the symbiosis. Combined with extensive bioinformatics analyses, our approaches have generated interesting findings which led to further laboratory verification, such as potential genes associated with alkaloid production.

P24: NMR database of flavonoids

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The improvements in separation and analysis of complex mixtures by LC-NMR during the last decade have shifted the center of gravity from data acquisition to data analysis. For adequate data analysis not only high quality datasets are necessary but adequate software and adequate databases for semi-automated or fully automated assignments are needed. Here we report on a database of over 220 flavonoids, both aglycones and conjugates. These flavonoid compounds have received considerable attention in the last decade because they are abundantly present in plants, with a whole array of functions. Identification of the different types of flavonoid molecules is hampered by the fact that LC–MS is not directly suitable for identification of flavonoids because of the possibility of different isomers. Only by using NMR, when necessary in combination with MS, identification of the different types of flavonoids can be achieved. As there are thousands of different flavonoids in nature, identification of a specific flavonoid molecule present in a plant mixture, can be extremely troublesome. The information about NMR based identification is scattered throughout the scientific literature with sometimes incorrect assignments, making identification of the flavonoid under study based on literature data difficult. We therefore set out to assemble an extensive NMR database of the most common flavonoids to be of use for the scientific community in order to make quicker and more automated assignments possible.

P25: quantitative structure retention relationship (QSRR) – a way towards improved identification ratios in GS–MS based plant metabolomics

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The objective in metabolomics is to quantify and identify all metabolites in a given biological system. In plant metabolomics gas chromatography coupled to electron impact mass spectrometry has during the last years become the most commonly used analytical technique, a technique which gives good structural information about the metabolites. However, many metabolites remains unknown since identification of the metabolites are done by comparisons with mass spectral libraries and the number of metabolites in the libraries are and will be a limiting factor (it will be impossible to produce a complete mass spectra library with all metabolites present in a plant cell). Despite the structural information obtained in mass spectral analysis, mass spectra alone will not give enough evidence for structure elucidation. Therefore retention indices are needed for adequate identification.

By describing compounds with chemical descriptors the molecule information can be transferred into discrete data. This information can be used to predict analytical behavior of the compound, i. e. retention time on a column, a method known as Quantitative Structure Retention Relationship (QSRR). A prediction model for retention indices (RI) can be used to eliminate or verify the suspected molecular structure and will together with interpretation of mass spectra improve identification ratios for the unknown metabolites.

We have used QSRR and multivariate methods to build up a model for prediction of GC retention indices. Approximately 380 compounds, commonly found in plant cells with known structures and retention indices, where selected from Max-Planck mass spectral library for the prediction model.

P26: ethnopharmacological evaluation of Radal (leaves of *Lomatia hirsuta*) through metabolite profiling, and isolation of 2-methoxyjuglone

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To evaluate the efficacy of the traditionally used herbal remedy Radal a metabolite profile of the original plant material was established. Radal is a tea decoction of the leaves of *Lomatia hirsuta* (Lam.) Diels ex Macbr. (Proteaceae). The tea is used in Chile among the Mapuche people for the treatment of bronchial disorders and infections.

2-Methoxyjuglone (1) was isolated through bio-guided fractionation from the EtOAc extract of the leaves and found to be active against the pathogenic fungus *Candida albicans* (MIC = 8 μg/ml). Cinnamic acid (2) and vanillic acid (5) were identified as the major constituents in the tea by GC–MS along with other phenolic derivatives. To the best of our knowledge this is the first time these compounds with antimicrobial properties have been found in *Lomatia hirsuta*.

The tea was found not to be toxic against *Artemia salina*. The non-toxic properties and the identification of phenolic derivatives with antimicrobial properties encourage further in vivo investigations in order to add further support for the traditional use.

P27: temporal dynamics of pathogenesis related metabolites and their metabolic pathways following inoculation of potato leaves with *Phytophthora infestans*.

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Metabolite profiles developed based on GC/MS analysis of polar and non-polar extracts of potato leaves, inoculated with water or P. infestans and sampled at 24, 48 and 96 h after inoculation, were used to study the temporal dynamics of metabolites. A total of 106 consistent (in five replicates) peaks were detected of which 95 metabolites were tentatively identified. Following pathogen inoculation, the abundances of 42 metabolites were significantly increased or decreased, and these metabolites were designated as Pathogenesis Related (PR)-Metabolites. Factor analysis, using principal component method, of abundances of 106 metabolites identified four host–parasite interaction functions: (i) homeostasis; (ii) primary defense; (iii) secondary defense; (iv) collapse of the defense responses. During the primary and secondary defense phases significant changes in the amino acids, known precursors of several plant defense related metabolites, were observed. The up-regulation of these families of amino acids and other secondary metabolites, involving satellite-networks of metabolic pathways, and their potential application in the evaluation of horizontal resistance in potato against late blight pathogen will be discussed.

P28: functional genomics analysis of the flavonoid pathway in *Arabidopsis* seed

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Arabidopsis thaliana seed has become a model for flavonoid metabolism and its regulation due to the availability of a wide range of flavonoid deficient mutants that have been identified on the basis of seed coat colour changes. However, functional characterization of the genes involved in this pathway requires in-depth analysis of seed flavonoid structure and composition. We have analysed the diverse and specific flavonoids that accumulate during seed development and maturation in wild types and mutants using HPLC–UV–ESI–MS–MS. Wild-type seed contained more than 26 different flavonoids belonging to flavonols, flavan-3-ol and proanthocyanidins. The nature and the linkage of the glycoside(s) in the most abundant flavonols were fully characterised using NMR spectroscopy. Flavonols were composed of quercetin, kaempferol and isorhamnetin aglycones that were glycosylated in position 3 and/or 7 with rhamnose and/or glucose. Unlike many other seeds, *Arabidopsis* used exclusively epicatechin monomers as the building block of proanthocyanidin oligomers that were individually detected with degrees of polymerisation up to 9. Interestingly, a novel group of four biflavonols that are dimers of quercetin-rhamnoside was also detected. Finally, eleven mutants affected in known structural or regulatory functions of the pathway and their three corresponding wild types were also studied. Flavonoid profiles of the mutants were consistent with previous predictions based on genetic and molecular data. In addition, they also revealed the presence of new products in seed and underlined the plasticity of this metabolic pathway in the mutants (Routaboul *et al.*, 2006). These results provide insight into critical steps in flavonoid biosynthesis that could be useful for designing strategies to modify related crop plants. They are also essential to characterise novel gene function (Pourcel *et al.*, 2005) or to explore natural variability.

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This work was supported by GENOPLANTE (NO2001 00045) and the European Commission (FOOD-CT-2004–513960 FLAVO)

P29: comparative metabolite profiling of GM rice and conventional rice plant leaves

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Metabolite profiling has been suggested as a useful tool for the total and comprehensive compositional analysis of crop and foods, allowing multi-parallel monitoring of low molecular weight compounds in them, utilizing GC/MS, NMR or LC/MS. Such metabolite profiling was conducted as potential tool for investigation of any new metabolites formed in the herbicide resistant GM rice leaves, compared to the conventional rice leaves. This unbiased approach may help to increase the chance to detect unintended effects due to the application of recombinant DNA techniques.

A glufosinate resistant GM rice and five conventional rice plant leaves were collected for the study. The profiling approach was based on consecutive extraction of lipids and polar compounds from rice plant leaves and subsequent fractionations of both extracts. Transesterification, solid phase extraction (lipids) and selective hydrolysis of silylated derivatives (polar compounds) were applied to separate major from minor constituents. Extensive investigation of fractions (Fatty acid, hydrocarbon, sugars, organic acid, amino acid etc) by gas chromatography/mass spectrometry (GC/MS) was conducted. More than 100 peaks were observed from each rice leaves and 41 compounds were identified through library search (NIST 1995) and metabolite mass spectra library of Max Plank Institute of Molecular Plant Physiology (MPI 2000). The PCA (principal component analysis) enabled the comparison of metabolic profile between rice varieties. As a result of comparison of six rice plants, any particularly interesting compounds were not found in GM rice plant leaves and that similar profile of metabolites was observed among rice plant species.

P30: comparative metabolite profiling of GM rice and conventional rice plant roots

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Metabolite profiling has been suggested as a useful tool for the total and comprehensive compositional analysis of crop and foods, allowing multi-parallel monitoring of low molecular weight compounds in them, utilizing GC/MS, NMR or LC/MS. Such metabolite profiling was conducted as potential tool for investigation of any new metabolites formed in the herbicide resistant GM rice roots, compared to the conventional rice roots. This unbiased approach may help to increase the chance to detect unintended effects due to the application of recombinant DNA techniques.

A glufosinate resistant GM rice and five conventional rice plant roots were collected for the study.

The profiling approach was based on consecutive extraction of lipids and polar compounds from rice plant roots and subsequent fractionations of both extracts. Transesterification, solid phase extraction (lipids) and selective hydrolysis of silylated derivatives (polar compounds) were applied to separate major from minor constituents. Extensive investigation of fractions (Fatty acid, hydrocarbon, sugars, organic acid, amino acid etc) by gas chromatography/mass spectrometry (GC/MS) was conducted. More than 100 peaks were observed from each rice roots and 41 compounds were identified through library search (NIST 1995) and metabolite mass spectra library of Max Plank Institute of Molecular Plant Physiology (MPI 2000). The PCA (principal component analysis) enabled the comparison of metabolic profile between rice varieties.

As a result of comparison of six rice plants, any particularly interesting compounds were not found in GM rice plant roots and that similar profile of metabolites was observed among rice plant species.

P31: metabolite profiling of volatile constituents from pepper using SAFE and GC/MS

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Recently, metabolite profiling has been suggested as a useful tool for the total and comprehensive compositional analysis of crop and foods, allowing multi-parallel monitoring of low molecular weight compounds in them, utilizing GC/MS, NMR or LC/MS. A significant parts of plant metabolome, volatile metabolites play an important role in fundamental process such as signaling mechanisms and interorganism interactions, and are major determinants of food and flower quality in terms of flavor and fragrance. SAFE and GC–MS approach has allowed comparative analysis of the volatile metabolites of ripe fruit of 25 pepper (Capsicum annum) cultivars.

Among several sample preparation methods, we employed the solvent assisted flavor evaporation (SAFE), because it is a high efficiency flavor extraction method introduced recently, isolating volatile metabolites from fresh pepper fruits after grinding in liquid nitrogen. The extracts were then, analyzed qualitatively and quantitatively by gas chromatography (GC-FID) and gas chromatography–mass spectrometry (GC–MS). The amount of total volatile components recovered from 20 g of fresh pepper fruits by SAFE ranged from 59.8 ± 1.0 to 10.8 ± 0.2 μg/g sample. The major volatile constituents were xylene (24.3 ± 12.3%), 3-methoxy-1,2-propanediol (17.4 ± 20.1%), ethylbenzene (13.5 ± 4.8%), toluene (10.0 ± 3.3%). The most volatile metabolites were analyzed in 'NS13' of 25 pepper cultivars.

