Soy 2004

10th Biennial Conference of the Cellular and Molecular Biology of the Soybean

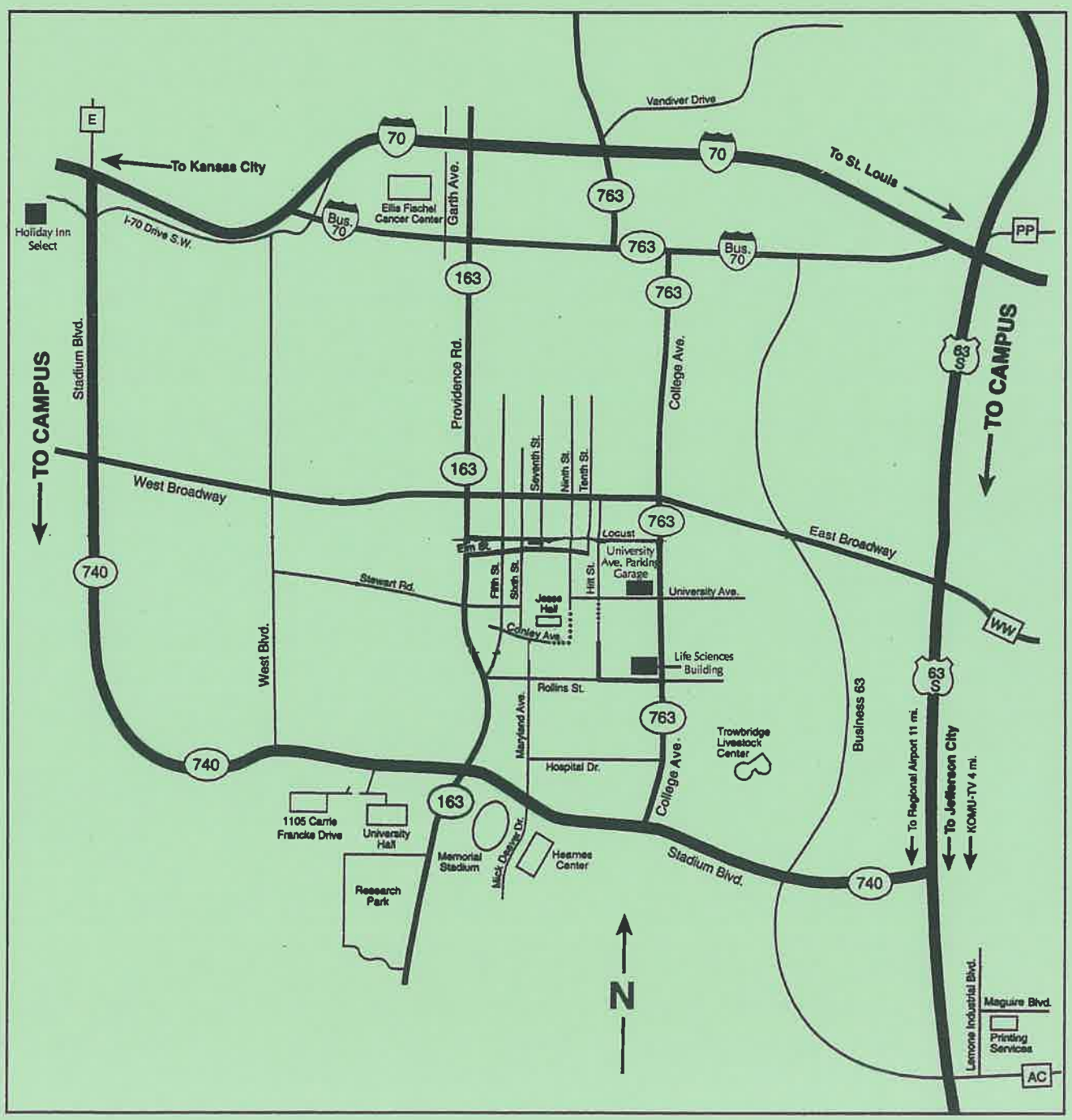
Program/Abstracts

August 8-11, 2004
Holiday Inn Select Executive Center
Columbia, Missouri

Hosted by:
University of Missouri-Columbia

Directions to the University of Missouri:

Travel south on Stadium Blvd. until you reach College Ave. (approximately 4.5 miles) turn left (north) onto College Ave; turn left (west) onto Rollins Ave. (second stop light) and the Life Sciences Building will be on your immediate right.



