



The Samuel Roberts Noble Foundation, Inc.

2510 Sam Noble Parkway Ardmore, Oklahoma 73401
Telephone 580/223-5810 FAX 580/221-7362

Dear Colleague,

Welcome to the NSF-VBI-Noble workshop focused on integrated functional genomics. This workshop is being conducted as part of the outreach component of a project funded by The National Science Foundation Plant Genome Research Program (#0109732). This project is focused on integrated functional genomics and entitled "An Integrated Approach to Functional Genomics and Bioinformatics in a Model Legume". The objective of this meeting is to gather together integrated functional genomics practitioners to discuss the current status and future directions of the field in the presence of those who wish to learn more about the science of integrated functional genomics. This meeting is intended to be a tutorial for those new to functional genomics and a discussion session for those who practice functional genomics. This workshop is made possible through financial support provided by The Samuel Roberts Noble Foundation and The National Science Foundation, and it is being held November 16-18th, 2005 at The Noble Foundation in Ardmore, OK. The program includes two days of lectures (Nov. 16-17, 2005) and a third day (Nov 18, 2005) for laboratory and instrumental demonstrations including data processing. The lecturers are divided into two general categories: 1) Technologies and 3) Applications. Following below is a program.

More information on the Noble Foundation and this project can be obtained via the internet.

Noble Home Page: <http://www.noble.org/>

NSF Project Description: <http://www.noble.org/medicago/NSF/Nsf.main.html>

Virginia Bioinformatics Institute: <http://mendes.vbi.vt.edu/tiki-index.php>

Previous Workshops: <http://www.noble.org/medicago/NSF/Workshops.html>

Contact Info: <http://www.noble.org/Admin/Contact/Index.html>

We look forward to meeting you and a productive meeting,

Lloyd W. Sumner
Noble Foundation
lwsurner@noble.org

Richard A. Dixon
Noble Foundation
radixon@noble.org

Pedro Mendes
Virginia Bioinformatics Institute
mendes@vbi.vt.edu

Gregory D. May
Noble Foundation
gdmay@noble.org

Joel Timothy Smith
Southeastern Oklahoma State University
tsmith@sosu.edu

NSF-VBI-Noble Integrated Functional Genomics Workshop
November 16 to 18th, 2005
The Noble Foundation, Ardmore, OK.



NSF-VBI-Noble Plant Integrated Functional Genomics Workshop
November 16-18, 2005
Ardmore, OK



Tuesday, November 15

Travel & Arrival
6:00-9:00 pm Mixer/Check-in/Registration/Dinner
Noble Foundation Conference Center

Wednesday, November 16

7:00-8:30am Breakfast at Conference Center

(Shuttles run 8-9 am from Conference Center to Kruse Auditorium)

TECHNOLOGIES: Genomics, Transcriptomics, Proteomics, Metabolomics, Mutagenesis, and Bioinformatics

9:00 – 9:15 am Michael A. Cawley, President & CEO, The Noble Foundation
Welcome & Introduction

9:15 – 10:00 am S1 - Bruce Roe, University of Oklahoma
“Sequencing the Gene-Rich Euchromatin of the Model Legume, Medicago truncatula”

10:00 - 10:45 am S2 – Michael Udvardi, Max Planck Institute
“Functional Genomics of Nodule Transport and Metabolism in Lotus and Medicago”

10:45 am Group photo- outside Kruse Auditorium – Broderick Stearns

10:45 – 11:15 am Break (coffee, sodas, water, bagels, assoc pastries)

11:15-11:50 am S3 – Marina Naoumkina, The Noble Foundation
“Microarray Technologies for Gene Discovery and Understanding Metabolism in Medicago truncatula Cell Suspension Cultures Responding to Yeast Elicitor or Methyl Jasmonate”

11:50 – 12:25 S4 – Ulrike Mathesius, Australian National University
“Proteome Analysis of Medicago truncatula to Study Root Responses During Nodulation”

12:30-1:30 pm Lunch – NF Cafeteria (coconut shrimp)

1:30 -2:00 pm S5 – B. Mark Lange, Washington State University
“Integration of Transcript, Protein and Metabolite Profiling Data Sets Using the BioPathAt Tool”

- 2:00 – 2:30 pm S6 – J. Tim Smith (SOSU, CE/MS & CE/LIF)
“Applications of Capillary Electrophoresis as a Tool in Metabolic Profiling”
- 2:30- 3:00 pm S7 – Vladimir Shulaev, Virginia Bioinformatics Institute
*“Insertional Mutagenesis as Functional Genomics Tool in Diploid Strawberry (*Fragaria vesca*)”*
- 3:00 – 3:30 pm Break (coffee, sodas, water, assoc cookies)
- 3:30 – 4:00 pm S8 – Kiran Mysore, The Noble Foundation
*“An Overview of *Medicago truncatula* Mutagenesis”*
- 4:00 – 4:30 S9 – Dong Xu, University of Missouri-Columbia
“Cellular Function Prediction and Biological Pathway Discovery through Mining Genome-Scale Data”
- 4:30 – 5:00 pm S10 – Gregory D. May, The Noble Foundation
“A Virtual Plant Information Network”
- 5:00 – 5:30 pm S11 – Pedro Mendes/Bharat Menrotra, Virginia Bioinformatics
“DOME, a Database and Analysis System for Systems Biology Experiments”
- (Shuttles run 5-6 pm from Kruse Auditorium to Conference Center)
- 5:45 -6:45 pm Posters – Conference Center
- 7:00 - 8:00 pm Dinner (southern barbeque) – The Noble Pavilion
- 8:00 - 9:00 pm Posters – Ping Pong Tournament (Pavilion, Zhentian Lei coordinator), TV, Card games, soda/beer.

Ping Pong Champion Dr. Dong Xu,
University of Missouri, Columbia



Thursday, November 17

7:00-8:30 am Breakfast at Conference Center

(Shuttles run 8-9 am from Conference Center to Kruse Auditorium)

INTEGRATED FUNCTIONAL GENOMICS APPLICATIONS: Richard Dixon (Chair, The Noble Foundation)

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| 9:00-9:45 am | S12 – Carl Douglas, University of British Columbia
<i>“An Integrated Approach to the Functional Genomics of Fiber and Wood Development in Poplar and Arabidopsis”</i> |
| 9:45-10:30 am | S13 – Kazuki Saito, Chiba University
<i>“Integration of Omics Towards Phytochemical Genomics”</i> |
| 10:30-11:00 am | Break |
| 11:00 – 11:35 | S14 – Maria Harrison, Boyce Thompson Institute
<i>“Transcriptional Profiling and RNAi to Dissect the Molecular Basis of the AM Symbiosis in Medicago truncatula”</i> |
| 11:35 – 12:10 am | S15 – Brett Tyler, Virginia Tech
<i>“Functional Genomics of the Soybean-Phytophthora Interaction”</i> |
| 12:10 – 12:45 pm | S16 – Daniel Schachtman, Danforth Center
<i>“Mineral Nutrient Signaling in Roots - From Microarrays to Function”</i> |
| 12:45 – 1:45 pm | Lunch – NF Cafeteria (fried/blackened catfish) |
| 1:45 – 2:20 pm | S17 –Richard A. Dixon, The Noble Foundation
<i>“Gene Annotation in Secondary Metabolism: A Perspective from Integrated Transcriptomics, Metabolomics and Protein Structural Analysis in Medicago truncatula”</i> |
| 2:20 – 2:55 pm | S18 – Lloyd W. Sumner, The Noble Foundation
<i>“Integrated Functional Genomic Studies of Medicago truncatula reveal new knowledge in the form of gene validations, gene discoveries, mechanistic insight, and hypothesis building”</i> |
| 3:00-3:30 pm | Break |
| 3:30 – 4:30 | FUTURE DIRECTIONS Open Discussion: |

- 4:30 – 5:15 pm Tour of Noble Foundation Campus/Facilities
(Shuttles run 5-6 pm from Kruse Auditorium to Conference Center)
- 5:30-6:30 pm Posters – Conference Center
- 6:30-7:30 pm Conference Dinner - (Conference Center)
- 7:30-9:00 pm Posters & Social – Billiards tournament (Satish Nagaraj coordinator)

Billiards Doubles Champions
Zhentian Lei & Corey Broeckling



[Friday, November 18](#)

FUNCTIONAL GENOMICS DEMONSTRATIONS

- 7:00-8:30 am Breakfast at Conference Center
(Shuttles run 8-9 am from Conference Center to Kruse Auditorium)
- 9:00 am Assemble in Kruse Auditorium
- 9:00-10:30 am **Workshop – Topic 1 – Metabolomics**
Lloyd W. Sumner – Overview
Ewa Urbanczyk-Corey Broeckling GC/MS
David Huhman (LC/MS)
Corey Broeckling (MET-IDEA)
Ewa Urbanczyk (GeneSpring)
- 10:30-11:00 am Break
- 11:00-12:30 pm **Workshop – Topic 2 – Proteomics**
Zhentian Lei – Overview
Bonnie Watson – 2DE
Zhentian Lei – MALDI & LC/MS/MS QTof
Satish Nagaraj - comparative data processing

12:30-1:30 pm	Lunch – NF Cafeteria (Taco Salad)
1:30-2:45 pm	Workshop – Topic 3 – Microarrays Yuhong Tang, Marina Naoumkina Experimental procedure and chemistry involved in microarray Experimental design Data collection and inspection Data Mining: Data presentation: Challenges Demo
2:45-3:15 pm	Break
3:15-5:00 pm	Workshop - Topic 4 – Informatics and Data Integration Pedro Mendes, Saroj Mohapatra, and Bharat Menrotra DOME - Overview of the functionality for project management, metadata support, data entry, and hands-on demonstration of the query and analysis capabilities of the system.
(Shuttles run 4:30 -5:30 pm from Kruse Auditorium to Conference Center)	
5:00-6:00 pm	Posters – Conference Center (please remove posters after 6pm)
6:00-9:00 pm	Video night in Kruse Auditorium (Movie - To Be Named with Soda/Beer/Pizza)

Saturday, November 19

7:00-12:00 am	Departure – Conference Center
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Abstracts

Oral Presentation

Sequencing the Gene-Rich Euchromatin of the Model Legume, *Medicago truncatula*

Bruce A. Roe¹, Nevin D. Young², Christopher D. Town³, and other members of our laboratories.