P32: metabolome analysis of Arabidopsis based on FT-MS spectra by using high-throughput comprehensive analyzing systems

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Determination of chemical structure of metabolites based on ultra-high mass resolutions obtained from available metabolomic experiments is an important issue. The requirements of establishing a software system for automatically assigning empirical formulae to separate peaks of mass spectrum data have recently been reviewed. For this purpose, we have developed a metabolomics experimental scheme comprising of an analytical tool for automatic high-throughput processing of mass spectrometric data from FT-MS analyses and a database system for searching available information on metabolites that are contained mainly in plants (KNApSAcK, http://kanaya.naist.jp/KNApSAcK/, 11,075 metabolites on 23th February 2006). In the present study, we applied our metabolomics scheme for profiling *Arabidopsis* metabolites. After confirming reproducibility of m/z values with intensities among multiple FT-MS experiments, we found that about 10% of the total ion species detected from the FT-MS analyses have fluctuated representing different growth conditions for Arabidopsis. Furthermore, we were able to identify metabolite candidates corresponding to those m/z values by using KNApSAcK database. Some of these metabolites were already reported in published research articles on *Arabidopsis*; however, many other of the m/z values of interest could not be assigned to any of known metabolites from Arabidopsis, and hence it is important to investigate their molecular structure because that would be essential for the complete realization of the cell system of *Arabidopsis*. We are further constructing an analytical data processing system that include MS/MS structural information together with a comprehensive metabolite database and would be helpful for understanding how plant metabolites work as an integrated system.

P33: metabolic clues of Kreb cycle regulation in root tissue

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Plant roots exude an abundance of low molecular weight compounds into the critical root–soil interface. Organic acids, primarily metabolised by the Kreb cycle, are a major constituent of these exudates. It has been hypothesised that these organic acids perform a myriad of functions, including nutrient acquisition, mineral weathering, chemotaxis and detoxification processes. In addition, Krebs cycling might also serve another significant purpose in non-photosynthetic root tissue by supplying the necessary energy to the ATP-consuming process of nitrogen fixation. Therefore, the function and regulation of Krebs cycling might have a distinct biological role in root tissue. In keeping with these observations recent analyses of plants with constitutive reduced expression of Krebs cycle enzyme activities show a significant decrease in root growth (Carrari *et al*., 2003, Nunes-Nesi *et al*., 2005). Here Krebs cycle metabolism was physiologically and metabolically probed in tomato (*Solanum lycopersicum*) roots with constitutive reduced expression of L-malate NAD(+) oxidoreductase (mMDH, E.C. 1.1.1.37); and fumarate hydratase (FUM, E.C. 4.2.1.2). Metabolic profiles and flux determinations in context of the physiological parameters of the endo and exometabolites of the root system will be presented.

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P34: metabolome-fluxome facility at the Plant Systems Biology Institute in Bordeaux, France

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The Metabolome-Fluxome Facility of the Plant Systems Biology Institute (IFR103) provides targeted or untargeted metabolite profiling of plant extracts using HPLC or $^{1}H/^{13}C$ NMR, quantification of metabolic fluxes or *in situ* analysis by NMR, and also extraction and identification of metabolites like polyphenols. It is more particularly involved in developing techniques and savoir-faire that allow metabolome studies and quantification of metabolic fluxes. Five HPLC systems and two NMR spectrometers and dedicated home-made libraries with growing collections of NMR spectra are present for metabolite analyses. The facility has developed a strong expertise providing fast and efficient response to the needs of national and international research programs on plant functional genomics, genetics, plant–pathogen or pest relationships, structural biology, ecophysiology and agronomy. The facility is also a training centre where students and scientists are trained in all aspects from sample extraction to data collection and statistic analysis. For bioinformatic aspects (signal pre-processing and analysis, data bases), IFR 103 and the Centre de Bioinformatique de Bordeaux (CBiB, http://cbi.labri.fr/) work in collaboration. The facility is being integrated as a "Metabolome pole" into the Functional Genomic Platform of Bordeaux (http://www.pgfb.u-bordeaux2.fr/eng//index.html). It is deeply involved in one national project (MetaboP "Plant metabolome for genetic resource analysis, mutant and transformant screening and quantitative trait loci determination" 2005–2006 in Génoplante) and two European projects (IP ISAFRUIT "Increasing Fruit consumption through a trans disciplinary approach leading to high quality produce from environmentally safe, sustainable methods" 2006–2009, STREP META-PHOR "Metabolomics for Plants, Health and OutReach" 2006–2008).

For further information see
http://www.bordeaux.inra.fr/umr619/UK_page_PF_metabolome.ht.

P35: development of an integrated platform technology for GC–TOF–MS based metabolomics

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GC–TOF–MS instruments and modern computing capacity provide an alluring platform technology for metabolomics with fast scanning rates, precision pneumatics control and microbore columns offering the potential for fast, high throughput, separations. This allure is tarnished by the twin bottlenecks of sample preparation and data processing, and further dulled by the absence of a suite of comprehensive, effective (i.e., that incorporate strategies to eliminate from the data, errors arising from well understood instrumental phenomena inherent to hyphenated-MS analysis) tools for data processing. To realise fully the platform's potential requires strategies to overcome these bottlenecks.

Here at The National Centre for Plant & Microbial Metabolomics we have effectively eliminated the sample preparation bottleneck by developing a straightforward rapid sample preparation method based upon direct two-step derivatization of lyophilized tissues to form O methyloxime/TMS derivatives. We have also incorporated instrumental solutions to both minimise instrument downtime/improve instrumental reproducibility and reduced our existing analysis times from 65 min to approximately 20 min without compromising chromatographic performance. Finally, we have developed an interim solution for rapid data processing that incorporates simultaneous bucketing and data export for multivariate statistical analysis.

P36: metabolic flux analysis for the study of the regulatory role of SuSy on carbon metabolism

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The development of non-photosynthetic tissues is closely related to sucrose import and metabolism. Sucrose is degraded by sucrose synthase (SuSy) or invertase to provide UDP-Glc or hexoses-P for the biosynthesis of structural or storage compounds and for ATP production. Although often suggested, the regulatory role of SuSy in sugar partitioning remains a matter of debate. Short and steady state labeling experiments (1–3) were used to analyze glucose metabolism in maize root tips of an inbred line and a mutant in two sucrose synthase genes derived from this line. Unidirectional rates of synthesis for storage compounds were determined by short labeling experiments using [U-^{14}C]glucose, and compared with net synthesis fluxes: the difference gives the unidirectional rate of degradation of these storage compounds, which also is the rate of glucose production from these compounds. Data obtained after steady state labeling with [1-^{13}C]glucose, [2-^{13}C]glucose and [U-^{13}C]glucose, were interpreted using the software C13-FLUX (4). About 30 fluxes in intermediary metabolism were quantified. The reduction of SuSy activity leads to a 52% increase in the wall synthesis flux. Most of the fluxes, in particular the substrate cycles, were not significantly modified.

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P37: rosmarinic acid biosynthesis in hairy root cultures of Korean mint, Agastache rugosa k

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Korean mint, Agastache rugosa Kuntze belonging to mint family (Labiatae) is a perennial herb and is widely distributed in the East Asian countries with important role in the traditional medicine of several Oriental cultures. One of the main active constituents of Korean mint is rosmarinic acid which contains attributes like antioxidant and allelochemical properties.

Agrobacterium rhizogenes 15834 was tested for its ability to induce hairy root formation on Agastache rugosa explants. Wounded leaves were highly susceptible to infection by *A. rhizogenes* 15834 and several hairy roots were produced from explants within four weeks. Hairy roots were excised from the necrotic explant tissues and subcultured on fresh agar-solidified MS medium containing 500 mg carbenicillin l-1. After repeated transfer to the solidified MS medium for two months, rapidly growing hairy root cultures of Korean mint were transferred to a liquid culture medium.

The hairy roots were cultured in MS liquid media supplemented with 3% sucrose for 15 days, and examined for the growth and rosmarinic acid production. Hairy roots grown in MS media had the highest levels of growth (45.7 mg dry wt/30 ml) and rosmarinic acid production (0.15g /g dry wt.). Further investigations for the improvement of rosmarinic acid production in hairy root cultures of Korean mint are progress in our laboratory.

P38: production of essential oil in hairy root cultures of *Valeriana officinalis* and *Angelica gigas*

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Agrobacterium rhizogenes is a genus of gram-negative soil bacteria belonging to the Rhizobiaceae. *A. rhizogenes* can transfer T-DNA, excised from Ri (root inducing)-plasmids several hundred kb in size, from the bacterial to the plant cell. It is the causal agent of /'hairy root/' diseases in plants, and has been used for the production of hairy root cultures from a multitude of species. Hairy root cultures from plants have attracted considerable attention because of their genetic and biochemical stability, rapid growth rate and ability to synthesize secondary products at levels comparable to the original plants.

Thus hairy root cultures could possibly serve as a potential system to study biosynthesis of important essential oils from roots of several herbs. Valeriana (*Valeriana officinalis* L) and Korean Angelica (*Angelica gigas*) produce essential oils from its roots. However, there have been no reports about essential oil production in hairy root culture of these herbs. We report on the production of essential oil in hairy root cultures of Korean Angelica and Valeriana.

P39: metabolomic methods for discovering novel low-abundance plant hormones

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Our work centres on discovering a novel mobile hormone that regulates shoot branching. Branching genes cloned from Arabidopsis (MAX3, MAX4) and pea (RMS1, RMS5) are members of the carotenoid cleavage dioxygenase (CCD) family, and recombinant enzymes exhibit cleavage activity. Because it is therefore likely that the novel hormone is an unknown apocarotenoid, chemical identification requires a comprehensive metabolomic screen. Based on branching models, we predict that biosynthetic mutants rms1 and rms5 will be depleted relative to wild-type, whereas the perception/signal transduction mutant rms4 will have normal or elevated levels.

Here we have attempted to overcome two major hurdles: (1) typically low abundance (ng per g) of plant hormones, and (2) lack of suitable metabolomic acquisition and data analysis methods for detecting low-abundance unknowns. First, we developed a procedure which removes major polar metabolites and enriches low abundance components. Further sample enrichment is gained by dissecting xylem tissue, because evidence points to signal synthesis and transport in the xylem. Second, we have refined strategies for forcing LC–MS–MS spectra of all compounds in a sample regardless of abundance, while ensuring ion suppression is minimised and sensitivity maximised. Daughter/parent ion pair lists from different genotypes are compared via custom MatLab scripts. Candidates exhibiting significant variation across genotypes are re-analysed by MRM and are assessed for presence of apocarotenoid signatures. Parallel approaches via GC–MS analysis generate complementary data sets.

As final steps, full structural elucidation will be attempted on top candidate peaks, together with testing for biological activity in one or more bioassay systems.