Soy2004: Cellular and Molecular Biology of the Soybean

Final Meeting Program

Sunday, August 8, 2004

4- 6 pm Registration, Holiday Inn Executive Center

5:30 pm (First vans depart from Holiday Inn for Opening and Reception)

6:15 pm Official Opening, Life Science Center, Univ. of Missouri, Campus

Opening Lecture: **Nicholas Kalaitzandonakes**

"Truths, Truisms, Half-Truths, and Myths in Biotech"

7:30-9 pm Reception, Life Science Center, University of Missouri, Campus

The Life Science Center is the newest research building on the Univ. of Missouri campus and the opening and reception will be one of the first, official activities to be held in the building. Be sure to attend to inaugurate this research facility. We will be arranging tours.

Monday, August 9, 2004

7:00 am Continental Breakfast

Plenary Session: Update on Soybean Technology
Chairperson Gary Stacey

8:00 am	Welcome	
8:05 am	**Perry Cregan**	Linkage disequilibrium and genetic association analysis for gene discovery
8:35 am	**Tom Clemente**	Transgenes in soybean: Methods and applications
9:05 am	**H. Roger Boerma**	Application of molecular markers to enhance the rate of soybean improvement
9:35 am	**Randy Shoemaker**	Structural genomics
10:05-10:35 am	Break	

Concurrent II: Stress I Chairperson: Melissa Goellner

1:30 pm	**Madan Bhattachariya**	Towards understanding the mechanism of *Phytophthora* resistance in soybean
1:50 pm	**Anne Dorrance**	New sources of resistance to *Phytophthora sojae*
2:10 pm	**David Lightfoot**	Genomic approaches to molecular breeding of resistance to soybean sudden death syndrome and cyst nematode in elite cultivars.

2:30 pm-3:00 pm Break

3:00 pm	**Eric Davis**	Getting to the roots of parasitism by cyst nematodes
3:20 pm	**Glen Hartman**	Soybean rust: present and future challenges
3:40 pm	**Jamie A. O'Rourke**	Functional Analysis of Iron Deficiency Chlorosis
4:10 pm	**Joseph A. Bedell**	The path to a complete soybean gene sequence: Gene enrichment by methyl filtration

4:30-6:00 pm **Poster Session**

6:30-8:00 pm Genomics Strategic Planning Session
Organized by Randy Shoemaker, SoyGEC chairman

Agenda:
Introduction - Randy Shoemaker, Chair SoyGEC, USDA-ARS, ISU
The Soybean Genomics Action Plan
Report from NSF-sponsored Workshop - Gary Stacey, U-MO
USDA-sponsored Bioinformatics (LIS/ISU)
The Soybean Genetics Executive Committee
Elected members

Concurrent sessions

Concurrent III: Metabolic Engineering Chairperson: Kristin Bilyeu

1:30 pm	**Eve Wurtele**	Integration of mciroarray, metabolomics, and metabolic flux in developing soybean seeds
1:50 pm	**Oliver Yu**	Transcriptional Regulation to metabolic channeling: Understanding the flavonoid and isoflavonoid pathway for metabolic engineering
2:10 pm	**Hari Krishnan**	Quality vs quantity: Constraints in soybean protein quality enhancement
2:30 pm-3:00 pm	Break	
3:00 pm	**Ed Cahoon**	The challenges of producing greasy molecules in soybean
3:20 pm	**Chris Todd**	Ureide degradation in two soybean cultivars
3:40 pm	**Victor Raboy**	Genetics of Seed Phytic Acid, Phosphorus and Minerals
4:10 pm	**Grace E. Byfield**	Effects of Temperature on Desaturase Allele Expression at Various Stages of Seed Development in Soybean
4:30-6:00 pm	**Poster Session**	

Concurrent IV: Stress II Chairperson: Henry Nguyen

1:30 pm	**Bob Sharp**	Proteomic analysis of the differential responses of root and shoot growth to water deficits
1:50 pm	**Larry Purcell**	Soybean yield and drought: can we realistically produce more yield with limited water?"
2:10 pm	**Tara Vantoai**	Tolerance of Soybeans to

10:30 am	**Wayne Parrott**	Beans & Genes: Public attitude and the future of GE crops
11:00 am	**Stanton Dotson**	Drought responses in soybean - new insights from transgenic studies
11:30 am	**Ed Ready**	Why the checkoff board supports genomics research?

Noon—meeting adjourns.