¹ Advanced Center for Genome Technology (ACGT), Stephenson Research & Technology Center, Norman, OK 73019 USA

² Dept of Plant Pathology, University of Minnesota, 495 Borlaug Hall, St Paul, MN 55108 USA

³ The Institute for Genomic Research (TIGR), 9712 Medical Center Drive, Rockville, MD 20850 USA

Medicago truncatula is a model for legume genomics because of its compact genome, simple genetics, short generation time, reasonable transformation, and well-characterized symbiotic relationships with rhizobia and mycorrhizae. The *M. truncatula* genome is highly syntenic with cool season legumes, like alfalfa, and moderately syntenic with soybean. We are collaborating with scientists in the European Union to sequence the gene-space of *M. truncatula*, with completion of 200 Mbp scheduled for December 2006. Cytogenetic evidence and extensive sequence data demonstrate that the *Medicago* genome is organized into distinct gene-rich euchromatin separate from pericentromeric heterochromatin, so most of the gene-space can be sequenced in a highly efficient manner. Our BAC-by-BAC sequencing effort will eventually result in pseudomolecules representing the 16 euchromatic chromosome arms of *M. truncatula*. A centralized website (www.medicago.org/genome) acts as a portal for the sequencing project with additional informatics found at the websites of the participating sequencing centers. As of October 2005, ~156 Mbp of non-redundant sequence was complete or in progress, with ~133 Mbp anchored to the genetic map, mostly by SSR markers. The International Medicago Genome Annotation Group (IMGAG; www.medicago.org/genome/IMGAG) is coordinating annotation and nomenclature, leading to community-wide standards and stable gene models. By comparing completed genome sequence with either ESTs or a set of low copy orthologs culled from the *Arabidopsis*, poplar, and rice genomes, we project that 250 to 300 Mbp will eventually be required to capture >95% of all *Medicago* genes.

Keywords: Euchromatin; Gene-space; Genome Sequencing; Legumes; *Medicago truncatula*; Model organisms

Functional Genomics of Nodule Transport and Metabolism in Lotus and Medicago

Michael Udvardi¹, Thomas Ott¹, Ombretta Montinari¹, Vera Voroshilova¹, Catrin Günter¹, Diana Sahid¹, Lene Krusell¹, Katja Krause¹, Guilhem Desbrosses¹, Gillian Colebatch¹, Joachim Kopka¹, Klementina Kakar¹, Armin Schlereth¹, Maren Wandrey¹, Tomasz Czechowski¹, Mark Stitt¹, Wolf-Rüdiger Scheible¹, Martin Crespi², Helge Kuester³, Richard Thompson⁴, Greg May⁵, Yongli Xiao⁶, Hank Wu⁶, Foo Cheung⁶, and Chris Town⁶

¹Max-Planck Institute of Molecular Plant Physiology, Germany;

²Institut des Sciences Végétales, CNRS, France;

³Center for Biotechnology, Bielefeld University, Germany;

⁴INRA Unité de Recherches en Génétique, Dijon, France;

⁵Noble Foundation, USA;

⁶The Institute for Genome Research, USA.

Legume root nodules contribute about 50 million tonnes of fixed-N to agriculture annually, via a symbiosis between plant and bacterial (rhizobia) cells. The intracellular symbiosis is stabilized by a plant membrane called the symbiosome membrane (SM) that surrounds one or more nitrogen-fixing bacteroids, forming an organelle called the symbiosome. The SM is strategically placed to control nutrient exchange between the plant and its microsymbionts, including dicarboxylate transport from the plant for ammonium and amino acids from the bacteroids. Development of a nitrogen-fixing root nodule involves massive changes in gene expression in both the plant and bacterial cells, which have been revealed by transcriptomics in our group and others. We have also used metabolomics to study the effects of these gene expression changes on nodule metabolism in *Lotus japonicus*. Integration of 'OMICS' approaches with forward and reverse genetics, has enabled us to define the physiological roles of several proteins that are important for nodule function, including symbiotic hemoglobins and the sulfate transporter, SST1. More recently, we have begun to produce gene-specific primers for all putative transcription factor genes in *Medicago truncatula* and *Lotus japonicus*, for qRT-PCR studies designed to identify key regulators of organ development and differentiation in these model legumes. Following my move to the Noble Foundation in 2006, I (Michael) hope to establish new collaborations with US and other groups in the areas of transport, metabolism, and genetic regulation of processes in Medicago nodules, seeds, and other organs.

Microarray Technologies for Gene Discovery and Understanding Metabolism in *Medicago truncatula* Cell Suspension Cultures Responding to Yeast Elicitor or Methyl jasmonate

Marina Naoumkina¹, Mohamed A. Farag¹, Corey Broeckling², David Huhman¹, Tang Yuhong¹, Lloyd W. Sumner¹, and Richard A. Dixon¹.

¹Plant Biology Division, Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401

²Dept. Horticulture and Landscape Architecture, Shepardson Building, Colorado State University, Fort Collins, CO 80523 -1173

DNA microarray technology is a powerful technique that was recently developed in order to analyze thousands of genes in a short time. As part of the NSF project “An Integrated Approach to Functional Genomics and Bioinformatics in a Model Legume” we performed transcript and metabolite analysis of *Medicago truncatula* cell suspension cultures elicited by yeast or methyl jasmonate.

As a legume, *M. truncatula* establishes symbiotic relationships with nitrogen fixing Rhizobia and arbuscular mycorrhizal fungi. Such complexes of interactions have resulted in the evolution of a rich variety of natural products. Particularly important are the isoflavonoids, with anti-microbial activity for plants and anticancer and other health-promoting effects for humans. This pathway has been well characterized in alfalfa, and in other legumes such as soybean and chickpea (Dixon 1999). Nevertheless, some areas of isoflavonoid metabolism, such as dehydration, glycosylation and hydroxylation reactions have yet to be elucidated at the molecular level (Dixon 1999).

Two different microarray techniques were applied for this research. Medicago 16K oligo array was used for a detailed kinetic analysis of 21 time points (yeast elicitor) and 11 time points (methyl jasmonate). Two critical time points (2 and 24 hours for both treatments) were re-analyzed with a 60K affymetrix array. The 16K oligo array detected a relatively small number of differentially expressed genes, but gave us general overview of expression kinetics and ideas as to which pathways were activated. The more sensitive Affy array technique detected a large amount of differentially-expressed genes and gave us a detailed view of the pathways activated in response to each treatment.

We have combined these microarray data with metabolite analysis to draw hypotheses on the functions of uncharacterized genes in isoflavonoid metabolism. Such an approach can be a powerful tool for gene discovery and prediction of function.

Proteome Analysis of *Medicago truncatula* to Study Root Responses During Nodulation

Ulrike Mathesius^{1,3}, Tursun Kerim^{1,2,3}, Susan Mulders², Rob Verbeek^{1,3}, Karsten Oelkers^{1,3}, W. Dietz Bauer⁴, and Barry G. Rolfe^{2,3}

¹School of Biochemistry and Molecular Biology, Australian National University, Canberra, Australia.

²Research School of Biological Sciences, Australian National University, Canberra, Australia.

³Australian Research Council Centre of Excellence for Integrative Legume Research.

⁴Horticulture & Crop Science Department, Ohio State University, Columbus OH, USA

The promise of proteome analysis is to obtain a complete picture of all the proteins present in an organism at a given time, to study changes in protein abundance and post translational modification in response to genetic or external factors. So far, no complete proteome has been studied in any organism because of the technical constraints involved in extraction, separation, identification and reliable differential display of thousands of proteins with individual properties. This talk focuses on the possibilities and difficulties in analyzing the proteome of the model legume *Medicago truncatula*, with the aim of identifying proteins involved in the response of roots to different signals from symbiotic bacteria.