P40: phenolic compounds modulate *Rhodococcus fascians* att locus expression

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Rhodococcus fascians, a Gram-positive Actinomycete bacterium, is pathogenic to a wide range of dicotyledonous and monocotyledonous plants. Its interaction with plants leads to the development of specific symptoms such as local proliferation of meristematic tissues resulting in a leafy gall formation (Goethals *et al*., 2001). Development of the symptoms relies on a genetic network involving several bacterial genes mapped on a large, conjugative, linear plasmid (pFiD188) that is required for symptoms induction (Crespi *et al*., 1992). Molecular characterization of this plasmid has underlined the importance of at least two loci:

- fas locus: essential for virulence and putatively involved in the biosynthesis of a cytokinin-like molecule;
- att locus: suggested to be involved in the synthesis of auto-inducing compounds acting on the expression of both loci (Maes *et al*., 2001).

The onset of a bacteria–plant interaction is characterized by a complex and intense exchange of signal molecules from both partners that induce expression of bacterial genes and that initiate response of the plant. In *Agrobacterium tumefaciens*, it is known that the expression of the vir genes is synergistically controlled by phenolics and sugars released by wounded plant cells. In *R. fascians*, sugars have also a synergistic effect on the in vitro induction of att expression by leafy gall extracts. This research shows that phenolic compounds present in leafy galls modulate *R. fascians* att locus and are therefore likely involved in the plant-*R. fascians* interaction.

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P41: metabolomics of tomato fruit volatiles during fruit development

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Fruits of four different developmental stages (mature green, breaker, turning, red) of six different genotypes have been analysed for volatile compounds using an untargeted approach. The approach was based on the SPME/GC/MS profiling, mass spectral alignment of GC/MS data sets using ''MetAlignTM'' software package and reduction of the complex data matrix derived using MMSR procedure (Tikunov et al., 2005). Three hundred and thirty putative mass spectrums derived have been subjected to a multivariate analysis, which revealed development driven changes in volatile composition of fruits. The volatiles determining the difference have been subjected to the putative identification using the NIST mass spectral library. Most of the volatiles could be divided into two main groups according to their accumulation dynamics: (1) the volatiles, which are gradually accumulated and (2) the volatiles, which decrease their concentration during fruit development. Research is in progress to determine the chemical identity of the key molecules in each group. The pattern of volatile accumulation appears also to be dependent not only on developmental stage but also on genotype. This suggests an existence of differential regulation mechanism of the pathway leading to the production of these compounds in different varieties.

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P42 – methodological advances in hetero-nuclear NMR-based metabolomics: stable isotope labeling of *Arabidopsis thaliana* towards metabolic flux analysis

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Metabolomics is an approach for measuring time-related biochemical responses and reactions. We have been developing new methodologies for plant metabolomics by combination of stable isotope labeling and multi-dimensional hetero-nuclear NMR analysis (Kikuchi *et al.*, 2004; Kikuchi, 2005). We have established hexafluoroacetonedeuterate/HEPES-d18 buffer system not only for comprehensive extraction of a variety of metabolites but also to obtain constant chemical shifts in NMR spectra for unambiguous assignment of each signal.

In addition to the aforementioned advances, we are also establishing a methodology for the uniform stable isotope labeling of plants. Highly stable isotope labeling considerably increases the number of detectable signals in hetero-nuclear NMR spectra. Furthermore, monitoring of intensity of each signal would afford detailed information of metabolic flux at atomic level. We analyzed the incorporation ratio of [$^{13}C_6$] glucose into *A. thaliana* at suitable time intervals by ^{1}H–^{13}C HSQC spectra. NMR signals of the plant extract, which is a complex mixture of metabolites, can be efficiently assigned by use of the system described above. Time-related changes in the peak height ratio of each carbon reflect enrichment by labeled glucose and dilution by atmospheric $^{12}CO_2$. Detailed time-course of labeling profile of primary metabolites, such as amino acids, TCA cycle intermediates and carbohydrates will be discussed in the conference.

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P43: steady-state flux analysis of the central carbon metabolic network in the alga *Chlamydomonas reinhardtii*

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The objective of our research program is to quantify the non-photosynthetic carbon flux through the central carbon metabolic network of the unicellular freshwater alga Chlamydomonas reinhardtii. The algae (wild type strain CC-125 mt+) are grown under continuous light (390 μmol/m^2/s PAR) in identical, temperature-controlled mini-chemostats (100 mL). Inorganic nutrient media is pumped into each chemostat at a rate of 83.5 μL/min using computer-controlled micropumps (Bio-Chem Valve, Inc. Series 120SP). Experimental treatments include a range of light intensities and induction of the glyoxylate pathway by exposing the algae in the chemostats to 10 mM acetate. For the steady state carbon labeling experiments micropumps supply ^{13}C-labeled substrate at a fixed rate. Depending on the experiment, either [1-^{13}C]-acetate or a mixture of [U-^{13}C]-D-glucose and [1-^{13}C]-D-glucose is used. Algae in the chemostat overflows are collected for 1-h periods up to 50 h after the start of the labeling period. Algal proteins are extracted, and hydrolyzed, and the amino acids derivatized with MTBSTFA and separated by GC–MS (Varian Saturn 2000 ion-trap). For each recognized amino acid fragment a normalized mass isotope distribution vector is calculated and corrected for naturally occurring isotopes using MatLab-based software (MSCorr, ^{13}C-Flux, or FiatFlux). The software subsequently calculates the mass distribution vector of the amino acid precursors in the central carbon metabolic network. Initial results indicate a measurable effect on the mass isotope distribution vectors of several amino acids fragments due to the induction of the glyoxylate pathway by acetate.

P44: using ^{1}H NMR screening to characterise carbohydrate biosynthesis mutants in *Arabidopsis thaliana*

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^{1}H NMR spectroscopy of crude solvent extracts from plant tissue offers a rapid method to generate fingerprints from large numbers of samples in automation. These fingerprints contain contributions from most of the organic compounds that are soluble in the extracting solvent, and can be considered as representative of the total soluble metabolome. In this project we have used ^{1}H NMR screening to characterise *Arabidopsis thaliana* carbohydrate biosynthesis mutants pgm1 (which encodes phosphoglucomutase activity and is involved in starch biosynthesis), adg1 and adg 2 (which encode ADP glucose pyrophosporylase small and large subunit protein, respectively) and sex1 (starch excess mutant). The poster will demonstrate how NMR fingerprints are collected and discuss the information content in terms the compounds that can be positively identified by reference to spectra from a library of standards constructed using the same solvents and instrument settings. The poster will also show how data can be exported and utilised in multivariate statistical analysis to highlight differences, between mutants and wild type, that can be further investigated by targeted analytical techniques. Using this method, structures can also be suggested for compounds responsible for differences between the crude plant extract samples.

Acknowledgements

The work presented here forms part of the HiMet (Hierarchial Metabolomics) project, funded by BBSRC. Collaborators in the HiMet project are The University of Wales, Aberystwyth, CNAP, York and The John Innes Centre, Norwich.

P45: oxylipin database – a tool for browsing the plant oxylipin pathway and downloading profiling results

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Plant oxylipins are products of polyunsaturated fatty acid oxidation and comprise a wide array of molecules (e.g., fatty acid hydroperoxides, volatile aldehydes, jasmonates). The formation of these metabolites may occur either by autoxidation or by the action of enzymes. The enzymatic formation of hydroperoxy fatty acids represents the first step in the synthesis of oxylipins. The hydroperoxides can be converted by enzymes within the so-called oxylipin pathway which seems to be the most prominent pathway of enzymatic lipid peroxidation in plants. In vivo oxylipins are involved in abiotic and biotic stress responses. Some of them have direct antimicrobial properties; others may act as regulators of plant defence gene expression.

In order to facilitate navigating the complexity of oxylipin biosynthesis, a web-based interface has been set up for browsing the plant oxylipin pathway. The pathway has been split into separate schemes according to the enzymes involved and the substrates converted. The respective schemes are interactive, and the formation of around 200 oxylipins can be displayed in detail together with mass- and UV-spectral information characteristic for the individual compounds. In addition, the interface is connected to an SQL database available to registered users, which is capable of managing, calculating, and graphically representing data derived from oxylipin profiling experiments. Detailed laboratory protocols are provided to perform the underlying analytical procedures. They consist of an organic extraction of frozen plant tissue followed by combinations of HPLC, GC and/or GC–MS separation steps. The procedures allow the monitoring and quantification of more than 150 metabolites of the oxylipin pathway in parallel and in the same sample.

P46: assessing spectral similarities in high resolution mass spectra – a tool for database queries

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Analytical chemists benefit from the constant development and improvement of instruments and especially in the advancement of performance. As a consequence repositories are becoming important tools for handling the huge amounts of data being generated, and they form the cornerstone of advanced data-mining.

The scope of this work is to propose a novel database search algorithm directed towards efficient comparison and classification of high resolution mass spectra. The method is illustrated by analyzing spectra from direct infusion mass spectrometry of crude fungal extracts, exploiting the full data quality in terms of high resolution and mass accuracy in relation to fungal chemotaxonomy. Moreover, the approach can easily be applied in e.g., metabolomics, sample screening/de-screening, and novelty discovery. Our approach is designed to work on both nominal and high-resolution data. Whereas existing library search methods and related data-mining techniques are all bound to process aligned (binned) variables, the method presented here is independent of any bin alignment.

The method relies on robust detection of peaks in the mass spectrum. From each peak, descriptive statistics, e.g., mass, intensity and peak width are used to compare and establish a correspondence between peaks between pairs of spectra. After all correspondences between peaks have been established, an overall similarity between the spectra is found. The similarity is then (1) used as a discriminative value between the peaks in the spectra to be compared, based on a simple peak model assumption, and then finally (2) combined to give an overall estimate of the similarity between whole spectra.

P47: metabolic responses to nitrogen starvation in *Arabidopsis thaliana*

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Nitrogen is an essential element for plant growth, and nitrogen stress triggers various responses at the level of metabolism, gene expression and development. Nitrogen starvation induces important changes in major C and N metabolism, but more detailed analysis of metabolite profiles are needed to understand the dynamic response of the metabolic network to N stress.

We established a model of N-starvation using *Arabidopsis thaliana* plants cultivated under hydroponic conditions. Plants were grown for 5 weeks on 6 mM nitrate in a hydroponic device and total nitrogen starvation was applied for either 2 days or 10 days, allowing the analysis of intermediate and long time responses to N starvation. Roots and shoots were sampled separately and extracted for metabolite profiling.

Plant extracts were derivatised with methoxyamine hydrochloride and MSTFA and their metabolic patterns analysed in both EI and CI mode on a GCT Premier (Waters). The exact mass data was deconvoluted and aligned and subjected to PCA analysis using MarkerLynx (Waters). Clear separation was observed between the starved and non-starved root and shoot metabolite profiles with the root samples after 2 days starvation clustering closer to the 10 day starvation samples than the corresponding 2 day shoot samples which were closer to the non-starved samples. This corresponds to a more rapid depletion of the nitrogen pool in roots. The main discriminating compounds were found to be amino acids, sugars and organic acids.