ABSTRACTS OF ORAL PRESENTATIONS

Transgenes in Soybean: Methods and Applications

Tom Clemente, Center for Biotechnology/Plant Science Initiative/ Dept. of Agronomy & Horticulture/U. of Nebraska

Genetic engineering of soybean is currently carried out using three distinct target explants, cotyledonary-node, embryonic axis and somatic embryos. The latter two have been successfully transformed using both Agrobacterium-mediated and microprojectile-derived protocols, while the first has only been reported in Agrobacterium-mediated protocols. With the recent advances in soybean transformation efficiencies are sufficient enough to utilize this tool in both basic and applied studies. This tool can be used to modulate endogenous gene expression via induction of post transcriptional gene silencing and/or transposon-based mutagenesis. In which the latter can be coupled with gene trap, enhancer trap, and activation tagging elements. These approaches can serve as a strong complement to soybean functional genomics programs. Roundup Ready soybean is an input trait introduced in to soybean germplasm via genetic engineering that provides growers with a convenient and effective weed management tool. Ongoing research endeavors in both public and private sector institutions have targeted other novel input and output traits for soybean. Input traits being investigated include alternative herbicide resistance and tolerance to both biotic and abiotic stresses, while output traits are primarily focused on modulating oil and protein composition in the seed. Current protocols in soybean transformation permit the introduction of these novel traits directly into elite soybean germplasm. In addition traits introduced through genetic engineering will behave as a dominant allele thereby facilitating subsequent breeding.

Structural Genomics of Soybean

Randy C. Shoemaker, USDA-ARS, Corn Insect and Crop Genetics Research Unit, Dept. of Agronomy, Iowa State Univ., Ames, IA

The evolutionary history of *Glycine max* (L.) Merr. includes at least two rounds of genome duplication. The homoeologous genomic regions resulting from these duplications are observable with hybridization-based comparative mapping. The observations of nested' duplications were the first clue to soybean's paleopolyploidy. Subsequent analyses of large numbers of transcripts put the timing of the duplication events at approximately 14 MYA and 44 MYA; the latter date probably corresponding to the most recent genome duplication in the model legume, *Medicago truncatula*. Following polyploidization events it is common for the duplicated genomes to diverge in the process of diploidization'. This can occur through accumulation of additions, deletions, point mutations, transposable element activity and rearrangements. These events, especially rearrangements, complicate the detection of macro-synteny with other plant genomes. Although colinearity has been implicated between regions of the soybean genome and regions of other plant genomes little is known about microsynteny. Currently, our research involves micro-syntenic analyses between homoeologous regions in soybean and between the soybean genome and other plant genomes.

Sequencing the Gene Space of the Model Legume, *Medicago truncatula*

Nevin Dale Young, Steven B. Cannon, Joann Mudge, Xiaohong Wang, Atif U. Ahmed, University of Minnesota

Medicago truncatula is a model legume with a genome of 470 Mb. Cytogenetics indicate most genes are localized in euchromatin separate from pericentromeric heterochromatin. Medicago has 190,000 ESTs, a dense genetic map, straightforward transformation, collections of natural ecotypes, and gene tilling/knockouts. Medicago also has a sequencing-ready physical map anchored by 400 SSRs. The international Medicago community has recently begun a BAC-by-BAC effort to sequence the gene-rich region of the genome, estimated at 200 Mb. This strategy provides the most utility as a reference genome for comparative genomics. Sequencing is taking place at Oklahoma (B Roe), TIGR (C Town), Sanger (J Rogers), and Genoscope (F Quetier), with physical mapping at UC-Davis (D Cook & DJ Kim) and bioinformatics at Minnesota (Young, Cannon & Retzel). As of June 2004, 934 BAC clones had been sequenced or in progress, representing 108 Mbp in total. Estimates of gene-space coverage range from 29 to 38, with one gene every 6.7 kb. Details are found at www.medicago.org/genome.

The effects of SCN resistance QTL and flanking regions on resistance and agronomic traits

Brian Diers, University of Illinois; Eileen Kabelka, University of Florida; Friedrich Kopisch-Obuch, Eric Brucker, Shawn Carlson, University of Illinois; Prakash Arelli, USDA-ARS

There is a need to confirm SCN resistance QTL and to study regions where they map. We have confirmed two major SCN resistance QTL from *G. soja* by backcrossing them into the background of a susceptible experimental line. Field tests show that these genes were associated with a positive or neutral effect on yield. To test the effect of the major SCN resistance gene *rhg*1 and flanking regions, populations of near isogenic lines (NILs) segregating for *rhg*1 from PI 88788 were developed. In the NIL populations, the resistance allele at *rhg*1 was associated with greater resistance, less field SCN reproduction, and greater yield under moderate to high SCN pressure than the susceptibility allele. Under low SCN pressure, the *rhg*1 resistance allele was associated with less yield in some populations.

Molecular Genetic Markers for Soybean Oil Content and Composition

Vincent R. Pantalone, University of Tennessee

Knowledge of soybean genetics has stimulated improvements of oil quality. Currently there are 16 linkage groups (LG) with loci controlling total oil biosynthesis. A major palmitic acid locus is near Satt684 on LG A1, which may help develop low-saturated fat oil. A major locus conditioning elevated stearic acid is located near Satt070 on LG B2, supporting a strategy for increasing a natural saturate, in order to reduce the amount of trans fat in semi-solid fat products such as margarines. Markers for oleic acid, a highly favorable fatty acid, have been found on LG A1, G, L, and M. Markers near the Fan locus on LG B2 will help reduce the level of linolenic acid, which will improve oxidative stability, and reduce the need for hydrogenation. A comprehensive understanding of oil genetic mechanisms will enable researchers to predict the outcomes of pyramiding multiple oil traits or combining specific oil traits with other desirable seed traits.