We have used two-dimensional gel electrophoresis to separate approximately 3500 root proteins from *M. truncatula* using both horizontal and vertical gel systems, with horizontal systems generally producing sharper and more reproducible gel patterns. Differential display of protein changes (mainly changes in abundance and changes in phosphorylation) have been done through comparison of silver- or Coomassie-stained gels, and recently through Difference Gel Electrophoresis (DIGE) using CyDyes. DIGE was found to produce more reliable comparisons of gels, a significant time saving for analysis of 2D gels and improved statistical analysis. Protein identification was done by peptide mass fingerprinting using MALDI-TOF mass spectrometry, or by tandem mass spectrometry which generates peptide sequences. Both techniques have yielded reliable identification of the proteins when protein data were searched against the *M. truncatula* EST database. In both bases it was difficult to identify post translational modifications. To detect protein phosphorylation, we have used Western blotting of 2D gels using antibodies against phosphorylated amino acids, as well as a phosphoprotein staining kit, both of which yielded some false positive results. Some examples of differential protein expression in response to bacterial signals and during nodulation will be presented.

Integration of Transcript, Protein and Metabolite Profiling Data Sets Using the BioPathAt Tool

B.M. Lange

Institute of Biological Chemistry and Center for Integrated Biotechnology, Washington State University

Research in the post-genomic era is focusing on studies to attribute functions to genes, their encoded proteins, and to describe the regulatory networks controlling biochemical, protein synthesis and signal transduction pathways. To facilitate the analysis of experiments using post-genomic technologies, new concepts for linking the vast amount of raw data to a biological context have to be developed. Visual representations of pathways help biologists to understand the complex relationships between components of metabolic networks. We have recently introduced the BioPathAt tool, a visual interface that allows the knowledge-based analysis of genome-scale data by integrating biochemical pathway maps with a manually scrutinized gene-function database for the model plant *Arabidopsis thaliana*. Using examples of our own experiments and those conducted with our collaborators, I will highlight the relevance of integrating different genomic data sets for the development of models describing the regulation of biochemical networks.

References

- [1] Lange, B.M. & Ghassemian, M. (2005) Comprehensive post-genomic data analysis approaches integrating biochemical pathway maps. *Phytochemistry* 66: 413-451.

Applications of Capillary Electrophoresis as a Tool in Metabolic Profiling

Joel T. Smith¹, Carson J. Cameron¹, Ron G. Workman¹, Jeff Hill¹, Amanda Beaubien¹, Brad Williams¹, Gary Rhodes¹, Pedro Mendes³, Richard A. Dixon², and Lloyd W. Sumner²

¹Department of Physical Sciences, Southeastern Oklahoma State University, Durant, OK.

²Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK.

³Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA .

A functional genomics data set for control and elicited cell cultures of *Medicago truncatula* is being developed. This data set encompasses expressed sequence information, mRNA, protein, and metabolite identities and relative concentrations. Our role has been to develop high throughput capillary electrophoresis (CE) methodologies to monitor key metabolites, including carbohydrates, inorganic and organic ionic species, and amino acids, in relationship to functional gene expression. The focus of this study was two-fold. Carbohydrates were monitored using laser-induced fluorescence (LIF) detection following derivatization. We found that 2-amino-acridinone to be the most suitable fluorophore to label the reducing carbohydrates because of its spectroscopic and mass spectrometric properties. Our CE-LIF method allows for more than 50 carbohydrates, including sugar phosphates, to be profiled in a 17 min analysis. We identified many of the components by spiking the sample with carbohydrate standards. A novel capillary electrophoresis-mass spectrometry (CE-MS) method was developed in an attempt to confirm the identity of suspected sugars and aid in the identification of the several unknowns. In a second set of analysis, the analysis of amino acids were determined means of CE-MS. Under our optimized conditions, 21 common amino acids could be completely separated in less than 12 minutes without prior derivatization. LODs were in the low- to sub-micromolar range with a 5.9 nL injection volume yielding low femtomolar mass sensitivity. Amino acid determination using CE-MS allows for rapid profiling of underivatized amino acids with minimal chance of interference and reduced sample preparation. In the summer of 2005, we were able to analyze more than 800 cell culture extracts in triplicate with this methodology the CE-MS technology. We applied the CE-LIF and CE-MS methodology to the analysis of free amino acids and carbohydrates in cell culture extracts of control and elicited cell cultures of *Medicago truncatula*. Following data analysis, the processed data is being uploaded to a database at the Virginia Bioinformatics Institute.

Insertional Mutagenesis as Functional Genomics Tool in Diploid Strawberry (*Fragaria vesca*)

Vladimir Shulaev

Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA

Rosaceae constitute the third most economically important plant family in temperate regions with the United States being a leading producer of almonds, apples, plums, peaches, pears, raspberries, sour cherries, sweet cherries and strawberries. Recently an effort has been made to develop a genomics platform for rosaceous crops using several model crop species, including peach, apple and strawberry. To date, insertional mutagenesis has not been utilized in any rosaceous species. Diploid strawberry (*Fragaria vesca*) is an attractive model for developing insertional mutagenesis in Rosaceae due to its small genome size, short reproductive cycle, efficient transformation, and facile vegetative and seed propagation. We have developed a high throughput platform for reverse and forward genetics in *F. vesca* based on insertional mutagenesis, gene identification and phenotype profiling. To facilitate the development of insertional mutagenesis we have developed a new and highly efficient transformation protocol that can be used for systematic production of T-DNA tagged insertional mutants. This protocol was used to produce a set of T-DNA-tagged lines in *F. vesca*. Screening of T-DNA insertional mutant lines for obvious morphological mutants resulted in several putative mutants with altered leaf shapes which were further characterized. Thermal asymmetric interlaced (TAIL)-PCR was used to amplify and sequence genomic regions flanking T-DNA insertions in transformants. The generation of a T-DNA mutant collection of strawberry will impact functional genomics research and gene discovery in Rosaceae and other fruit crops.

An Overview of *Medicago truncatula* Mutagenesis

Kiran Mysore

Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK.

Legumes are second only to grasses in economic importance worldwide and understanding legume molecular genetics is vital to facilitate breeding of important grain and forage legumes. Over the past decade, *Medicago truncatula* has been selected as a model plant to study biological processes that are unique and pertinent to legumes which cannot easily be studied in *Arabidopsis thaliana*. International efforts are underway to sequence the gene-space of all the eight chromosomes of *M. truncatula* by 2007. The currently available functional genomic resources of *M. truncatula* and the resources that are under development will make *M. truncatula* a successful model legume. I will comment on the utility and recent advancements of the most common tools for introducing and analyzing genetic mutations in *M. truncatula*. Since transformation and regeneration are still bottlenecks in a legume species, large-scale insertional mutagenesis poses a major challenge in *M. truncatula*. I will discuss the tobacco retrotransposon, Tnt1, as a viable and attractive option to introduce multiple independent insertions per plant for saturation mutagenesis.

Cellular Function Prediction and Biological Pathway Discovery Through Mining Genome-Scale Data

Dong Xu, Yu Chen, and Trupti Joshi

Digital Biology Laboratory, Computer Science Department and Life Sciences Center, University of Missouri-Columbia, Columbia, MO.

Characterizing protein functions and biological pathways at the genome scale is one of the most important and challenging tasks in the post-genomic era. To address this challenge, we have developed an integrated probabilistic approach, which combines high-throughput data of protein-protein interactions, protein complexes, microarray gene-expression profiles, and genomic sequences. We quantified the relationship between functional similarity in the GO biological process and high-throughput data, and coded the relationship into a “functional linkage graph”, where each node represents one gene and the weight of each edge is characterized by the Bayesian probability of function similarity between the two connected genes. Then we used Boltzmann machine and simulated annealing to perform optimization for assigning gene functions based on the global information of the functional linkage graph. We also integrated the evolution and protein subcellular localization information into the prediction. We have implemented our method into the package GeneFAS. We used GeneFAS to systematically assign 1802 out of 2280 unannotated proteins in yeast. We have also assigned function for 4451 out of the 19,717 unannotated proteins in Arabidopsis using microarray gene expression data and predicted protein interactions.

In addition, we used a functional linkage graph for functional module identification and pathway inference. A functional module, defined as a substructure of a network involving a group of genes related by interaction or regulation, is identified through clustering a functional linkage graph using the Markov Cluster Algorithm. 86 modules were identified in the yeast, each having homogenous function among component genes. For building a pathway model, the path between two proteins (e.g., the sensor of a signal and the corresponding transcriptional factor) is constructed using the Dijkstra’s algorithm. We have constructed global pathway models for the yeast amino acid transport pathway and the signaling pathway of lipid as a second messenger in Arabidopsis.