The data show the wide-ranging effects of plant metabolism to moderate and severe N starvation in roots and shoots.

P48: effect of different inorganic nitrogen sources on the metabolite profiling of spinach (*Spinacea orelacea* L.) with gas chromatography/mass spectrometry

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The evaluation of the internal quality of leafy vegetables is normally based on several specific compounds, such as vitamins, sugars, nitrate, etc. Former researches revealed that many environmental factors could affect on the contents of these compounds and hence the regulation of quality control has been prompted, but the integrated relationship among these compounds and environmental factors are still ambiguous. Metabolic profiling, however, can be used to find out the physiological backbone of changing the contents of these compounds.

In the experiment, we use spinach (*Spinaceae Oleacea* L.) with different nitrogen source (ammonium and nitrate) and compared the change between two cultivars. Leaf was collected and metabolite profiling was made by using GC–MS with automated derivatization by CombiPAL. The metabolite profile was clearly distinguished by different nitrogen sources, while the difference between cultivars was rather small.

P49: revealing host metabolome reprogramming by the economically important fungal plant pathogens *Botrytis cinerea* and *Magnaporthe grisea*

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Fungal plant pathogens impose a consistent economic penalty on agronomy through a reduction in crop yield and/or quality. Many fungal pathogenic mechanisms aim to reprogramme the host metabolome to suppress or avoid defences and mobilise nutrients. Our work focuses on two economically significant plant pathogens. Magnaporthe grisea has a wide host range, but is best known as the causal agent of Rice-Blast disease. Botrytis cinerea is the causal agent of Grey Mould which causes significant losses of soft fruit crops.

The model grass species *Brachypodium distachyon* is an alternate host for *M. grisea* and responses appear very similar to those in rice. Metabolomic approaches were used to elucidate changes during susceptible and resistant interactions of *Magnaporthe grisea* with *B. distachyon*. Experimental reproducibility was demonstrated using a metabolic fingerprinting approach employing Fourier-transform infrared (FT-IR) spectroscopy. Subsequently, extracts of *M. grisea* challenged *B. distachyon* were directly injected into an electrospray ionisation mass spectrometer (ESI–MS). Following data mining, discriminatory analytes (m/z) were shown to be phospholipids (PL) by ESI–MS–MS. We are now moving to correlate transcriptional and metabolomic changes, concentrating on PL-based regulation.

The model plant *Arabidopsis thaliana* has been shown to be susceptible to *Botrytis cinerea* and offers mutants, particularly associated with ethylene signalling, which exhibit either enhanced or compromised defence. Cytological events associated with *B. cinerea* been extensively characterised as a preliminary to metabolomic analysis. FT-IR fingerprinting has revealed distinctive changes between *B. cinerea* challenged Arabidopsis wild type plants and ethylene mutants. Metabolites giving rise to variation seen with FT-IR are being identified using ESI–MS.

P50: using ^{1}H NMR to study the metabolomic profile over the life cycle of *Arabidopsis thaliana*

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The plant species, *Arabidopsis thaliana* has long been used as the standard for creating base-line molecular and genetic studies, and in plant metabolomics it continues to be a model species. For this project, we have used ^{1}H NMR spectroscopy and pattern recognition multivariate statistics to generate a profile of the metabolome throughout the complete growth cycle of wild-type *A. thaliana* 'Columbia', one of the ecotypes in common use in molecular genetics. All of the plants were grown from a single seed stock – thus sampling was taken from a single generation of plants that were grown according to our standard sampling methods (www.metabolomics.ac.uk), over the natural 2 month (approx.) life cycle. Twenty-three harvest points were selected, based on either rosette size (early stages) or distinct growth points such as imbibition, bolting, or flowering. Each stage harvested was photographed, producing a comprehensive record as well as a library of *A. thaliana* samples covering the whole life cycle. In accordance with our established sampling procedures, tissue harvest occurred at the same time point in the day–night cycle for all harvesting points to limit the influence from natural and environmental conditions (such as temperature or diurnal patterns).

NMR spectra collected from this experiment provide a model of the patterns and changes in the metabolites of *A. thaliana*. The resulting patterns suggested many complex and interesting metabolite relationships throughout the life cycle.

This base-line study suggests growth stage – metabolome relationships that should be considered when selecting sampling times for not only the study of specific metabolites within the species, but general research as well. As such this work should prove an important starting point for many future studies.

P51: metabolomics pipeline for investigation of silent plant phenotypes by combination of multivariate analysis and functional metabolic network analysis

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The goal of plant metabolomics is to provide comprehensive information that could help to optimize metabolic engineering strategies by discovering more information about metabolic pathways and the interaction between them. Although *Arabidopsis thaliana* is the most widely studied as a model plant, functions of the genes and the structure of gene-to-metabolite networks are largely unknown. Among gene modified plants, silent phenotypes do not show apparent phenotypic changes when compared with parental lines under given physiological conditions. They undergo, however, subtle but systematic metabolic changes that are difficult to detect by traditional targeted metabolite analysis. To analyze silent phenotypes, we developed a research pipeline of metabolic profiling by gas chromatography-time-of-fright mass spectrometry (GC-TOF/MS) followed by a combination of multivariate analysis and metabolic network analysis. We applied this pipeline to the well-studied Arabidopsis mutants (tt4 and mto1-1) and double knock-out mutant of Serat genes (Serat2;1 and Serat2;2). In our metabolome analysis, five biological replicates of roots, aerial parts, and whole samples of wild-type (WT) and same number of each mutant were extracted and analyzed using GC-TOF/MS. When multivariate analysis was applied to the non-processed GC/MS data matrix, the partial least squares projection-discriminate analysis (PLS-DA) score separated the groups of WT samples clearly from the mutants. With the multivariate statistical tool, we can both classify samples, and identify the variables that explain the differences between the samples. Lastly, the robust correlations were extracted in comparison with significance of correlations in each metabolic network for roots, aerial parts and whole samples.

P52: high-throughput functional genomic approach by integration of transcriptome coexpression analysis with metabolomics approach

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After the completion of the whole genome sequence of Arabidopsis, it is now possible to determine gene-to-gene and gene-to-metabolite correlation through the comprehensive analysis of metabolite accumulation and gene expression. Nevertheless, only half of Arabidopsis genes have functional annotation based on sequence similarity to the characterized genes, and the function of only about 11% has been confirmed experimentally. To execute the systems biology and elucidate a regulatory network of metabolite accumulation mechanism, functional annotation of all gene functions are required. In this research, we integrated metabolomics and transcriptome coexpression analysis for the high-throughput functional genomic approach of Arabidopsis.

The coexpression gene search is now possible in a web site released by ATTED (*Arabidopsis thaliana trans*-factor and *cis*-element prediction database) with using AtGenExpress GeneChip data. We inferred a coexpression framework model of flavonoid, proanthocyanidin and lignin pathway genes, and subsequently speculated each gene function. Metabolic profiling of wild-type plant and the targeted gene T-DNA insertion mutants was performed using high-throughput analysis based on the sensitivity and selectivity of mass spectrometry (MS) with reverse-phase HPLC coupled to UV photodiode array detection. Detailed analysis of metabolite changing in the mutant revealed the changes in metabolo-types, indicating the gene functions. Subsequently, three gene functions were speculated and partially proved by this approach. These results suggested that the functional genomics approach through the integration of coexpression analysis of transcriptome with targeted metabolite profiling provides an efficient means of identifying novel gene functions involved in plant metabolism.

P53 – PRIMe: Platform for RIKEN Metabolomics

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The last decade has seen a rapid progress in the field of functional genomics. Transcriptomics, proteomics and metabolomics are genomics technologies with great potential in plant science. Although *Arabidopsis thaliana* is the most widely studied model plant, functions of those genes and the structure of gene-to-metabolite networks are largely unknown. The metabolomics-based approach is regarded as a direct way to reveal the function of genes involved in metabolic processes and gene-to-metabolite networks. We have constructed a web-based portal, "PRIMe (Platform for RIKEN Metabolomics)" which contains databases and tools to analyze gene co-expression and mass spectral data. It has been developed with the main aim of facilitating integrated analysis for transcriptomics and metabolomics. The contribution of PRIMe is that it assists the biologists by providing information resources and analysis tools for metabolomics and transcriptomics projects. Already available are (1) Metabolite Database (KNApSAcK), (2) BL-SOM Analysis Tool (Batch-Learning Self-Organizing Map), (3) Coexpression Gene Search (coexpression database), and (4) Tree extracting tool (data from AtGenExpress) which are the major constituents of PRIMe. We are motivated to build a research platform with an aim to improve plant production capabilities in both quantitative and qualitative terms, based on integrated genome sciences founded on metabolome research. We perform comprehensive analyses of gene expression and metabolites using model plants (*A. thaliana*), and plants in practical use, such as crops and medicinal plants. PRIMe is available as a web-based service at http://www.prime.psc.riken.jp/.

P54: metabolite profiling of transgenic tomato plants with altered carotenoid content

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Besides, the aesthetic properties conveyed by carotenoid pigments, a number of health-promoting benefits have been attributed to the consumption of carotenoid-rich diets (Fraser and Bramley, 2004). Tomato fruit and its products are one of the principal sources of carotenoids in the western diet.

In order to further our understanding of carotenoid formation during tomato fruit development and ripening, transgenic tomato plants have been generated in which enzymatic steps in the pathway have been amplified. Fruit colour has been altered in these varieties due to increases in lycopene (2-fold increases) (Fraser *et al*., 2002) and beta-carotene (provitamin A) content (up to 3.5-fold increases) (Romer *et al*., 2000). Detailed characterisation of these transgenic plants using a metabolite profiling approach has indicated that the manipulation of carotenoid formation did not have adverse effects on the content of other health promoting phytochemicals, although some re-partitioning of precursors within related pathways was observed.

Acknowledgements

Financial support from the EU, BBSRC, Royal Society and Syngenta (Zeneca) is gratefully acknowledged.