Assignment of molecular linkage groups to soybean chromosomes

J.J. Zou, Department of Crop Sciences, University of Illinois, Urbana, IL 61801; J. Lee, Genetic Resources Division, National Institute of Agricultural Biotechnology, South Korea; S.J. Xu, USDA-ARS, Fargo, ND 58105; P.B. Cregan, USDA-ARS, Beltsville, MD 20705; S.J. Clough, University of Illinois and USDA-ARS, Urbana, IL 61801; T. Hymowitz, Department of Crop Sciences, University of Illinois, Urbana, IL 61801

Singh and Hymowitz (1988) published the first soybean chromosome map based on pachytene chromosome analysis. This laid the foundation to identify primary trisomics in the soybean (Xu et al. 2000). We have assigned 11 molecular linkage groups to soybean chromosomes by using primary trisomics and SSR markers. Primary trisomics were hybridized with *G. soja* in the greenhouse, F_1 plants with different chromosomes were identified cytologically and 41 chromosome plants were selfed. A deviation from 1:2:1 ratio in the F_2 population suggests a marker is associated with a chromosome. The relationships between soybean chromosomes and molecular linkage groups are: 1-D1, 3-N, 5-A1, 8-A2, 9-K, 13-F, 14-C1, 17-D2, 18-G, 19-L and 20-I. Updated information on gene expression profiling (ca. 10,000 genes) using primary trisomic and tetrasomic, which chromosomes were counted by Dr. Singh, to study single and double chromosomes effects will be presented.

SNP Detection and Genotyping in Soybean

Suk-ha Lee, Seoul National University, Korea

Direct sequencing of PCR products using various soybean genotypes identified a total of 472 SNPs (single nucleotide polymorphisms). Degenerate oligonucleotide primed PCR is also an efficient SNP detection method for whole genome amplification without initial sequence information. Sequencing of amplified representatives of whole genome in six soybean cultivars is in progress. Single nucleotide amplified polymorphism (SNAP) marker was developed for rapid screening of the trait, such as supernodulation (GmNARK) and beany flavor (lipoxygenase-2). These two functional SNP markers are superior to random DNA markers due to complete linkage with trait locus alleles and can be also developed to facilitate marker-assisted selection. Non-gel based SNP genotyping assays were tested. Several genotyping methods were compatible with Luminex, whereas Victor 3 can be used only for single base extension method.

New Sources of Resistance to *Phytophthora sojae* in Soybean

Anne E. Dorrance, Stuart Gordon, Dept. of Plant Pathology; Steven K. St.Martin, Dept. of Horticulture and Crop Science, The Ohio State University

Phytophthora sojae is the leading cause of yield losses in soybeans in some states and second in many others. Based on several regional surveys, it is apparent that *P. sojae* populations have adapted to the most widely deployed *Rps* genes. In an earlier study, we identified 32 putative new sources of resistance to *P. sojae* in soybeans. We are currently in the process of characterizing and mapping all of the *Rps* genes in these sources. Resistance from one of the 32 PIs is segregating as a single dominant gene and based on marker analysis and has been designated *Rps*8. More recent data suggests that this *Rps* gene now lies on MLG F. In many of the other PIs 2 or 3 *Rps* genes are present. Efforts are underway to determine if the resistance in these PIs is conferred by two or more novel genes, or by a novel gene combined with one or more previously identified *Rps* genes.

Getting to the roots of parasitism by cyst nematodes

Eric L. Davis, North Carolina State University

The soybean cyst nematode (SCN) is a major pathogen of soybean that has evolved a sophisticated interactive relationship with host cells in soybean roots to sustain its parasitic habit. Over fifty 'parasitism genes' expressed specifically within the esophageal gland cells of SCN have been identified that encode a potential arsenal of different secreted parasitism proteins from the nematode that may have direct effects on recipient host plant cells. The effects may include cell wall modifications and interactions with signal transduction receptors in the extracellular space, as well as direct introduction of secretions into host cells to influence cellular metabolism, cell cycle, selective protein degradation, localized defense response, and regulatory activity within the host cell nucleus. Specific disruption of these vulnerable points in the SCN parasitic cycle may provide novel targets for management of SCN in soybean.