A Virtual Plant Information Network

Gregory D. May¹, Susan Baxter² and Damian Gessler²

¹Plant Biology Division, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401 USA; ²National Center for Genome Resources, 2953 Rodeo Park Drive East, Santa Fe, NM 87505 USA

Today's biological research involves translation of genetic, genomic, transcript, proteomic, metabolic, and phenotypic information. To do this, plant biologists who focus on crop species typically rely on integrative and comparative analyses using model and reference species. This information is accessible to plant biologists through dozens of autonomous web-enabled plant information resources. While the proliferation of distributed, autonomous and often ephemeral information resources is a logical outcome to the distributed nature of the publicly supported plant biological research enterprise, it is also a source of logistical frustration to biologists. Biologists have to search multiple, often unlinked web sites to find data, information, and analysis tools to support their research. A Virtual Plant Information Network (VPIN) based on emerging middleware technology platforms such as BioMOBY will significantly empower plant biological research by providing powerful bioinformatics capabilities for integrative and comparative methods. We propose to develop a Virtual Plant Information Network (VPIN) consisting of data and analysis tools from TAIR (www.arabidopsis.org), DragonDB (antirrhinum.net), LIS (www.comparative-legumes.org), Gramene (www.gramene.org), TIGR's Genome Annotation (www.tigr.org/tdb/e2k1/mta1) and Medicago Gene Indices (www.tigr.org/tigrscripts/tgi/T_index.cgi?species=Medicago), and the CGIAR's Generation Challenge Program via IRIS (www.iris.irri.org) and IWIS (www.cimmyt.org/research/wheat/iwisfol/IWISFOL.htm).

DOME, a Database and Analysis System for Systems Biology Experiments

Pedro Mendes and Bharat Mehrotra

Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA

The convergence of development of multi-parallel experimental technologies, informatics, and theoretical and computational biology has made feasible the study of phenomena at the level of entire cells or tissues.

Experimentally, these systems biology approaches are characterized by an attempt to measure extensive intracellular molecular profiles of the cells of interest after appropriate perturbations, usually in a time course. Each of these experiments generates hundreds to thousands of data files, amounting to several hundred gigabytes and become a serious problem to manage and query. Here will present DOME, a system that we have built to manage and analyze experiments of this type. DOME supports data from RNA, protein and metabolite profiling, and its main objective is to integrate these different data types and allow for cross-queries that can start to uncover the relationships between these levels of biological organization.

We will describe the analyses enabled by DOME, and directions for its future development and improvement. Examples will be given with data from our NSF-funded *Medicago truncatula* project.

An Integrated Approach to the Functional Genomics of Fiber and Wood Development in Poplar and Arabidopsis

Carl J. Douglas¹, Michael Friedmann¹, Eryang Li¹, Margaret Ellis¹, Lee A. Johnson¹, Bjoern Hamberger¹, David M. Johnston¹, Dustin Lippert², Juergen Ehling³, Steve Ralph², Erin Gilchrist¹, Collin Kelleher², Jun Zhuang², Rick White, Natalie Forneris⁴, Rod Savidg⁴, Kermit Ritland⁵, Joerg Bohlmann² and Brian E. Ellis²

¹Department of Botany, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

²Micheal Smith Laboratories, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

³Institute for Plant Molecular Biology, Centre National de la Recherche Scientifique, 67000 Strasbourg, France

⁴Faculty of Forestry and Environmental Management, University of New Brunswick, Fredericton, NB E3B 6C2

⁵Department of Forest Science, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

In a Genome Canada-funded project, we have used a suite of genomic tools in Arabidopsis, poplar and spruce to study biological questions related to wood development and forest health. This talk will focus on the combined use of genomics and proteomics approaches in poplar and Arabidopsis to identify metabolic and regulatory genes involved in secondary wall formation, fiber and secondary xylem (wood) development, and cell expansion. In Arabidopsis, experiments involved the use of a full genome longmer microarray for expression profiling and the use of both 2-D gel and ITRAQ proteomics approaches to study global changes in protein abundance. In parallel poplar work, we generated over 100,000 poplar EST sequences, manufactured a 15.4K poplar cDNA microarray, and participated in the sequencing of the poplar genome. We used these combined poplar genomics resources, and comparative genomics approaches between poplar and Arabidopsis, in experiments and bioinformatics to identify candidate genes involved in secondary wall formation and fiber/xylem differentiation in poplar. I will also discuss approaches such as activation tagging, transgene over-expression/RNAi knockdown, and high throughput SNP discovery and genotyping that have been added to the poplar functional genomics repertoire. When integrated with other genomic approaches, these functional tools have the potential to generate new insights into the genes that control key traits in forest trees and to probe the allelic diversity that underlies variation in these traits in wild tree populations.

Integration of Omics Towards Phytochemical Genomics

Kazuki Saito

Graduate School of Pharmaceutical Sciences, Chiba University, CREST of JST, Chiba; RIKEN Plant Science Center, Yokohama, JAPAN

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 (*Curr. Opin. Biotechnol.*, **16**, 174-179 (2005)). To identify the function of particular genes and to find new molecular networks in *Arabidopsis thaliana*, we investigated global gene expression profile by DNA microarray and metabolite profile by combination of different mass spectrometric technologies including LC-MS, GC-MS and FT-MS.

Nutritional stress of nitrogen and sulfur resulted in global change of metabolome that could be correlated with the modulation of gene expression, indicating the presence of several gene-to-metabolite networks. In particular, glucosinolate biosynthesis was notably modified by these stresses, and thus the genes showing similar pattern of expression were identified as the candidates involved in glucosinolate biosynthesis (*Proc. Natl. Acad. Sci. USA*, **101**, 10205-10210 (2004); *J. Biol. Chem.*, **280**, 25590-25595 (2005)). The metabolite profiles of *pap1-D* mutant and *pap1* cDNA transgenic lines over-expressing a *Myb* gene in *A. thaliana* indicated that anthocyanin content increased specifically. The comprehensive gene expression and metabolite profiles of these lines indicated the function of novel genes that are responsible for modification and storage of anthocyanins. Some of those candidate genes were functionally identified by analysis of the T-DNA insertion lines and recombinant proteins (*Plant J.*, **42**, 218-235 (2005)). In addition, co-expression analysis of genes accumulated in a transcriptome database could provide the clues for functional identification of unknown genes, in particular, when combining metabolome analysis.

This strategy of integrated analysis of metabolome and transcriptome will be applicable not only for a model plant *A. thaliana*. It can be also applied to phytochemical genomics of any exotic plants, for example, camptothecin biosynthesis in *Ophiorrhiza pumila* root cultures (*Plant Physiol.*, **134**, 161-170 (2004)). Recent our efforts to identify genes, intermediates and pathways will be presented.

Transcriptional Profiling and RNAi to Dissect the Molecular Basis of the AM Symbiosis in *Medicago truncatula*

Maria J. Harrison¹, Steve Gantt², Jeon Hong¹, Sergey Ivashuta², Janelle Jung¹, Jinyuan Liu¹, Melina Lopez-Meyer¹, Ignacio Maldonado-Mendoza¹ and Colby Starker²

¹Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, New York, 14853, USA,

²Department of Plant Biology, University of Minnesota, Saint Paul, MN 55108, USA.

Plants live in symbiosis with a broad array of microorganisms but perhaps the most widespread mutualistic interaction is the arbuscular mycorrhizal symbiosis, formed between arbuscular mycorrhizal (AM) fungi and vascular flowering plants. These associations develop in the roots where the fungus colonizes the cortex to obtain carbon provided by the plant. In addition to growth within the root cells, the fungus establishes a network of hyphae in the surrounding soil, via which it assists the plant with the acquisition of mineral nutrients, particularly phosphorus. Fossil evidence indicates that plants have been associated with AM fungi since they first colonized land and today, AM symbioses are formed by almost all vascular flowering plant species and exist in ecosystems throughout the world. The symbiosis is a stable partnership and development requires the coordinate differentiation of both symbionts to create specialized interfaces over which nutrients are exchanged. We are using a model legume, *Medicago truncatula* and AM fungus, *Glomus versiforme*, to study the molecular events that underlie development and nutrient exchange in the AM symbiosis. Our current approaches include the use of transcriptional profiling to identify mycorrhiza-regulated genes and RNAi to down-regulate their expression. By integrating transcriptional profiling information with reverse genetics approaches we have been able to identify a number of plant genes whose expression is important for development of the AM symbiosis. Recent progress in these areas will be discussed.

Functional Genomics of the Soybean-Phytophthora Interaction

Brett M. Tyler¹, Lecong Zhou¹, Santiago X. Mideros², Trudy-Torto-Alalibo¹, Dianjing Guo¹, Sucheta Tripathy¹, Yongcai Mao¹, Hua Li¹, Stefano Costanzo², Steven K. St. Martin², M. A. Saghai Maroof¹, Pedro Mendes¹, Ina Hoeschele¹, and Anne E. Dorrance²

¹Virginia Polytechnic and State University

²Ohio State University

Phytophthora sojae is a eukaryotic pathogen of soybean. It is an oomycete, a fungus-like organism in the kingdom Stramenopiles. *P. sojae* kills soybean seedlings and damages roots of older plants, causing \$1-2b in losses to the worldwide soybean crop each year. Genetic resistance in soybean against *P. sojae* is provided by single major resistance genes and by quantitative trait loci. We have used Affymetrix GeneChips carrying probes for 15,800 *P. sojae* and 38,400 soybean genes to carry out transcriptional profiling of both host and pathogen during infection of eight soybean cultivars with varying levels of quantitative resistance. The levels of resistance varied from poor (OX20-8 and Sloan), to moderate (Williams, PI291327) to high (Conrad, General, V71-370 and Athow). Pathogen gene expression levels were analyzed after estimating the level of total pathogen mRNA in the infection samples by reference to pathogen RNA spiked into the soybean RNA from mock-inoculated samples. The results show that more than 70% of detectable soybean and *P. sojae* mRNAs show statistically significant changes in response to infection, indicating that both organisms undergo extensive adjustments in their metabolism during their battle for supremacy. We are exploring a variety of mathematical and statistical modeling approaches to build an understanding of how large numbers of potential pathogenicity genes contribute jointly to the outcome of a host infection at a “many-genes-to-many-genes” level.