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P55: metabolite "annotation" with milli-mass values and fragmentation patterns using liquid chromatography Fourier transform-ion cyclotron resonance mass spectrometry

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One of the major constraints to metabolic profiling is the lack of easy identification techniques of the metabolites subjected to the analysis. A number of plant metabolic profiles have been obtained using chromatography-coupled mass spectrometry (MS). However, only a limited number of metabolites in the analyses were identified using authentic compounds or speculated as known compounds from the MS/MS fragmentation patterns. Toward having a comprehensive database of plant metabolites with possible formulas deduced from accurate mass values and chemical structures speculated from MS/MS fragmentation patterns, we take advantage of liquid chromatography Fourier transform-ion cyclotron resonance mass spectrometry (LC–FT-ICR–MS). It gives mass values with 1 ppm accuracy, which is enough to draw a few or a single formula for most compounds. MS/MS fragmentation allows us to speculate chemical structures of compounds. Ion suppression, which is often seen in infusion analyses of crude biological materials with FT-MS, is greatly reduced after liquid chromatography separation. We have been analyzing metabolites of tomato fruit and working on preparing a tomato metabolite database. We found several novel glycoalkaroids and flavonoids during LC–FT-ICR–MS analyses. Such information databases are not necessarily enough for identification of compounds in the structural level, but rather for "annotation" of metabolites, similar to "gene annotation" in genome sequence projects. Metabolite annotation databases will serve as one of pivotal resources for metabolomics.

Acknowledgements

This work was partly supported by a grant from Research and Development Program for New Bio-industry Initiatives (Tokyo, Japan).

P56: metabolic approach of the interactions and communications in the rhizosphere

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The presented work is an analytical part of a collaborative research approach between all environmental science oriented institutes at the GSF-Research Center of Environment and Health of the Helmoltz Gemeinschaft in Münich/Germany (IBB, IBÖ, ARB, BIOP, IÖC). Plant health and quality is challenged by the attack of soil-borne pathogens and increased environmental stress. The ability to respond to environmental challenges and signals is essential for all organisms. Molecular mechanisms involved in organismic interactions (pathogenesis and symbiosis) as well as innate immunity response systems are highly conserved. To characterize the interface of plant–microbe and microbe–microbe interactions in the rhizosphere and its implication for plant health and quality, a functional genomics and metabolomics approach is applied. Bacterial signalling compounds such as in instance *N*-Acyl-homoserine lactones (AHL) involved in quorum sensing are important signalling molecules in the rhizosphere and are candidates to trigger a plant response cascade. Aim of the project is the detailed understanding of the quality and importance of signalling compounds and metabolites involved in direct biological control of pathogens in the rhizosphere.

The analytical tools and strategies for the characterization of the signalling molecules exemplified with AHL and metabolites involving high resolving chemical analyses are presented in the poster. The platform technology consists of complementary and hyphenated techniques, such as capillary separation techniques (CE, CEC, μLC, nanoLC, GC, UPLC), classical ion trap MS and ultrahigh resolution (FT/ICR–MS) mass spectrometry (12 Tesla Bruker Daltonics ApexQ, first magnet of this class in Europe) and higher dimensional and multinuclear NMR spectroscopy.

P57: high resolution profiling of plant secondary metabolites capillary liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry

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There is an urgent need for LC–MS based metabolite profiling to improve coverage of metabolomes. This need is even more pressing in plant science. A highly rich and diverse secondary metabolism is a hallmark of plant biology. Lacking the ability to avoid or to retreat from unfavorable conditions or potential foes, plants have evolved an enormous metabolic plasticity, which allows them to dynamically respond to environmental changes through the synthesis and/or degradation of particular compounds. We developed a platform for the highly sensitive profiling of mostly secondary metabolites, employing capillary liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry (CapLC–ESI–QTOF–MS). This approach achieves a very good coverage of Arabidopsis secondary metabolism. A recent compilation listed six biosynthetic classes: nitrogen-containing compounds, phenylpropanoids, benzenoids, polyketides such as flavonoids, terpenes and fatty acid derivatives. Metabolites of five of these classes can clearly be detected by CapLC–ESI–QTOF–MS. Furthermore, in-source fragmentation and targeted tandem MS analysis allow to obtain structural information on unknown compounds. This is of paramount importance given, for instance, the conservatively estimated 5000 metabolites in *Arabidopsis thaliana* of which maybe 500 are annotated today. Databases for LC–MS spectra and for known and ''theoretically'' occurring compounds in the Brassicaceae help in structural elucidation and in cataloguing the Arabidopsis metabolome. A systematic evaluation of matrix effects has shown that the good separation achieved allows reproducible quantification. The platform and examples of application for the elucidation of plant interactions with the biotic and abiotic environment will be presented.

P58: improvements of the web-based analysis tool KaPPA-view for integration of metabolome and transcriptome data on plant pathway maps

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To identify gene functions and to find metabolic changes and novel metabolic pathways, integration of metabolomic data generated by analytical instruments with transcriptomic data obtained by microarray experiments attracts much attention of plant researchers in recent years. To facilitate to depict the metabolic features and to find key-components affecting the biological systems, we developed a web-based analytical tool, KaPPA-View (http://kpv.kazusa.or.jp/kappa-view/) (Tokimatsu *et al.*, 2005). Here we report the recent improvements of KaPPA-View. (1) KaPPA-View can represent the ratio of gene expression and metabolite accumulation by color gradation of the symbols on the pathway maps. Previous version could only process a pair of data simultaneously, and users had to re-select the data set to view another comparison. In the present version, multiple data sets such as those from time-course experiments can be treated. Once selecting the data sets at the beginning, users can quickly access to each result from the tabs appeared on the window. (2) Lines connected among the symbols for genes or metabolites for representing the relationships such as correlation coefficients of co-expression of the genes can be overlay on a pathway map, for clear recognition of the gene and/or metabolite networks. (3) In the new version, we can access directly to the information pages of individual genes, metabolites, and maps through link URLs described with systematic IDs for these elements. This interface gives KaPPA-View much flexibility to be integrated in other tools or databases.

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P59: metabolic fingerprinting of crude plant extracts using Q-TOF mass spectrometry

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When fully developed, this method is thought as a screening tool to detect plants with root-uptake of an herbicide at sub-lethal concentrations due to i.e., soil pollution. The main purpose of this work was to obtain metabolic fingerprints of crude plant extracts without any cleanup of the crude extracts prior to analysis, and to identify changes in the metabolic fingerprints, determined from mass spectral data. Crude methanol extracts of plants (*Brassica napus* L. cv. Pollen – shoots) grown in hydroponic nutrient solutions containing varying levels of the herbicide glyphosate, were analyzed using a novel method for metabolic fingerprinting of crude plant extracts. The method is based on full-scan mass spectrometry, using a quadropole-time-of-flight mass spectrometer (Q-TOF MS) with direct infusion and electrospray ionization (ESI) in the negative ion mode. Mass spectral data in the mass range 100–1500 was obtained. Shikimic acid, a well-known metabolite of the shikimate pathway accumulating due to glyphosate inhibition of the EPSPS enzyme, was identified in the crude extracts of glyphosate treated plants. The identification is based on exact masses of shikimic acid determined by time-of-flight mass spectrometry. Other compounds correlating to glyphosate exposure were found and will be identified in future studies by the use of multivariate statistical methods such as principal component analysis (PCA) and partial least squares (PLS) regression.

P60: effects of phenylpropanoid depletion in Arabidopsis detected by metabolomics

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The aim of this work was to characterise the changes in metabolites resulting from disturbance to the phenylpropanoid pathway in *Arabidopsis thaliana* leaves. To generate the phenylpropanoid-depleted plants the Pseudomonas fluorescens HCHL gene was introduced, as its expression in tobacco was known to convert *p*-coumaroyl-CoA type intermediates to hydroxybenzaldehydes thus leading to a depletion in phenylpropanoids. Numerous transgenic lines were generated, two of which (16N, 20A) showed marked morphological phenotypes. Northern blots showed that these lines had the highest level of HCHL transcript, together with another line with no morphological phenotype change (1F). Microarray analysis showed that 16N & 20A also had the highest number of genes with altered expression levels. Proteomic analyses on 16N and 1F showed altered expression profiles between transgenic and control plants.

To characterise metabolite changes, two approaches were used: (1) ^{1}H-NMR was used as a rapid, non-targeted technique, and (2) HPLC and LC–MS/MS were used to look at more specific classes of secondary metabolites.

NMR lent itself well to this study, as clear signals were obtained from both novel and naturally occurring phenolics. Multivariate analysis of whole ^{1}H-NMR spectra showed that 16N and 20A were the two most affected lines, followed by 1F. Several differences were identified between the transgenic lines, in particular the nature and relative amounts of the novel hydroxybenzoic acid derivatives and the partially depleted kaempferol and sinapoyl derivatives could be deduced. These results were confirmed by the more sensitive HPLC and LC–MS/MS approaches.

Acknowledgement

Funded by the UK Food Standards Agency (G02004).

P61: metabolite profiling of ascorbate-deficient vtc mutants of *Arabidopsis thaliana*

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Ascorbate (vitamin C) is an abundant antioxidant and is also a cofactor for 2-oxoglutarate-dependent dioxygenases in plants. A range of ascorbate deficient *Arabidopsis thaliana* mutants have been isolated (Conklin *et al.*, 2000), which have proved useful in investigating the pathway of ascorbate biosynthesis and the functions of ascorbate in plants. For example, some of the mutants are hypersensitive to ozone and high light stress. Three of these mutants (vtc1, 2 and 4) are affected in enzymes of the D-Man/L-Gal ascorbate biosynthesis pathway, while vtc3 is not yet identified. Metabolite profiling or fingerprinting offers the potential of identifying specific metabolic consequences of each mutation, as well as the metabolic consequences of ascorbate deficiency itself (which should to some extent be common to all the mutants). An extensive ^{1}H NMR analysis of the vtc mutants was carried out. Principal components analysis clustered the mutants into distinct groups from the wild type plants. vtc2 and 4, which are affected in neighbouring enzymes of the D-Man/L-Gal biosynthetic pathway, cluster together while vtc1, which is affected in an enzyme earlier in the pathway clusters separately. vtc3, whose function is so far unidentified, is similar to vtc1. Further analysis of this data set, along with LC analysis of aromatic compounds will be presented.

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P62: bioinformatics resources for the study of plant metabolomics

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In the frame of a French network on plant metabolome, MetaboP (see Poster by Moing *et al.*), we develop bioinformatics resources. Our activities involve: (i) the design and development of an infrastructure for storage and mining of plant metabolome data, (ii) the definition of standards for the sharing of protocols and analytical results, (iii) the development of means for the integration of metabolomics results and data derived from other "omics" approaches.

A first version of the MetaboP database has been implemented. Its data model is compliant with the standards ArMet (http://www.armet.org/) and SMRS (http://www.smrsgroup.org/). Our database is designed to handle the minimal set of information necessary to fully describe metabolic experiments : experimental data and analytical results for 1H RMN and GC–MS.

For controlled vocabularies related to taxonomy, plant structure, growth and developmental stages and metabolites, we decided to rely as much as possible on available standards, that is, respectively, NCBI taxonomy, Plant Ontology Consortium (POC) for *Arabidopsis thaliana* and maize, and KEGG Ligand database. Collaboration with the POC (http://www.plantontology.org/) was initiated in order to extend the ontology for tomato.