The path to a complete soybean gene sequence: Gene enrichment by methyl filtration

Joseph A. Bedell, Orion Genomics, LLC, Saint Louis, MO; Sandra W. Clifton, Lucinda Fulton, Deana Pape, Washington University Genome Sequencing Center, Saint Louis, MO; Henry Nguyen, Gary Stacey, National Center for Soybean Biotechnology, University of Missouri, Columbia, MO; Andre Nunberg, Muhammad A. Budiman, Nathan Lakey, Orion Genomics, LLC, Saint Louis, MO

Glycine max has a tetraploid genome of approximately 1.1 Gb. The large size and polyploid nature of the genome make it especially challenging for standard genomic technologies. For example, the 1.1 Gb genome would require more than 10 million whole genome shotgun reads to obtain a 6x coverage. We undertook a pilot project to assess methyl filtration as a means to enrich for the gene-rich regions of the soybean genome. Methyl filtration exploits the genome architecture of plants in which genes are concentrated in the hypomethylated regions. Preliminary results suggest a 2.5-fold reduction of the soybean genome using methyl filtration, bringing the accessible gene-rich portion to 450Mb -- the size of the rice genome. Another facet of the pilot is the production of several thousand whole genome shotgun reads which will be used to assess and identify the repetitive content of the genome. With the pilot data in hand, we will present a comprehensive, cost-effective approach to rapidly elucidate the full gene complement of soybean and to efficiently anchor it to the physical map. With the expected completion of the cv. Williams 82 physical map by the end of the year, this strategy would provide an immediate and cost-effective way to sequence the soybean genome.

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

Lloyd W. Sumner, Corey Broeckling, David Huhman, Zhentian Lei, Mohamed Farag, Aaron Elmer, Lahoucine Achnine, Naveed Aziz, Gregory D. May, Richard A. Dixon, The Samuel Roberts Noble Foundation; Joel T. Smith, Southeastern Oklahoma State University; Pedro Mendes, Virginia Bioinformatics Institute

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

Isolation and Characterization of the *Rpg*1-b disease resistance gene from soybean

Roger Innes, Tom Ashfield, Indiana University; Steven Cannon, Nevin Young, University of Minnesota; Laura Ong, Indiana University

We have been studying the evolution of disease resistance (R) genes in *Arabidopsis* and soybean that confer resistance to bacterial blight disease. Isolation of the *Arabidopsis RPM*1 gene and the soybean *Rpg*1-b gene revealed that both belong to the CC-NBS-LRR class of R genes, but that these genes evolved independently. Several genes that are highly similar to *Rpg*1-b are found adjacent to *Rpg*1-b in the soybean genome, suggesting that recombination may have played a role in the evolution of *Rpg*1-b. To test this hypothesis, we have embarked on a large-scale comparative genomics project in which approximately 1 megabase of DNA sequence flanking *Rpg*1-b will be compared to the equivalent (orthologous) region in wild relatives of soybean, including *Medicago truncatula*. Preliminary data indicate that this region has undergone dramatic changes in R gene number, while the content and order of non-R genes has been mostly maintained.

Comparing soybean genomic responses to pest and pathogens

Steven J. Clough, USDA-ARS; Jijun Zou, University of Illinois

Soybean plants are attacked by a wide variety of pests and pathogens requiring effective means of defense. Gene activation is a major mechanism to reduce the degree of damage caused by pests and pathogen invasion as it leads to the rapid, regulated production of toxins and enzymes in addition to structural reinforcements. We used soybean cDNA microarrays to measure differential gene expression in response to soybean aphid (*Aphis glycines*) and against the causal agents of three different diseases. *Pseudomonas syringae*, with or without *avr*B, allowed for the comparison of the hypersensitive defense response to susceptibility. Inoculating differential lines with *Sclerotinia sclerotiorum* allowed the study of quantitative resistance in stem infections. Treatment of fresh cuttings with sterile culture filtrate allowed the study of soybean response to mycotoxin released by *Fusarium solani*. Expression similarities and differences between these soybean-pest/pathogen interactions will be discussed.

INTEGRATION OF MICROARRAY, METABOLOMICS, AND METABOLIC FLUX IN DEVELOPING SOYBEAN SEEDS

Eve Syrkin Wurtele
Department of Genetics, Development and Cell Biology, Iowa State Universtity, Ames IA 50014.