Mineral Nutrient Signaling in Roots - From Microarrays to Function

Daniel P. Schachtman, Ryoung Shin and R. Howard Berg

Donald Danforth Plant Science Center, 975 N. Warson Rd., St. Louis, MO 63132

Plants adapt to changes in soil mineral concentrations to ensure an adequate supply of essential nutrients, but it is not well understood how plant root cells sense or signal the changes that occur upon the onset of mineral deficiency. To understand root response to K⁺ deficiency, we conducted microarray analyses using *Arabidopsis* roots (1) and found that many genes involved in reactive oxygen species (ROS) metabolism were induced. We also showed that H₂O₂ levels increased after K⁺ deprivation *in planta* and ROS accumulated in a discrete region of roots. Suppression of an NADPH oxidase (*rhd2* mutant), which is involved in ROS production, prevented the up-regulation of several genes that are normally induced by K⁺ deficiency; however, the induction of high affinity K⁺ transport activity was unchanged. Biochemical and physiological studies verified that responses to K⁺ deficiency in *Arabidopsis* roots are mediated by ROS.

To determine the role of ROS in plant response to nitrogen and phosphorus deficiency, studies were conducted using wild type *Arabidopsis* and several root hair mutants (2). The expression of several nutrient responsive genes was determined by northern blot and ROS were quantified and localized in roots. The genes that were monitored varied in intensity and timing of expression, depending on which nutrient was deficient. In response to nutrient deprivation ROS concentrations increased in specific regions of the *Arabidopsis* root. Changes in ROS localization in wild type *Arabidopsis* and in a set of root hair mutants, suggest that the root hair cells are important for response to nitrogen and potassium. In contrast, the response to phosphorus deprivation occurs in the cortex where an increase in ROS may be involved in signaling and also in altering lateral root growth that emerge from the pericycle.

Specific candidate genes were chosen from the results of the microarray study. Overexpression lines were created and knockout lines were isolated. The phenotypes of these lines were characterized. Plants overexpressing one gene encoding a Myb transcription factor and one gene overexpressing an SNF kinase showed interesting phenotypes. The functional characterization of the proteins encoded by these genes will be described.

1. Shin, R. & Schachtman, D. P. (2004) Hydrogen peroxide mediates plant root response to nutrient deprivation *Proc Natl Acad Sci U S A* **101**, 8827-8832.
2. Shin, R., Berg, R. H. & Schachtman, D. P. (2005) Reactive oxygen species and root hairs in *Arabidopsis* root response to nitrogen, phosphorus and potassium deficiency *Plant Cell Physiol* **46**, 1350-7.

Gene Annotation in Secondary Metabolism: A Perspective from Integrated Transcriptomics, Metabolomics and Protein Structural Analysis in *Medicago truncatula*.

Richard A. Dixon, Marina Naoumkina, Lahoucine Achnine, Jack Blount, Bettina Deavours, Mohamed A. Farag, Xian-Zhi He, David Huhman, Hui Shao, Lloyd W. Sumner, Yuhong Tang, and Xiaoqiang Wang

Plant Biology Division, Samuel Roberts Noble Foundation, Ardmore, OK.

Functional genomics has the goal of discovering the biochemical functions of all the genes within an organism. In plants, a significant number of genes are involved in modification of secondary metabolites. Problems associated with promiscuity in relation to in vitro substrate specificity, or genetic redundancy, may complicate attempts to assign functions to the many hundreds of genes of secondary metabolism accessible to researchers through various genome and EST sequencing projects.

We discuss the above problems in relation to our attempts to integrate transcriptome and metabolome data in *Medicago truncatula* cell suspension cultures undergoing genetic reprogramming for secondary metabolism. In many cases, neither sequence identity nor in vitro biochemical functional assays are sufficient to assign a true in vivo function to genes annotated as being involved in specific biochemical pathways. We argue that true functional annotation may require combined approaches involving expression analysis, tissue-selective metabolite profiling, and structure determination/prediction in addition to the standard functional genomics tools of sequence comparisons, analysis of recombinant protein activities and genetic dis-regulation. Specific examples to be discussed include secondary metabolite glycosyl- and *O*-methyl-transferases.

Integrated Functional Genomic Studies of *Medicago truncatula* Reveal New Knowledge in the Form of Gene Validations, Gene Discoveries, Mechanistic Insight, and Hypothesis Building

Corey D. Broeckling,^{1,2} Mohamed A. Farag,^{1,3} David V. Huhman,¹ Ewa Urbanczyk-Wochniak,¹ Zhentian Lei,¹ Bonnie S. Watson,¹ Satish Nagaraj,¹ Marina Naoumkina,¹ Bettina Deavours,¹ Gregory D. May,¹ Pedro Mendes,² Joel T. Smith,³ Richard A. Dixon,¹ and Lloyd W. Sumner¹

¹Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK.

²Current address: Colorado State University, Fort Collins, CO.

³Current address: University of Kentucky, Lexington, KY.

⁴Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA .

⁵Department of Physical Sciences, Southeastern Oklahoma State University, Durant, OK.

An integrated functional genomics approach to study the relationships between gene expression, protein levels and metabolites in the model legume *Medicago truncatula*, following biotic and abiotic elicitation will be described. *M. truncatula* suspension cell cultures were separately treated with methyl jasmonate, yeast cell wall extract, or UV light. Samples were collected at 21 time points following each elicitation and analyzed at the metabolite, protein, and mRNA levels. Significant changes were observed in both primary and secondary metabolism. Multiple examples of data that is yielding new knowledge will be provided in the form of gene validation, gene discovery, mechanistic insight, and hypothesis building. Yeast elicitation induced known accumulation in the phytoalexin medicarpin; thereby validating the experimental methods and liquid cell cultures. More interestingly, yeast also induced multiple and previously unreported phenolics. The integrated data were further queried to yield putative novel genes. These candidates were expressed in *E. Coli* and enzymatic assays performed to validate new gene discoveries. Methyl jasmonate induced several amino acids and correlation analyses reveal dramatic changes in serine, glycine, and threonine biosynthesis. The data provide experimental evidence for a threonine aldolase previously unreported in plants. However, queries of the *M. truncatula* ESTs reveal a highly similar threonine aldolase to that in yeast and *E. Coli*, thus the data validates the presence of this enzyme in plants. Methyl jasmonate has not been traditionally associated with the induction of the phenylpropanoid pathway; however medicarpin accumulated following MeJa elicitation and the integrated data suggest an alternate mechanism in response to this stress. A large number of other primary metabolites were also induced in response to MeJa and suggest a major reprogramming of metabolism. The cumulative responses were used to suggest a model or hypothesis for the mechanism of carbon repartitioning from primary metabolism to secondary metabolism.

ABSTRACTS

Poster Presentations

High-throughput Functional Genomic Approach by Integration of Transcriptome Coexpression Analysis with Targeted Metabolite Profiling

Takayuki Tohge,¹ Keiko Yonekura-Sakakibara,¹ Takeshi Obayashi,^{2,3} and Kazuki Saito^{1,2,3}

¹RIKEN Plant Science Center, Yokohama, Japan

²Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan

³CREST, JST (Japan Science and Technology Agency), Chiba, Japan

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. However, 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 database is constructed from Affymetrix GeneChip data of AtGenExpress, which is multinational consortium for Arabidopsis transcriptomics. The coexpression gene search is now possible in a web site released by ATTED (*Arabidopsis thaliana* trans-factor and cis-element prediction database). We inferred a coexpression framework model of phenylpropanoid 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 metabolite-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.

A Strategy for Finding Silent Phenotypes in *Arabidopsis* Using FOX Hunting System

Miyako Kusano,¹ Takanari Ichikawa,² Miki Nakazawa,² Motoaki Seki,² Minami Matsui,² Kazuo Shinozaki,¹ Par Jonsson,³ Thomas Moritz,³ Hideyuki Suzuki,⁴ Daisuke Shibata,⁴ and Kazuki Saito^{1,5,6}

¹RIKEN Plant Science Center, Kanagawa, Japan; ²RIKEN Genome Science Center, Kanagawa, Japan; ³UPSC, Dept. of Forest Genetics and Plant Physiology, SLU, Umeå, Sweden; ⁴Kazusa DNA Research Institute, Chiba, Japan; ⁵Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan; ⁶CREST, JST (Japan Science and Technology Agency), Chiba, Japan

Silent phenotypes are genetically modified organisms that do not show apparent changes in morphology, yield, or growth rates 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. For investigating such global changes in metabolic conditions, we introduced metabolomics concept using “FOX (Full-length cDNA Over-expressor gene) hunting system” of model plants, *Arabidopsis thaliana*. The system has been developed by RIKEN GSC plant functional team⁽¹⁾ and each transgenic line is over-expressing full-length cDNA constitutively throughout the genome. Therefore, screening against FOX lines will be certainly resulted in the genomic-wide gene hunting. By using the system we are trying to make a rapid and useful method to find the “silent phenotypes” by GC-TOF/MS.