P63: evaluation of HILIC in a metabolomic setup comparison with a reversed-phase type of column

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Nowadays, metabolomic research is a booming section in the functional genomics world. More and more, LC–MS is considered as the indispensable tool for metabolomics, due to a combination of sensitivity, discrimination and identification power, and applicability to a broad range of metabolites. The optimisation of chromatography is a first vital step in developing an LC–MS tool. In plant extracts, chemical compounds have predominantly polar characteristics that complicate the separation on conventional reversed phase columns. Here, we describe the use of hydrophilic interaction chromatography within the development of a metabolomics tool. A comparison was made between a HILIC column, namely the TSK Gel Amide 80 column (Tosoh Bioscience) and an Atlantis dC18 column (Waters), which is specially designed to retain polar compounds. Our setup consists of *Arabidopsis thaliana* extracts, spiked with known amounts of metabolites. These compounds cover a broad polarity range (log *D* [pH 3] from –7.85 to 6.63) and belong to several metabolite classes, thus representing the complex content of plants. Chromatographic parameters such as capacity factor and selectivity are discussed as well as reproducibility of retention time in complex mixtures, the latter being an important issue in metabolomic data sets. Relative variation on retention time is for most compounds below 1%, thus compatible with data bunching in data mining post-processing steps. Retention is superior for polar compounds and the spread of the extended polarity difference compound set occupies much more of the chromatographic time frame. As a conclusion, hydrophilic interaction chromatography is a worthy alternative of reversed phase column and gives an excess value in the analysis of polar plant extracts.

P64: LC–MS based metabolomics and accurate mass measurements in complex extracts

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Metabolomics represents the in-depth profiling of metabolites. A key part is identifying differences in metabolite patterns. Accurate mass measurements of the compounds defining a pattern are beneficial for the construction of the profiles and for the subsequent structural elucidation of key metabolites. Here, we discuss accurate mass measurement on a QTOF micro (Waters) equipped with a Lockspray. *Arabidopsis thaliana* extracts were spiked with a range of metabolites encompassing diverging compound classes, and representing various chemical properties. A comparison was made between leucine–enkephaline, widely used as a lockspray compound, and a mixture of five lockspray molecules covering the mass range from m/z 50 to 800. The Masslynx® software enables unattended batch correction of metabolite masses, using the multi-compound lockmix, thus correcting compounds with a reference generally closest in m/z. In extracts, mass errors of less than 50 ppm are obtained. The magnitude of the mass errors is related to ion intensity. The latter is a well-known issue in accurate mass measurements on time-of-flight (and FTMS) mass spectrometers and varying ion intensities are inevitable in plant extracts. Nevertheless, this type of mass accuracies can be used in a first part of the metabolomics workflow, namely the data mining step where bunching of accurate mass and retention times for further statistical analysis takes place anyhow. On identifying a limited subset of metabolites responsible for differences in metabolite pattern, a more accurate mass measurement can, however, be obtained from the same dataset. Mass correction for a single, e.g., peak tail, scan with an ion intensity close to the intensity of the reference, produces accurate mass measurements within 10 ppm.

P65: spatially resolved metabolic profiling of soybean leaves using non-aqueous fractionation, GC/MS and UPLC/MS

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Metabolic profiling has been traditionally used to study whole organisms even though metabolite distributions vary among different organs or subcellular organelles. However, we are currently using non aqueous fractionation (NAQF), GC/MS, and UPLC to provide higher spatial resolution metabolomics data. NAQF of lyophilized soybean leaves was achieved using ultracentrifugation and CCl_4–*n*-Heptane gradient mixtures. Lyophilized leaves were used to avoid further metabolic changes during the procedure. (Gerhardt *et al*., 1984; Stitt *et al*., 1989). NAQF yielded six fractions. Chlorophyll content, and marker enzyme activities, phosphoenolpyruvate carboxylase (PEPC) and -mannosidase, were assessed as stroma, cytosol and vacuole markers, respectively. Metabolic profiling of each fraction was performed by GC–MS after TMS derivation (Broeckling *et al*., 2005). The distribution of chemical compounds identified was determined to be different among fractions and are being used to better understand the specific biochemical functions of the enriched subcellular organelles. For example, the distribution of hexoses and sucrose provide relative information on the availability of substrates for starch biosynthesis in soybean leaves prior to translocation. The results support that NAQF and GC–MS are useful tools for the metabolite profiling at the subcellular level.

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P66: effect of plant hormones on trichome initiation and secondary metabolism in *Solanum* sp.

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Solanum sp. have up to seven different anatomical types of trichomes. They can be subdivided into glandular and non-glandular types. Glandular types have been shown to contain several secondary metabolites. We are investigating the relationship between the presence and density of these glands with the production of some of these compounds such as terpenoids and acylsugars. In *S. esculentum*, it was found that glandular type VI produces and stores mono and sesquiterpenoids. The composition of this mixture in each variety varies in quality and quantity. We also found a second possible glandular type not described in *S. esculentum* before, similar to types IV from *S. hirsutum*. We are currently testing its production of secondary metabolites against the known compounds that are produced by these glands in the other species, i.e., *S. pennellii* type IVs produce acylsugars. Using GC–MS and LC–MS to study the production of secondary metabolites will allow us to describe secondary metabolite production in these glands in different developmental stages and in response to plant hormones.

The main purpose is to study how trichome initiation (for each type of trichome) and the production of secondary metabolites (terpenes and/or acylsugars) are influenced by gibberellins (GA) and jasmonates (JA), and the interaction between these two hormones. Our preliminary results show that the non-glandular type V is up regulated by GA in the same way than *Arabidopsis trichomes* (non-glandular). Glandular type VI initiation, which has been described to respond to JA, is not up regulated by GA. We are currently studying the levels of mono and sesquiterpenoids in GA treatments. Also we are setting metabolites baselines to study the interaction of JA and GA in trichome secondary metabolism and initiation using tomato GA mutants (pro and gib-1).

P67: the application of plant metabolomics in the assessment of field grown GM wheat

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The safety of novel foodstuffs, in particular those derived from genetically modified crops, is of public concern. One criterion that has been put forward for assessing the safety of novel foodstuffs is that of substantial equivalence. The principle behind this being; that if the composition of the novel foodstuff does not differ in a meaningful way from a traditional variety(s), it is safe. For obvious reasons, a major part of this compositional analysis should be directed at the chemical composition of the material, i.e., the metabolites (the metabolome).

We have applied metabolomic technologies (principally NMR) combined with multivariate statistics to asses the substantial equivalence of three lines of genetically modified wheat (two non-GM parent lines and one null transformant were used for comparison). The wheat was engineered with altered sub-unit composition of glutenin, thereby modifying its bread-making properties. The material to be investigated was produced in field trials at two geographically different sites over 3 years, allowing the natural variability of the crop to be to be taken into account. Some consistent differences between one of the GM lines and its parent were observed (principally in disaccharides); however, for all of the other lines no differences could be observed against the natural variability of site and year.

P68: following Columbus's explorations for routes to eugenol in the glands of basil

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The phenylpropene class compounds (eugenol, isoeugenol, chavicol and their derivates) play an important role in the ecology of some plant species, either for defense or for attraction of insects for pollination. These compounds are recognized as the main contributors to the characteristic aroma of some of the most important spices that shaped human history (e.g., cloves and allspice). Sweet basil (*Ocimum basilicum*) was shown to synthesize and accumulate these beneficial compounds in the peltate glands on the surface of its leaves. A genetic approach that includes ethyl methane sulfonate (EMS) mutagenesis of a eugenol producing basil line combined with high-throughput screening for scent mutants is applied for unraveling elusive steps in the specialization from the common phenylpropanoid (lignin) pathway to phenylpropenes. Proteomics analysis of the glands from two independent scentless mutant lines identified a single protein that was underrepresented in the mutants glands. Heterologous expression of the encoding gene in *E. coli* indicate that this gene, namely EUGENOL SYNTHASE 1 (EGS1), plays a major role in this metabolic specification. Furthermore, this novel protein forms a distinct clade of enzymes with orthologs from different plant species that synthesize similar compounds. Functional analysis of one of these orthologs, ISOEUGENOL SYNTHASE 1 (IGS1) from petunia, clearly indicate a common ancestry to these genes. This study highlights the power of combining genetics and proteomics for the study of specialized metabolism in plants.

P69: are cyanogenic glucosides, rhodiocyanosides and other cyanoalk(en)yl glucosides found in cyanogenic plants produced by a single promiscuous multienzyme complex?

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The rhodiocyanosides A and D belong to a group of cyanoalkenyl glucosides structurally related to cyanogenic glucosides (CNglc). In contrast to the wide spread CNglc's, cyanoalkenyl glucosides are not cyanogenic. Rhodiocyanosides A and D were first isolated from Rhodiola used in Chinese traditional medicine, where they are proposed to function as inhibitors of histamine release in allergic response. The model legume Lotus japonicus contains high levels of rhodiocyanosides A and D as well as the CNglc's linamarin and lotaustralin. Rhodiocyanosides are derived from the amino acid L-isoleucine as is lotaustralin, indicating that they are synthesized in the same multienzyme complex, able to hydroxylate and dehydrate a putative nitrile intermediate in their biosynthesis at different positions. LC–MS metabolite profiling of Lotus, Rhodiola and also Ribes species demonstrated that rhodiocyanosides usually co-occur with lotaustralin and revealed several unidentified peaks with m/z-values identical to those of rhodiocyanosides and lotaustralin and one identical to that of a hydroxylated rhodiocyanoside, e.g., sarmentosin, previously identified in Sedum sarmentosum. Ion trap LC–MS/MS demonstrated that the unidentified compounds are non-cyanogenic glucosides, corresponding to compounds possibly derived from 2-methyl-butyronitrile, an intermediate in lotaustralin biosynthesis. This was substantiated by administration of radiolabelled amino acids to leaves from Ribes uva-crispa which gave rise to radiolabelled products derived from isoleucine, but not from leucine. The unidentified components are now being isolated for structure determination. A similar complex profile is known from barley, which in addition to the leucine-derived cyanogenic glucoside epi-heterodendrin contains the non-cyanogenic cyanoalk(en)yl glucosides osmaronin, dihydroosmaronin, epidermin, and sutherlandin.

P70: systems approaches to understand the isoprenoid pathway regulation and integration into the cellular gene network

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Plant isoprenoids represent the largest group of biologically active metabolites with at least 30 000 chemical compounds. Plant isoprenoids have essential functions in physiological and biochemical processes (e.g., photosynthesis, respiration, membrane fluidity, pathogen defense, key plant hormones -cytokinin, brassinolides, gibberellic acid, abscisic acid; protein prenylation). Many plant isoprenoids also have substantial commercial, pharmacological and agricultural value (e.g., essential oils, anticancer drug taxol, antimalarial drug artemisin, polymers in rubber, antibiotics, carotenoid and tocopherol antioxidants, herbivore repellents). Understanding the regulation of the plant isoprenoid biosynthetic pathway and its integration into the cellular metabolic and signal transduction network is therefore not only of scientific but also of commercial interest.