MetNet is a suite of publicly available emerging software tools designed for analysis of genome-wide expression profiling data (transcriptomics, proteomics, metabolomics) and metabolic flux data combined with a metabolic and regulatory network map.
MetNetDB (http://get.gdcb.iastate.edu/) is a database map of entities (metabolites, genes, RNAs and proteins) and interactions (including enzymatic, transport, positive and negative regulation) between these entities. Other information includes references, synonyms, subcellular location, and confidence. The map is exported in an xml format, and can be visualized together with gene expression data using a multivariate graphic capability based on the visualization software GeneGobi and statistical analysis R (http://www.public.iastate.edu/~dicook/GeneGobi/GeneGobi.html). Multi-dimensional profiling data can be projected in two or three dimensions; the user can overlay statistical analysis. FCModeler is a graph visualization and modeling tool that uses the MetNetDB map together with experimental profiling data and several modeling approaches. The graph display interface supports extensive user interactions with user-designated views of the network graph. Graph theoretic analysis is used to extract pathways and cycles from the network. Fuzzy methods are used for modeling networks, and the results are interpreted using simple fuzzy cognitive maps. FCModeler is intended to provide a modeling lattice for assessing the large amounts of data captured by high-throughput gene expression experiments. The text mining tool, PathBinder, searches the PubMed database when the user clicks on any two entities in MetNetDB, or FCModeler; all abstracts that contain sentences with those two terms are retrieved. The MetNet tools are designed to provide a framework for the formulation of testable hypotheses regarding the function of specific genes, and in the long term to provide the basis for identification of metabolic and regulatory networks that control plant composition and development. MetNet will be illustrated with an example from Arabidopsis and from soybean seeds.

Quality vs. quantity: Constraints in soybean protein quality enhancement

Hari B. Krishnan, USDA-ARS and Department of Agronomy, University of Missouri, Columbia, MO 65211

Soybeans are an excellent source of quality protein for both human and animal consumption. Soybeans in general contain between 35 to 40 protein. In an effort to increase seed protein, elite cultivars are crossed with those containing elevated protein content. Even though higher protein is a desirable characteristic, whether such an increase will be accompanied by enhanced protein quality is not known. Soybean protein quality could be significantly improved by increasing the concentration of the sulfur-containing amino acids, cysteine and methionine. To ascertain if a correlation existed between protein quantity and quality, a comparison of the sulfur amino acid of soybeans differing in protein content was made. Soybean cultivars with higher protein content had a lower percentage of sulfur amino acids, while those with lower protein exhibited a higher content of total cysteine and methionine. Nitrogen application elevated the protein content but lowered that of the sulfur amino acids. Two abundant proteins with molecular weights of 19 and 10 kDa contain significant amounts of sulfur amino acids. Accumulation of these proteins was diminished when plants were fertilized with nitrogen. These results indicate a negative correlation exists between total protein and sulfur amino acid content.

Ureide Degradation in Two Soybean Cultivars

Christopher D Todd, Joe C Polacco, University of Missouri, Columbia, MO

During nitrogen fixation in symbiotic soybean plants the majority of xylem borne nitrogen is delivered to the shoot as the ureides allantoin and allantoate. Once in the leaf tissue allantoin and allantoate are ultimately broken down to ammonia, carbon dioxide and glyoxylate. The mechanism of this conversion is still under debate. Two different enzymes have been proposed to hydrolyze allantoate: allantoate amidohydrolase and allantoate amidinohydrolase. The amidohydrolase releases ammonia and carbon dioxide directly from allantoate whereas the amidinohydrolase instead produces urea, which can then be converted to ammonia and carbon dioxide by the nickel metalloenzyme urease. It has been suggested that both enzymes are present in the soybean germplasm and that different cultivars exclusively use one pathway over the other. This difference is thought to have agronomic importance as the amidinohydrolase is thought to continue to degrade allantoate under water limiting conditions, preventing accumulation of ureides and feedback inhibition of nitrogen fixation. Conversely, the amidohydrolase is believed to become inactive under water-deficit, ultimately contributing to inhibition of nitrogen fixation. We compared the breakdown of ureides in two soybean cultivars: Williams 82, a variety whose nitrogen fixation is sensitive to water deficit and which is believed to degrade allantoate through the amidohydrolase, and Maple Arrow, an insensitive cultivar, suggested to assimilate ureide nitrogen through the combined action of allantoate and ureidoglycolate amidinohydrolases and urease. We observed no biochemical support for the exclusivity of the two different pathways in these two cultivars and provide evidence for urea and ammonia generating activities in both varieties.

Effects of Temperature on Desaturase Allele Expression at Various Stages of Seed Development in Soybean

Grace E. Byfield, Microbiology Department; Robert G. Upchurch, ARS Soybean and Nitrogen Fixation Unit; Huiqin Xue, Crop Science Department, North Carolina State University, Raleigh, NC 27695