Five seeds from every FOX hunting lines were grown on the MS agar medium containing 20mg/l of hygromycin as the 1st screening. The aerial part of each 25 days-old FOX line was harvested. Samples were extracted and analysed by GC-TOF/MS, and thereafter the non-processed GC-MS files were subjected to multivariate analysis^(2, 3). The PLS-DA (partial least squares projection-discriminate analysis) results indicated that several over-expressing lines had metabolic differences compared with those of other lines. The compounds which made differences contained both primary and secondary metabolites. The selected lines were grown with wild-type (col-0), and then analysed using the same method. When multivariate analysis was applied to the non-processed GC/MS files, the PLS-DA score separated the groups of WT clearly from the lines. Then, candidate metabolites in each line that underwent significant change were selected by 99% confidence. Lastly, three lines could be selected as silent phenotypes by using this screening method. In the poster, we will present the method of sample cultivation, extraction and the result of multivariate analysis using chemometrics.

(1) Seki M, *et al.*, *Science*, 296, 141-145, 2002.

(2) Jonsson P., *et al.*, *Anal. Chem.*, 76, 1738-1745, 2004.

(3) Jonsson P., *et al.*, *Anal. Chem.*, 77, 5635-42, 2005.

Proteomics of Cassava Roots: Protein Identification and Differential Expression

Jeanne Speichinger¹, Nigel Taylor², Claude Fauquet² and Sixue Chen¹

¹Proteomics and Mass Spectrometry Facility, ²International Laboratory for Tropical Agricultural Biotechnology, Donald Danforth Plant Science Center, 975 N. Warson Road, St. Louis, MO 63132

Cassava supplies a major source of dietary energy for more than 700 million people, mostly in the developing countries. This tropical and subtropical crop is cultivated mainly for its edible storage roots, which are a source for a variety of food stuffs, animal feed and industrial products. To date no systematic studies have been undertaken to identify the proteins present in the cassava roots. Our research is to find traits of biological, nutritional and agronomic importance using proteomics tools. Using high-resolution two dimensional gel electrophoresis, we resolved proteins extracted from fibrous and tuberous root tissues of three month old cassava plants. Gel image analysis revealed an average of 1467 electrophoretically resolved spots on the fibrous gels and 1595 spots on the tuberous gels. Protein spots from both sets of gels were digested with trypsin. The digests were subjected to rapid protein identification using nanoelectrospray quadrupole time-of-flight (QTOF) tandem mass analysis. Currently, we have obtained 299 protein identifications for 292 gel spots corresponding to 237 proteins. The proteins span various functional categories from energy, primary and secondary metabolism, disease and defense, destination and storage, transport, signal transduction, protein synthesis, cell structure and transcription to cell growth and division. Gel image analysis has shown unique, as well as up- and down-regulated proteins, present in the tuberous and the fibrous tissues. Quantitative and qualitative analysis of the cassava root proteome identifies targets for further functional characterization. The biological significance and application implications in cassava biotechnology will be discussed.

Proteomics of Maize Root to Shoot Signaling and Root Growth Under Drought

Daniel P. Schachtman¹, Sophie Alvarez¹, Jinming Zhu², Ellen Marsh¹, Sixue Chen¹, Yajun Wu³, Robert E. Sharp²

¹Donald Danforth Plant Science Center, St. Louis, MO; ²Division of Plant Sciences, Univ. Missouri, Columbia, MO; ³Dept. Plants, Soils and Biometeorology, Utah State Univ., Logan, UT

Under conditions of drought, roots are able to sense soil drying and send chemical signals via the xylem to shoots to regulate water loss and growth. To identify protein based changes in xylem sap after drought stress, we created a map of the maize xylem sap proteome. The most abundant proteins in the xylem sap were characterized using two-dimensional electrophoresis followed by tandem spectrometry. One hundred fifteen gel spots out of 174 analyzed were identified and appear to be mainly involved in the xylem differentiation. These include proteins involved in cell wall metabolism, lignification and cell death. In addition we found plant defense proteins in sap and showed that the xylem sap had antifungal activity. Proteins in the xylem sap could be present as a result of xylem differentiation in roots or may be actively secreted and then transported to function in shoots. .

Kinematic studies have revealed different responses of cell elongation to water deficit in distinct regions of the growth zone of the maize primary root. In the apical 3 mm, elongation is maintained at well-watered rates under severe water deficit; the 3-7 mm region exhibits maximum elongation in well-watered roots but progressive inhibition of elongation in roots under water deficit. Previous work indicated that cell wall proteins (CWP) may play important roles in maintaining cell elongation in the apical region despite reduced turgor pressure. We used a proteomics approach to gain a comprehensive understanding of how CWP abundance changes in association with water deficit in the different root regions. We examined water soluble and loosely ionically-bound CWP using 2-D gels and mass spectrometry. The results of this work will be described.

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Integrated Proteomics Approach for Analysis of *Brassica napus* Seed Filling – High Protein Identification Rate with Limited Database Resources

Martin Hajduch¹, Jill E. Casteel¹, Katherine E. Hurrelmeyer¹, Zhao Song², Ganesh K. Agrawal¹ and Jay J. Thelen¹

¹Department of Biochemistry, University of Missouri, Columbia, MO

²Computer Science Department, University of Missouri, Columbia, MO

Proteomics is a tool of post-genomics area and is still under rapid development. The success of any proteomics analysis is much influenced by availability of technological and bioinformatics resources. In many organisms, especially plants, availability of comprehensive database is still limited. Therefore creative thought might help to increase identification frequency. Here we present approach for relatively high identification efficiency for *B.napus*. This plant might seem to be unsuitable for proteomics studies due to limited database resources. The National Center for Biotechnology Information (NCBI) non-redundant protein database contains only 2207 entries for *Brassica* species, as in March 2005. Most comprehensive public accessible database for *B.napus* is available at The Institute for Genomic Research (TIGR) and contains 5,568 tentative consensus (TC) entries. To overcome problem we decided to maximize use of available resources. Presented approach is based on simultaneous use of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) and liquid chromatography tandem mass spectrometry (LC-MS/MS). Furthermore, obtained MS data were searched using two different search engines (Protein Prospector and BioWorks 3.1) against two different databases (NCBI and TIGR). After application of threshold criteria during two steps data consolidation, this integrated approach finally resulted into 65.1% protein identification efficiency. Presented results confirmed, that LC-MS/MS is superior over MALDI-TOF. However MALDI-TOF is more successful in some cases. Additionally, level of agreement between these two MS methods will be presented.

Monitoring Carbohydrate Metabolism in *Medicago truncatula* using Capillary Electrophoresis

Jeff Hill¹, Amanda Beaubien¹, Brad Williams¹, Gary Rhodes¹, Carson J. Cameron¹, Pedro Mendes³, Richard A. Dixon², Lloyd W. Sumner², and Joel T. Smith¹

¹Department of Physical Sciences, Southeastern Oklahoma State University, Durant, OK.

²Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK.

³Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA .

A functional genomics data set for control and elicited cell cultures of the model legume *Medicago truncatula* are being generated by a collaborative effort. This global data set is being used for an integrated approach to functional genomics for this model legume. Our role has been to develop high throughput capillary electrophoresis (CE) methodologies to monitor key metabolites, including carbohydrates, inorganic and organic cations and anions, and amino acids, in relationship to functional gene expression. The highly expressed simple carbohydrates and many simple inorganic anions and cations are determined using indirect UV detection with CE. Carbohydrates accumulated at lower concentrations are monitored using laser-induced fluorescence (LIF) detection following derivatization. We found that 2-amino-acridinone to be the most suitable fluorophore to label the reducing carbohydrates because of its spectroscopic and mass spectrometric properties. Our CE-LIF method allows for more than 50 carbohydrates, including sugar phosphates, to be profiled in a 17 min analysis. We identified many of the components by spiking the sample with carbohydrate standards. Our laboratory has performed the extraction, sample preparation, and analysis on more than 800 cell culture samples. Following data analysis, the data is being uploaded to a public domain database at the Virginia Bioinformatics Institute.

Monitoring Amino Acid Metabolism in *Medicago truncatula* Using Capillary Electrophoresis-Mass Spectrometry

Carson J. Cameron¹, Ron G. Workman¹, Pedro Mendes³, Richard A. Dixon², Lloyd W. Sumner², and Joel T. Smith¹

¹Department of Physical Sciences, Southeastern Oklahoma State University, Durant, OK.

²Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK.

³Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA .

A functional genomics data set for control and elicited cell cultures of *Medicago truncatula* is being developed. This data set encompasses expressed sequence information, mRNA, protein, and metabolite identities and relative concentrations. Our role has been to develop high throughput capillary electrophoresis (CE) methodologies to monitor key metabolites, including carbohydrates, inorganic and organic cations and anions, and amino acids, in relationship to functional gene expression. The main focus of this study was the analysis of amino acids by means of CE-MS. Mass spectrometric parameters including sheath flow composition, drying gas temperature and flow rate, and ion optics potential were examined. It was found that mass spectrometric parameters played a critical role in the detection of the amino acids and that these parameters varied among the basic, neutral, and acidic groups of amino acids. Under our optimized parameters, the 20 common amino acids could be completely separated in less than 12 minutes. LODs were in the low- to sub-micromolar range with a 5.9 nL injection volume yielding low femtomolar mass sensitivity. We have applied this methodology to the analysis of free amino acids in cell culture extracts of control and elicited cell cultures of *Medicago truncatula*. Amino acid determination using CE-ESI-MS allows for rapid profiling of underivatized amino acids with minimal chance of interference and reduced sample preparation. In the summer of 2005, we were able to analyze more than 800 cell culture extracts in triplicate with this methodology.

Metabolic Profiling of *Medicago truncatula* Saponins Using Capillary LC/QTOF/MS

David V. Huhman; Zhentian Lei; Lloyd W. Sumner

The Samuel Roberts Noble Foundation, Ardmore, OK

Legumes are important agriculture and commercial crops and *M. truncatula* has been chosen as a model legume species based on its small diploid genome and fast regeneration time. To gain a better understanding of secondary metabolism and biosynthesis in legumes, metabolic profiling of triterpene saponins in *M. truncatula* is underway. Previously, we reported on the metabolic profiling of saponins using an HPLC coupled to an ion trap MS for the identification of over 29 saponins. Currently, a new method has been development utilizing capillary LC coupled to a quadrapole time-of-flight mass spectrometer with improved sensitivity, resolution, and mass accuracy. The new method is being used for comparative profiling of various ecotypes of *M. truncatula*.

Medicago truncatula ecotypes were grown in turface under identical light and fertilization regiments. Six week old roots were harvested, lyophilized, and extracted in 80% methanol. Capillary HPLC was performed using an LC Packings Ultimate HPLC coupled to a Famous autoinjector and Switchos valve. Capillary LC/MS was optimized based on column material and length, flow rate, and acid modifier using a known Soyasapogenol B standard mix.

A capillary LC/MS/MS method for saponin profiling has been established using a 300 μm id x 250 mm length, 5 μm C18 column (LC Packings) operated at 4 $\mu\text{L}/\text{min}$ and in the negative ESI mode. This method yielded an separation efficiency of 97,000 which is approximately twice that previously reported (Huhman and Sumner, 2002, *Phytochemistry*, 59, 347-360). The limit of detection was determined for soyasaponin 1 to be <1pmol. This optimized method was applied to the comparative profiling of saponin content in various ecotypes of *M. truncatula* and the comparative analysis results will be discussed.

Comparative Proteomics of Yeast Elicited *Medicago truncatula* Suspension Cell Cultures

Zhentian Lei¹, Bonnie S. Watson¹, Satish Nagaraj¹, Richard A. Dixon¹, Pedro Mendes² and Lloyd W. Sumner^{1*}

¹Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK.

²Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA .

Legumes are rich in proteins, carbohydrates, and many other natural products including alkaloids, isoflavonoids and saponins. Many of these natural products have documented antimicrobial and anticarcinogenic properties and are commonly induced in response to external stress. To advance our understanding of legume biology, *Medicago truncatula* (common name barrel medic and close relative to alfalfa) suspension cell cultures were subjected to yeast elicitation to mimic a fungal attack and two-dimensional electrophoresis (2-DE) was used to investigate the response of suspension cell cultures at the proteome level. The differentially accumulated proteins will be presented and discussed. Proteomic analyses of *M. truncatula* suspension cell cultures at 0, 12, 24 and 48 hrs following elicitation reveal that multiple proteins were differentially accumulated in response to yeast elicitation. These proteins were associated with a variety of cellular functions and included primary metabolism, energy production, disease/defense responses and natural product biosynthesis. At least 26 proteins were found to be differentially expressed 24 hours after yeast elicitation with 18 of them showing significant increase. A great portion of the up-regulated proteins were related to secondary metabolism such as isoflavonoid biosynthesis. These proteins include vestitone reductase and chalcone-flavonone isomerase. The induction of the isoflavonoid pathway was also confirmed using metabolic profiling analyses that demonstrated a fifteen fold increase in medicarpin, a major isoflavonoid in legume formed through the reduction of vestitone. Up-regulated proteins involved in other secondary metabolism and defense/disease response can be exemplified by 12-oxophytodienoate reductase, quinone oxidoreductase, In2-1 protein and glycosyl hydrolase family 51. Interestingly, it was found that at 0 time point all differentially displayed proteins were associated with protein synthesis. These proteins include eukaryotic protein synthesis initiation factor 5A-2, ribosomal protein, chaperonin CPN60-2, elongation factor and heat shock protein 70. While this may be attributed to the time delay in sampling cells after elicitation, it could also be due to the fact that cell cultures are difficult to synchronize.

Analysis of Lipid Molecular Species Using Electrospray Ionization Tandem Mass Spectrometry

Giorgis Isaac, Alexis Sparks, Richard Jeannotte, Ruth Welti

Division of Biology, Kansas State Lipidomics Research Center, Kansas State University, Manhattan, KS 66503

Lipidomics is the comprehensive profiling of the lipids of a cell or organism. Lipidomics can be utilized to identify lipid alterations correlated with physiological changes or with the function of specific gene products. Conventional analysis methods of lipid molecular species require laborious procedures including separation by column, argenation thin-layer chromatography or LC detection after pre- or post- column derivatization. Mass spectrometry is the leading technology for quantitative lipid profiling with sensitivity and high throughput. Recent developments in mass spectrometry make possible the identification and quantification of lipid molecular species directly from crude lipid extracts. It involves analysis both of lipid classes (with class being defined by a common head group) and of groups of complex lipids defined by acyl species in common. The Kansas Lipidomics Research Center (KLRC) has developed an electrospray ionization tandem mass spectrometry based method to quantitatively profile plant membrane lipid molecular species and some of their metabolites. Here we present the procedures and data processing involved at KLCR during lipid molecular species profiling.

Integration, Visualization and Comprehensive Analysis of Diverse Metabolite and Transcript Datasets.

Ewa Urbanczyk-Wochniak, Xinbin Dai, Patrick Zhao and Lloyd W. Sumner

Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK.

The advancement of multi-parallel ‘omics’ technologies have expanded the opportunity for greater insight into the biological systems. The application of DNA array technology in transcriptome analysis and chromatographic separation techniques coupled with mass spectrometry for metabolomic analysis have resulted in the generation of qualitative and quantitative data useful for understanding the plant metabolism. However, the large quantity of data generated by modern techniques urgently requires more effective 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 upon plant metabolic pathway maps. All metabolites currently detected using multiple *x*C-MS techniques (unidentified and identified) were tabulated, and putative biological functions assigned. In parallel, all transcript elements contained on the recently released *Medicago* Affymetrix chip were annotated and grouped into functional categories using MIPS terminology. The information was combined into a master table (“genome”) and imported into a commercial software package GeneSpring, which includes a statistical analysis platform. Furthermore, gene annotations were applied to predict the legume specific metabolic pathways by using the MetaCyc database (see URL <http://MetaCyc.org>), which comprises a collection of metabolic pathways and enzymes from a wide variety of organisms including primarily microorganisms and plants. The automated alignment was manually curated and *Medicago*-specific pathways such as isoflavonoid biosynthesis added. The resulting pathways will be implemented into GeneSpring for automatic visualization of transcript and metabolomics experiments. The presentation of data in this manner will provide improved visualization and interpretation of integrated results.

Functional Genomics Workshop Attendees/addresses

<p>Beaubien, Amanda Southeastern Oklahoma State University 1405 N. 4th P. O. Box 4025 Durant, OK 74701</p>	<p>Douglas, Carl J. Professor and Head Department of Botany 6270 University Blvd. University of British Columbia Vancouver, BC V6T 1Z4 CANADA Phone: (604) 822-3554 FAX: (604) 822-6089 email: cdouglas@interchange.ubc.ca</p>
<p>Broeckling, Corey Department of Horticulture and Landscape Architecture Shepardson Building Colorado State University Fort Collins, CO 80523-1173 e-mail: cbroeckl@lamar.colostate.edu</p>	<p>Hajduch, Martin Biochemistry Department University of Missouri-Columbia 204 Life Science Center Columbia, MO 65211 Phone: 573-884-5979 e-mail: hajduchm@missouri.edu</p>
<p>Cameron, Carson Southeastern Oklahoma State University 1405 N. 4th P. O. Box 4025 Durant, OK 74701</p>	<p>Harrison, Maria Boyce Thompson Institute for Plant Research Tower Road Ithaca, NY 14853-1801 Phone: 607-254-6472 Fax: 607-254-6779 e-mail: mjh78@cornell.edu</p>
<p>Chen, Sixue Proteomics and Mass Spectrometry Facility Donald Danforth Plant Science Center 975 N. Warson Road St. Louis, MO 63132 Phone: 314-587-1224 Fax: 314-587-1324 e-mail: schen@danforthcenter.org</p>	<p>Hill, Jeff Southeastern Oklahoma State University 1405 N. 4th P. O. Box 4025 Durant, OK 74701</p>
<p>Cheng, Hui Virginia Bioinformatics Institute 18880 Pratt Drive Blacksburg, VA 24072 Phone: Fax: e-mail:</p>	<p>Huhman, David Plant Biology Division The Noble Foundation P. O. Box 2180 Ardmore, OK 73402 Phone: 580-224-6159 Fax: 580-224-6692 e-mail: dvuhman@noble.org</p>
<p>Dixon, Richard Director Plant Biology Division The Noble Foundation P. O. Box 2180 Ardmore, OK 73402 Phone: 580-224-6601 Fax: 580-224-6692 e-mail: radixon@noble.org</p>	<p>Isaac, Giorgis Division of Biology Ackert Hall Kansas State University Manhattan, KS 66506-4901 Phone: 785-532-5756 Fax: 785-532-6653 e-mail: giorgis@ksu.edu</p>