In our laboratory, we are using systems approaches to understand the isoprenoid pathway regulation as well as pathway integration into the cellular genetic network. To this end, using variety of publicly available genome-wide gene expression data sets, we modeled the isoprenoid pathway network using sparse Gaussian graphical modeling (Wille *et al.*, 2004). This analysis revealed some novel unexpected dependencies between the genes in the pathway that can be further investigated.

Another approach that we are undertaking to understand the pathway integration into the cellular network is the molecular analysis of responses upon systematic perturbation of the isoprenoid pathway using Arabidopsis loss-of-function mutants and plants ectopically expressing specific enzymes. This approach, as well as preliminary results will be discussed in more details at the meeting.

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P71: functional genomics by integrated analysis of metabolome and transcriptome

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Plants produce a wide diversity of compounds used for foods, flavors, medicines and industrial materials. To improve the productivity of plants by modification of the genes involved in synthesis of useful compounds or by the strict control of plant's growth condition, it is essential to understand plant's metabolic process and its regulatory mechanisms as a whole. Novel technologies for comprehensive analysis of the transcripts, proteins and metabolites open the door for elucidation of a whole metabolic system. In this presentation, we introduce our strategy for systems-based analysis of plant metabolism, which leads to functional genomics concerning the production of useful secondary metabolites.

Metabolomics of Arabidopsis was carried out by combining non-targeted analysis using FT-ICR–MS and targeted analyses by capillary electrophoresis and HPLC. Transcriptomics of the identical samples was also carried out by commercially available microarrays. Time-course metabolome and transcriptome data of sulfur-starved Arabidopsis were merged and analyzed by Batch Learning-Self Organizing Mapping to classify the metabolites and genes based on the time-dependent pattern of accumulation and expression in response to sulfur starvation. The genes involved in glucosinolate (GLS) biosynthesis were classified into a single cluster. Assuming that the genes belonging to the same cluster are also involved in GLS biosynthesis, we predicted the functions of several genes, for example, as desulfoGLS sulfotransferase. The predicted functions were proved by in vitro enzymatic assay using recombinant gene products.

Our strategy is applicable to non-model plants, crops and medicinal plants by analyzing the transcriptome using cDNA-AFLP, differential display, PCR-select subtraction, and so on.

P72: discovering how tomatoes work through metabolomics

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Surveys have highlighted the characteristics of tomatoes that consumers most appreciate, but the challenge is to understand how these characteristics can be bred into plants without introducing undesirable properties. Using our in-house metabolomics platform, we have determined the levels of endogenous metabolites which impart the desired characteristics and have related these back to the genetic constitution of the plant. Combining metabolic data with other "omic" information, we can determine the parts of the tomato genome which control the quality characteristics we are interested in.

P73: development of a UPLC method for high throughput plant metabolomics

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HPLC–MS is widely used in the metabolomics laboratory at the Syngenta Jealott's Hill Research Laboratory for the non-targeted analysis of complex plant extracts. With the demands placed on expensive instrumentation in any research laboratory increasing throughput is important not only to feed back into the discovery process more rapidly, but also to enable greater access to instrumentation by multiple users. Current established analytical procedures utilising traditional HPLC methods result in run times of ca 60 min. With the advent of UPLC–MS technology, it has been shown to be possible to reduce the cycle time for the assay to ca 15 min enabling higher sample throughput whilst maintaining chromatographic resolution. This was demonstrated following a non-targeted approach to the analysis of wheat and barley extracts using the established HPLC–MS and UPLC–MS methodologies.

P74: methodology for profiling of the *Arabidopsis metabolome* using UPLC linked electrospray ionization quadrupole time of flight mass spectrometry

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Liquid chromatography (LC) linked mass spectrometry is an important tool for the rapid profiling of ionisable biochemicals present in the plant metabolome. We report the use of ultra performance LC- QTOF mass spectrometry to profile plant metabolites in *A. thaliana* plants grown in controlled environments. Plants were grown from seed for 30 days until they reached 14–20 rosette leaf stage. Above ground shoot and leaves were homogenised in liquid nitrogen and extracted in a chloroform methanol water (2:6:2) mixture and heated at 600 °C for 15 min prior to storage at –700 °C. Plant extracts were divided into a further three preparations prior to LC–MS analysis: an aliquot was evaporated under vacuum and redissolved in acetonitrile water; chloroform was added to another aliquot and the resulting aqueous and chloroform phases analysed separately. All plant extracts were analysed in positive and negative modes by TOF MS followed by PCA analyses to identify marker metabolites. QTOF and GC–MS analyses were also used to identify structures of biomarkers. Analyses of the different types of plant extracts revealed that evaporation of the extracts resulted in little loss of metabolites as detected by LC–MS; however, partition into chloroform resulted in a significant loss of over 100 lipophilic metabolites. Data on the effects of plant position and small changes ($< 10\%$) in light intensity during plant growth on the resulting plant metabolome are presented. Our work shows that UPLC separation in combination with high resolution TOF analyses allows the profiling of over 5000 lipophilic and polar plant metabolites.

P75: metabolite profiling as a tool for investigation of the metabolic response of soybean to infection by *Phytophthora Sojae*

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The phytoalexin response of soybean (*Glycine max*) to infection by *Phytophthora sojae* has been well characterized over many years of investigation. Other influences of infection on soybean metabolism are not as well understood. We employed untargeted metabolite profiling of infected and non-infected soybean hypocotyls of both resistant and susceptible cultivars to explore the effect of infection on soybean metabolism more generally. Non-polar and polar extracts were analysed by GC–MS. Principal components analysis of chromatographic results unambiguously clustered all sample types separately.

Simple sugars and certain amino acids tended to decrease upon infection in both resistant and susceptible interactions, suggesting that the increasing rate of metabolism to fuel defence responses burns up simple sugars. A large increase in lactic acid in both resistant and susceptible interactions was consistent with an increase in anaerobic metabolism to provide energy for the defence response. Large amounts of the isoflavonoid phytoalexins were observed in both susceptible and resistant interactions (approximately three times more in the resistant than the susceptible interaction). An increase in the level of salicylic acid (greater for the susceptible than the resistant interaction) suggests that the pathogen may subvert host defences by channelling metabolism into SA-based (biotrophic) defence mechanisms, which would impair host defence during the necrotrophic phase of *P. sojae* infection.

P76: metabolomic characterization of lavender plant species by profiling volatile metabolites from living flowers using SPME-GC/MS

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Flowering plants (angiosperms) comprise approximately 90% of the Kingdom Plantae. Flower characteristics play a significant role in the taxonomy of flowering plants. Volatiles emitted from living plant flowers, mainly low molecular weight molecules, may represent part of plant metabolite network and reflect various genetic traits of plants. A previously developed SPME-GC/MS method has been used to profile volatile metabolites from the flowers of 12 different lavender plant species and 10 different cultivars. The chromatographic results and subsequent statistical analysis showed that the metabolic profiling of volatiles distinctly differentiate the species, which is consistent with the morphological characters. It is suggested that the metabolic profiling of volatiles from living flowers should be integrated into a metabolomics network, and SPME-GC/MS may be a useful technique in volatile metabolomics that can be used as a platform to further investigate the characterization of plants, and together with the complementary genomic and proteomic techniques, ultimately to gene-function determination.

P77: using metabolomics to decipher functions of Arabidopsis genes in the context of metabolic and regulatory networks

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A multi-institutional consortium of labs is developing metabolomics as a new functional genomics tool for elucidating the functions of Arabidopsis genes that are currently annotated as having an "unknown function". Approximately one third of the Arabidopsis genes are so annotated. The consortium utilizes five distinct analytical platforms that couple different separations methods (GC, LC, and CE) to mass-spectroscopic detection systems. These analytical platforms are used in both "non-targeted" and targeted metabolomics analyses, which in combination detect approximately 2,000 metabolites, of which 700 are chemically defined. The consortium is applying these platforms to reveal changes in the metabolome associated with knockout mutations in genes of unknown function and comparing these to similar mutants in genes of known functions. We will discuss initial data generated from a small set of exemplar mutants. These data indicate that metabolomics can reveal metabolic changes in mutants that are otherwise "silent" in phenotype. These data are being interpreted via two strategies: (1) as a "fingerprint" of the metabolic consequence of each mutation, which can be used to functionally cluster genes; and (2) by mapping metabolite changes on metabolic and regulatory network maps, such as AraCyc and MetNetDB, to identify specific functions that are affected by each mutation. Thus, metabolomics, in combination with other "-omics" technologies promises to be a new resource for determining the function of Arabidopsis genes.

P78: high-throughput fingerprinting of Arabidopsis metabolism mutants by flow-injection electrospray mass spectrometry with machine-learning data analysis

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Metabolomics using hyphenated analytical systems (GC–MS, LC–MS, etc.) produces the highest quality of chemical information, but is demanding in machine time and post-run data deconvolution. This can impede progress of metabolomic projects with large numbers of samples to process. We have been exploring high-throughput analytical methodologies to provide preliminary information on the characteristics of large batches of plant samples, and hence to guide metabolomics project planning – an approach we term 'hierarchical metabolomics' (Catchpole *et al.*, 2005). Particular success has been obtained using non-chromatographic flow-injection electrospray mass spectrometry, on materials extracted with simple one-tube solvent mixes. The analytical data is highly dimensional, and to extract maximal information we have used machine-learning tools including Random Forests and Support Vector Machines. Within the BBSRC Exploiting Genomics Initiative, we have compared the metabolome fingerprints of putative metabolic function mutants of Arabidopsis, originating from conventional or insertion (T-DNA or single copy dSpm) mutagenesis. This methodology discriminated the metabolomic fingerprints of a number of mutants in putative lipid or carbohydrate metabolism genes for which no phenotypes have previously been demonstrated. While the metabolic information in the high-throughput fingerprints is far from comprehensive, they do have interpretative potential. For example, using an appropriate extraction procedure, predictable changes in the glycolipid profiles of characterized lipid mutants can be discerned.

Reference

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P79: detection and quantitation of resveratrol in tomato fruit (*Lycopersicon esculentum* mill.)

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Resveratrol is a stilbene phytoalexin well known for its presence in grapes, wine, and peanuts. As a result of its antioxidant and chemopreventative properties, it has gained much attention in the functional food field. A GC–MS method for the detection of resveratrol, its glycosylated product piceid, and the *cis* isomers of both compounds has been developed and used to quantitate the levels of these compounds in the skin of commercially available tomato fruits (*Lycopersicon esculentum* mill.). A time course study revealed that total resveratrol concentration remains relatively stable during fruit maturation, reaching a maximum concentration in the skin of 18.0 μg/g dry weight at 4 weeks post-breaker. No stilbenes were detected in the flesh of tomato fruits. The biosynthetic origin of resveratrol in tomato is under investigation.