Fatty acid desaturases (FADs) play an important role in determining the fatty acid composition of soybean oil during seed development. Soybean lines have been developed that produce oil containing 50 or greater oleic acid (C18:1) This higher level of oleic acid is nutritionally desirable and beneficial for human health. The stable expression of the mid oleic trait in soybean, however, is significantly affected by plant growth temperature. In an effort to document and understand the effect of temperature on fatty acid composition of the oil, we conducted experiments to examine the potential interaction between temperature and the expression of seven desaturase alleles at various stages of seed development in five soybean cultivars. Specific primers were designed and used to survey the genomes of selected soybean lines for the presence of FAD alleles using polymerase chain reaction (PCR). Work in progress and published work have identified the presence of alleles for stearoyl acyl-carrier protein desaturase (SACPD) two potential alleles, FAD2-1 two alleles and FAD3 three alleles. Plants were grown under controlled temperatures and beans harvested at various growth stages between R5 and R6. Total RNA was isolated and allele-specific primers used to quantify transcript accumulation of the various alleles by reverse transcription, Real Time PCR. Results suggest that there is significant interaction between temperature and transcript accumulation in all the lines investigated. Differences were also observed in transcript accumulations for the various stages that were analyzed.

Soybean Yield and Drought: Can We Realistically Produce More Yield with Limited Water

Larry Purcell, University of Arkansas

Soybean growth, development, and yield depend the capture and utilization of essential resources. When nutrients and solar radiation are not limiting growth, crop yield is linearly related to the seasonal quantity of water transpired. Increasing yield under drought depends upon increasing the quantity of water that is available for transpiration or bending (not breaking) the tight biochemical and biophysical relationships between crop yield and transpiration. Genes that only prolong crop survival under severe water deficits have little impact on increasing transpired water and, therefore, have little economic relevance. Specific traits that may be important in increasing transpirable water during a drought include deep rooting, slower crop growth under well-watered conditions, and an increased sensitivity of stomatal closure to water deficits. Modeling and physiological studies also show that increasing the tolerance of N_2 fixation to drought will increase yields, but that increasing the tolerance of photosynthesis to drought will decrease yield in many environments.

Heat Stress in the Early Soybean Production System

James R. Smith; Felix B. Fritschi; Jeffery D. Ray; Alemu Mengistu; Lee Daughtry; Robert Paris; Randall L. Nelson, USDA/ARS

The Early Soybean Production System (ESPS) has improved seed yield in the Midsouth. However, the quality of seed produced in the ESPS is typically poor. The reduction in seed quality observed in the ESPS is likely affected by the fact that seed-filling period, seed maturation, and harvest all occur during periods of high heat and humidity. Efforts are underway to identify soybean genotypes that produce high quality seed under high-heat conditions. Seed quality data will be presented for selected plant introductions grown and evaluated in the ESPS. Also, preliminary growth, physiological, and proteomic data will be presented for heat-affected isogenic lines subjected to differential heat treatments.

Utilization of RNA interference to Confer Resistance to the Soybean Cyst Nematode, *Heterodera glycines*.

Harold N. Trick, Ryan Steeves, Tim C. Todd, Kansas State University

The soybean cyst nematode, *Heterodera glycines*, is an important pest in soybean production throughout the United States and the world. Efforts to control soybean cyst nematode (SCN) have traditionally focused on plant breeding. However, with the discovery of RNA interference (RNAi), new methods of control may be possible. In this presentation, we report our results of expressing a RNAi construct directed to a male fertility (MS) gene of SCN. Transgenic soybean embyrogenic calli were transformed via biolistics with the MS construct and over 23 events were recovered. Twelve events were analyzed for smRNA accumulation and in eleven events 21-23 nts RNAs which hybridize to the MS gene were detected. Bioassays showed a marked increase in SCN resistance as demonstrated by both reduced cyst and reduced egg production on transgenic plants when compared to non transgenic controls.

Recent Developments in Intellectual Property Protection for Soybean Innovation--Law and Public Policy

Jay P. Kesan, Professor, University of Illinois at Urbana-Champaign

In this talk, I will provide an overview of recent developments in intellectual property (IP) protection for soybean innovations. In the past few years, the legal landscape governing IP protection for plant innovation has been changing rapidly. More robust IP protection, such as utility patents for plant innovation, has been permitted by the U.S. Supreme Court. In addition, enforcement of these patent rights through bag-tag or label licenses and technology license agreements have been upheld in various federal appellate decisions. The domestic and international public policy implications of these decisions for scientists engaged in soybean innovation in the private sector and in universities and for soybean producers will be discussed.

MODIFYING SOYBEANS FOR HEALTH AND NUTRITION

Anthony J. Kinney, Pioneer Crop Genetics

Consumption of soy protein has been correlated with many health benefits, including a reduced risk of cardiovascular disease. Improving the quality of soybean oil may result in complementary health benefits and further enhance the nutritional properties of soybeans. Initial approaches focused on changing the existing ratios of the fatty acids in soybean oil. The result has been the production of oils with a reduced saturated fatty acid content and oils with a greatly reduced acid ratio of omega-6 to omega-3 fatty acids, thus improving the balance of polyunsaturated acids in the diet. More recent efforts to mine the biodiversity of the plant and microbe kingdoms has uncovered new genes which can be used to produce novel fatty acids in plant oils. Examples of these include oils containing conjugated fatty acids and plant oils enriched in bioactive polyunsaturated fatty acids.