<p>Jeonotte, Richard Kansas Lipidomics Research Center Division of Biology 510 Ackert Hall Kansas State University Manhattan, KS 66506-4901 Phone: 785-532-5756 e-mail:</p>	<p>May, Gregory Plant Biology Division The Noble Foundation P. O. Box 2180 Ardmore, OK 73402 Phone: 580-224-6650 Fax: 580-224-6692 e-mail: gdmay@noble.org</p>
<p>Jha, Ajah Kumar Graduate Research Assistant Department of Botany 104 Life Science East Oklahoma State University Stillwater, OK 74078-3013 Phone: 405-744-5378 e-mail: ajay.jha@okstate.edu</p>	<p>McClure, Thomas D., Ph.D. Product Manager, Hybrid Instruments Scientific Instruments Division Thermo Electron Corporation 355 River Oaks Parkway San Jose, CA Phone: 408-965-6046 Fax: 408-965-6117 e-mail: thomas.mcclure@thermo.com</p>
<p>Kusano, Miyako Research Scientist Metabolomics Research Group RIKEN Plant Science Center RIKEN 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama City Kanagawa, 230-0045 JAPAN Phone: 011-81-45-503-9442 Fax: 011-81-45-503-9489 e-mail: Mkusano005@psc.riken.jp</p>	<p>Mendes, Pedro Virginia Bioinformatics Institute 18880 Pratt Drive Blacksburg, VA 24072 Phone: 540-231-3469 Fax: 540-231-2606 e-mail: mendes@vt.edu</p>
<p>Lange, Bernd Assistant Professor Institute of Biological Chemistry Center for Integrated Biotechnology Washington State University P. O. Box 646340 Pullman, WA 99164-6340 Phone: 509-335-3794 Fax: 509-335-7643 e-mail: lange-m@wsu.edu</p>	<p>Menrottra, Bharat Virginia Bioinformatics Institute 18880 Pratt Drive Blacksburg, VA 24072 Phone: 540-231-3469 Fax: 540-231-2606 e-mail: bmehro@vbi.vt.edu</p>
<p>Lei, Zhentian Plant Biology Division The Noble Foundation P. O. Box 2180 Ardmore, OK 73402 Phone: 580-224-6156 Fax: 580-224-6692 e-mail: zlei@noble.org</p>	<p>Mittal, Shipra Oklahoma State University e-mail: shipra.mittal@okstate.edu</p>
<p>Mathesius, Ulrike School of Biochemistry and Molecular Biology Australian National University Canberra ACT 0200 Phone: 011-61-2-6125-2840 Fax: 011-61-2-6125-0313 e-mail: ulrike.mathesius@anu.edu.au</p>	<p>Mohapatra, Saroj Virginia Bioinformatics Institute 18880 Pratt Drive Blacksburg, VA 24072 Phone: Fax: e-mail:</p>
	<p>Muralla, Rosanna 104 Life Science East Department of Botany Oklahoma State University Stillwater, OK 74075 Phone: 405-744-9560 Fax: 405-744-7074 e-mail: muralla@okstate.edu</p>

<p>Mysore, Kiran The Noble Foundation Plant Biology Division P. O. Box 2180 Ardmore, OK 73402 Phone: 580-224-6740 Fax: 580-24-6692 e-mail: kmysore@noble.org</p>	<p>Schachtman, Daniel P. Principal Investigator and Associate Member Donald Danforth Plant Science Center 975 N. Warson Road St. Louis, MO 63132 Phone: 314-587-1421 Fax: 314-587-1521 e-mail: dschachtman@danforthcenter.org</p>
<p>Nagaraj, Satish Plant Biology Division The Noble Foundation P. O. Box 2180 Ardmore, OK 73402 Phone: 580-224-6152 e-mail: snagaraj@noble.org</p>	<p>Schubert, Karel Vice President Technology Management and Science Administration Donald Danforth Plant Science Center 975 North Warson Road St. Louis, MO 63132 Phone: 314-587-1211 Fax: 314-587-1311 e-mail: kschubert@danforthcenter.org</p>
<p>Naoumkina, Marina Plant Biology Division The Noble Foundation P. O. Box 2180 Ardmore, OK 73402 Phone: 580-224-6128 Fax: 580-224-6692 e-mail: manaoumkina@noble.org</p>	<p>Shulaev, Vladimir Virginia Bioinformatics Institute 18880 Pratt Drive Blacksburg, VA 24072 Phone: 540-231-3469 Fax: 540-231-2606 e-mail</p>
<p>Richerson, Kristy The Noble Foundation Plant Biology Division P. O. Box 2180 Ardmore, OK 73402 Phone: 580-224-6107 Fax: 580-24-6692 e-mail: klricherson@noble.org</p>	<p>Smith, Tim Associate Professor Southeastern Oklahoma State University 1405 N. 4th P. O. Box 4025 Durant, OK 74701 Phone: 580-745-2444 Fax: 580-745-7488 e-mail: tsmith@sosu.edu</p>
<p>Roe, Bruce Advanced Center for Genome Technology Department of Chemistry and Biochemistry Stephenson Research and Technology Center 101 David L. Boren Boulevard, Room 2107 University of Oklahoma Norman, OK 73019-0370 Phone: 405-325-4912 Fax: 405-325-7762 e-mail: broe@ou.edu</p>	<p>Sparks, Alexis Research Assistant Kansas Lipidomics Research Center Division of Biology 510 Ackert Hall Kansas State University Manhattan, KS 66506-4901 Phone: 785-532-5756 e-mail: aasparks@ksu.edu</p>
<p>Saito, Kazuki Graduate School of Pharmaceutical Sciences Chiba University RIKEN Plant Science Center Yokohama, Japan e-mail: ksaito@faculty.chiba-u.jp</p>	

<p>Sumner, Lloyd Assistant Scientist & Head, Biological Mass Spectrometry Plant Biology Division The Noble Foundation P. O. Box 2180 Ardmore, OK 73402 Phone: 580-224-6710 Fax: 580-224-6692 E-mail: lwsumner@noble.org</p>	<p>Urbanczyk-Wochniak, Ewa Plant Biology Division The Noble Foundation P. O. Box 2180 Ardmore, OK 73402 Phone: 580-224-6151 Fax: 580-224-6692 e-mail: urbanczyk@noble.org</p>
<p>Tang, Yuhong Plant Biology Division The Noble Foundation P. O. Box 2180 Ardmore, OK 73402 Phone: 580-224-6645 Fax: 580-224-6692 e-mail: ytang@noble.org</p>	<p>Watson, Bonnie Plant Biology Division The Noble Foundation P. O. Box 2180 Ardmore, OK 73402 Phone: 580-224-6154 Fax: 580-224-6692 e-mail: bowatson@noble.org</p>
<p>Tohge, Takayuko Research Scientist Metabolomics Research Group RIKEN Plant Science Center RIKEN 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama City Kanagawa, 230-0045 JAPAN Phone: 011-81-45-503-9442 Fax: 011-81-45-503-9489 e-mail: cupdtohge@psc.riken.jp</p>	<p>Workman, Ron Southeastern Oklahoma State University 1405 N. 4th P. O. Box 4025 Durant, OK 74701 Phone: 580-745-2444 Fax: 580-745-7488</p>
<p>Tyler, Brett Virginia Bioinformatics Institute 18880 Pratt Drive Blacksburg, VA 24072 Phone: 540-231-3469 Fax: 540-231-2606 e-mail</p>	<p>Xu, Dong 271C Life Sciences Center 1201 East Rollins Road University of Missouri-Columbia Columbia, MO 65211-2060 Phone: 573-882-7064 E-mail: xudong@missouri.edu</p>
<p>Udvardi, Michael Molecular Plant Nutrition Group Max Planck Institute of Molecular Plant Physiology Am Mühlenberg 1 14476 Golm Germany Phone: 011-49 331 5678149 Fax: 011-49 331 5678250 E-mail: Udvardi@mpimp-golm.mpg.de</p>	<p>Zou, Jijun Ph.D. Research Scientist, Functional Genomics Analytical and Genomics Technologies Pioneer Hi-Bred International, Inc. 7300 NW 62nd Ave. Johnston, IA 50131 Phone: 515-270-3699 Fax: 515-270-3367 e-mail: Jijun.Zou@pioneer.com</p>

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