P80: comparing the performance of LC/MS processing software

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In the last years, mass spectrometry (both GC–MS and LC–MS) has emerged as an important technology to solve functional genomics challenges. For high-throughput analysis, e.g., in Arabidopsis and other plants, several (complementary) protocols have been developed.

Several methods have been developed to preprocess and align the raw data such that prominent peaks are recognised across the runs, and a shift profile aligns the retention times onto a common basis. The alignment is only based on the exact mass of related peaks, without the need for identification. This approach is used by e.g., the MetAlign software and the XCMS package, which is part of the open source Bioconductor project (www.bioconductor.org).

We have used different peak models, e.g., the empirically transformed gaussian model to fit peaks from actual measured data, and obtained several classes of peaks through clustering in the model parameter space. From these model peaks, we can create synthetic LC/MS data and compare the performance of MetAlign, XCMS and other software with regard to the parameters of the created synthetic data.

P81: structure classification of flavonoids

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Flavonoid is a class of secondary metabolites with C6–C3–C6 skeleton derived from phenylpropanoid-acetate pathway; Beneficial effect of fruits and vegetables is often attributed to its anti-inflammatory and antioxidant activity, which have drawn much attention in food manufacturers. We present hierarchical classification of flavonoid structure based on its biosynthetic pathway and introduce a database of over 6000 natural molecules with information on biological species. The classification has three levels. The top class includes eight major groups based on biosynthetic origin (chalcones, flavones, flavonols, flavans, anthocyanidins, isoflavonoids, neoflavonoids, and biflavonyls). Subclass and subsubclass are based on chemical modifiers (O-glycoside, C-glycoside, etc.) and hydroxylation patterns of carbon skeletons. Each subsubclass contains up to 400 structures. Each molecule is assigned with a unique ID number representing its classes using fixed number of digits.

The structural classification with species information helps identify evolutional origin of each flavonoid category, and predict flavonoid presence in food. The database and related software tools for classification will be available online in April.

P82: exploring the generation of the tomato flavour metabolome

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The aroma profile of tomatoes has two major components, compounds present in the intact fruit and those generated enzymically upon homogenisation. For the consumer, the balance between these two groups of compounds will depend on the extent of tissue disruption as fruit is eaten. Sample preparation for chromatographic analysis of the flavour metabolome was designed to generated an aroma profile which mimicked that observed in the breath during eating.

Sensory evaluation of taste and flavour attributes of a range of tomatoes was performed in parallel with volatile analysis. This showed that some of the enzymically generated aroma compounds (most notably (Z)-3-hexenal) were important for the intensity of perceived flavour.

Hexenal is generated from the degradation of the fatty acid linolenic acid through the formation and cleavage of its 13-hydroperoxide (HPO). The key question was, why did tomatoes vary in the amount of hexenal they generated? Further studies analysed substate availability, the different enzyme isoforms (lipoxygenases) and hydroperoxides formation (13 versus 9-HPO's).

Overall, it appeared that the amounts of linolenic acid could be a limiting factor in hexenal generation, this will have a significant impact on the flavour metabolome and the quality of tomato flavour as perceived by the consumer.

P83: microarray services available at the Norwegian Arabidopsis Research Centre (NARC)

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NARC is a national technology platform funded through the Functional Genomics (FUGE) initiative of the Norwegian Research Council. The centre is represented at three universities – Norwegian University of Science and Technology (NTNU) at Trondheim, University of Oslo (UiO) and Norwegian University of Life Sciences (UMB) at Ås. At NTNU, we offer plant scientists the opportunity to do microarray experiments, with guidance and support from skilled personnel at all stages of the analysis.

Two oligonucleotide sets form the basis for microarray production at NARC. One set embodies whole gene families for nutrition uptake, various stress responses and transport processes, and totals approximately 2200 genes. The other set is Operon's full-genome collection, totalling approximately 26,000 genes, and covering the entire nuclear genome of Arabidopsis. NARC's 34K chip comprises both oligonucleotide sets and is printed on aminosilane-covered glass slides, in collaboration with the Norwegian Microarray Consortium (NMC). For experiments, we use 15 μg of total RNA (from test and control plants) as starting material.

NARC's mission statement is to provide the plant science community with service and expertise in microarray analysis for the identification of gene function and comprehensive analysis of combined omics data. Our initial service activity is based on the principle of "RNA in, raw data out" – however, we offer extended expertise in all areas (e.g., plant growth and harvesting, experimental design and advanced analysis) and thus, seek to engage in scientific collaboration with platform users.

P84: investigation of the total chemical composition of leaf extracts by 'chip-based' Nanospray mass spectrometry

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The chemical composition of plants is usually investigated by GC–MS and/or LC–MS. This restricts the analysis to compounds amenable to these techniques or to the specific solvent systems being used. The aim of this study is to develop a routine methodology using nanospray for direct infusion mass spectrometry minimising the need for sample clean-up and removing the dependence on chromatography and sample derivatisation.

Analyses were performed in positive and negative ion modes using a Nanomate HD 'chip-based' (automated) nanospray system (Advion BioSciences, Norwich, UK) coupled to a QStar XL (Q-Tof hybrid) mass spectrometer (Applied Biosystems, Warrington, UK). Tandem mass spectrometry (MS/MS) was performed on all major ions to facilitate structural elucidation. Leaves from mature specimens (1 year old) of *Trifolium Repens* L, *Ranunculus Repens* and *Caltha Palustris* (grown under a controlled environment) were collected, freeze dried, powdered and solvent extracted (MeCN, MeOH, EtOAc, DCM, water). The extracts were diluted to approximately 1 mg/mL with the addition of (0.1%) formic acid and/or 50% MeOH as appropriate.

Using this methodology, a substantial volume of data was quickly and easily obtained. Five microliters infusions provided 15 min of stable signal, enabling multiple MS/MS experiments to be performed. In some cases spectra contained over 250 individual ions, many only at a few % intensity compared to the base peak. Good MS/MS was usually obtainable on ions observed above 10%. More intense signals often gave better MS/MS spectra than those at lower intensity or high masses. Negative ion spectra were much simpler than positive spectra and were probably dominated by various acids. Different solvent extractions produced very different spectra. The spectra from different leaves were also very different (as expected). Using this methodology, we have been able to identify membrane glycolipids and phospholipids, pigmentation compounds, oligosaccharides, carotenoids and chlorophyll.

P85: resolving *p*-hydroxybenzoic acid biosynthesis by in vivo blocking of phenylpropanoid pathway in *Daucus carota* L.

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Despite the simple structure of benzoate and its widespread occurrence in plants, the enzymatic steps of the biosynthesis of benzoic acid and its close relative *p*-hydroxybenzoic acid are still unresolved. This phenolic compound finds application in food, pharmaceutical and polymer industries. As has been demonstrated in several studies, *p*-hydroxybenzoic acid formation requires the cleavage of two carbons from the C3 side chain of its precursor *p*-coumaric acid. Until now, only a few reports are available on the enzymatic route to *p*-hydroxybenzoic acid formation. Evidence for the non-oxidative pathway has been studied with cell culture of Daucus carota in earlier years, but failed to demonstrate the chain-shortening enzyme activity. In this communication, we aim to elucidate the biosynthetic route to *p*-hydroxybenzoic acid formation in elicited hairy root cultures of *D. carota* by using selective metabolic inhibitors of plant phenylpropanoid pathway, namely, aminooxyacetic acid (AOAA), piperonylic acid (PA) and 3,4-methylenedioxycinnamic acid (MDCA), which are known to inhibit PAL, C4H and 4CL, respectively, the three early enzymes of phenylpropanoid metabolism. We anticipate that selective inhibition of these enzymes in vivo may provide information on the metabolic route to *p*-hydroxybenzoic acid formation. Selective inhibition of C4H by PA (100 μM–800 μM) and PAL by AOAA (200 μM–1 mM) results in a decrease of *p*-hydroxybenzoic acid accumulation in the cell wall. Interestingly, *p*-hydroxybenzoic acid accumulation remains unchanged with 4CL inactivation by increasing concentrations of MDCA, indicating that *p*-HBA biosynthesis is CoA-independent. In vitro conversion of *p*-coumaric acid to *p*-hydroxybenzoic acid has been demonstrated in cell extract of *D. carota* hairy roots, which provides an unequivocal support to the inhibitor experiments.

P86: elucidation of the molecular basis of heterosis via integrative analysis of metabolome and transriptome

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Although heterosis has been widely exploited in agriculture and breeding, very little is known about molecular mechanisms underlying this phenomenon. In our group, we use the model plant *Arabidopsis thaliana* to investigate the genetic basis of hybrid vigour.

Hybrids derived from two different Arabidopsis ecotypes Co1-0 and C24 exhibited elevated biomass production already 6 days after sowing (DAS). Difference in gene expression and metabolite levels before and during the occurrence of visible heterotic effects should give insight into molecular basis of heterosis. The parents and their reciprocal hybrids were analyzed in an integrative approach for their metabolome and transcriptome ate different points: 4,6 and 10 DAS. Additionally, we measured metabolite levels of the above mentioned genotypes at further time points: 15, 20, 25 and 30 DAS.

The pattern of differentially expressed genes were primarily dominant and intermediate at all time points. Metabolite levels in the hybrids as compared to the parents, however, were intermediate at 4 DAS and heterotic at 10 DAS. This tendency was even more pronounced at later time points.

Candidate genes extracted after combined analysis of metabolome and transcriptome were verified by RT-PCR. Additionally, differences in gene expression and metabolites are verified in RIL test crosses and further crosses between different ecotypes showing negative and positive heterosis.

P87: metabolome analysis of the colorless fruit peel tomato mutant (Y) by the use of Ultra Performance Liquid Chromatography (UPLC)–QTOF–MS

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The peel of fruit acts as a protective barrier and as a medium for the exchange of gases and water. We used UPLC–QTOF–MS to examine the metabolic alterations in a colorless peel tomato mutant termed "y". Compared to the wild-type (WT) ripening fruit, the "y" mutant peel lacks a yellow compound and the fruit displays a more pink and less glossy phenotype. Extracts of WT and "y" peels derived from four stages of fruit development were analyzed by the ES(+) and ES(–) modes. PCA analysis showed that most of the metabolic differences between "y" and the WT peel are in the late stages of fruit development. The high resolution and mass accuracy obtained by the UPLC–QTOF–MS analysis was subsequently used for the detection of differential mass peaks in between WT and "y" peel tissues and for structural elucidation of unknown compounds. Apart from a dramatic decrease in the levels of the yellow color flavonoid Naringenin Chalcone, levels of various derivatives of Naringenin and Naringenin Chalcone (hydroxylated, glycosylated and methoxylated) were reduced in the "y" mutant peel. We are currently continuing with the structural elucidation of differential mass peaks showing elevated levels in the "y" mutant peel by MS–MS analysis (in collaboration with PRI-Wageningen) and generating equivalent data at the Transcriptome level.