Drought responses in soybean - new insights from transgenic studies

Jingrui Wu; Karen Gabbert; Jacqueline Heard; Jaishree Chittoor; Stan Dotson, Monsanto Corp.

Water availability is the major limitation to crop yields and the availability of water is the major restriction for agricultural production. Globally, agriculture is responsible for 67 of freshwater withdrawal (UNESCO, 2000). Studies on crop responses to water stress have revealed key biochemical and physiological pathways, which provide strategies to enhance water utilization and efficiency. Functional genomics provides a complimentary approach to identify novel insights in water use biology. By screening transgenic Arabidopsis plants over-expressing a broad array of transgenes, a family of transcription factors, previously not associated with water stress tolerance, has been discovered which confer resistance to water stress. Transformation of soybean plants with transgenes from this family results in drought tolerance in both green house and field screens, suggesting the function of this TF family is conserved across plant species. The transgenic soybean plants are providing novel insights in the biology of water utilization.

POSTER ABSTRACTS ON

SOYBEAN TECHNOLOGY

2 Soybean Research Status at MU Plant Transformation Core Facility

Zhanyuan Zhang, Plant Transformation Core Facility and Department of Agronomy, University of Missouri-Columbia, Columbia, MO 65211 (zhangzh@missouri.edu; www.psu.missouri.edu/muptcf)

Our current soybean research focuses on the development of a high-throughput *Agrobacterium*-mediated transformation system, improvement of the quality of transgene integration and sufficient gene regulation to meet the needs of functional genomics and crop improvement. The need for research in the above areas has become a pressing issue due to the complexity of soybean genome. More specifically, we are now developing strategies for both efficient and high quality T-DNA integration using recombination systems and down-regulation or silencing of soybean genes using RNAi. We are also trying to understand functions of soybean genes at genome scale using T-DNA-based mutagenesis. This latter effort has been in collaboration with other research groups including two groups at MU (Gary Stacey's and Henry Nguyen's groups), the Plant Transformation Research Core Facility at University of Nebraska-Lincoln (Tom Clemente's groups), the Department of Agronomy and Plant Genetics at University of Minnesota (David Somers's groups), and the Plant Transformation Facility at Iowa State University (Kan Wang's group). For soybean trait improvement, our specific objectives are to regulate several economically important genes conditioning soybean seed polyunsaturated fatty acids and short chain sugars, secondary metabolites, abiotic stress, virus resistance, etc. Some of these studies are conducted as collaborations with other research teams. Certain preliminary results will be presented.

4 Regeneration from EMS-treated immature embryo culture in soybean

Hyun-Ju Jang, Suk-Ha Lee, School of Plant Science, Seoul National University, Seoul 151-742, Korea; Jung Suk Bae, Institute for Bioresources Research, Korea

In soybean, immature embryos are frequently used for efficient induction of somatic embryogenesis and organogenesis to form multiple shoots. This study was to determine optimal conditions for ethylmethane sulfonate (EMS) treatment and establish an efficient method for EMS mutagenesis from immature embryo culture. Using six different concentrations of EMS for 0 to18 hr with 3 hr interval, Sinpaldalkong 2 and Jack showed high efficiencies of somatic embryogenesis and regeneration regardless of EMS concentrations, whereas fairly low efficiency or no survival was observed in Jinju 1 and Iksannamulkong. Sinpaldalkong 2 showed good embryogenesis and regeneration even in much higher concentration of EMS under a short period of time. Thus, Sinpaldalkong 2 and Jack are good soybean genotypes for somatic embryogenesis and a total of 110 and 113 M_1R_0 plants were regenerated, respectively.

POSTER ABSTRACTS ON

BREEDING AND GENETICS

7 Variation and Classification of Canopy Type Characters in Korean Soybean Varieties

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This study was aimed to obtain information on agronomic characteristics of 75 Korean soybean varieties classified by utilization. Length of canopy was the longest in soybean varieties for bean sprout, followed by those for sauce and paste, and cooking with rice, while it was the shortest in those for vegetable and summer type. On the other hand, canopy width was the broadest in soybean varieties for cooking with rice, followed by those for sauce and paste and bean sprout, while it was the narrowest in those for vegetable and summer type. Canopy width/length ratio was the greatest in the order of soybean varieties for cooking with rice, sauce and paste, and bean sprout, and vegetable and summer type. Soybean varieties could be classified into 12 types based on canopy characters, i.e. 6 groups (I-VI) for stem height, number of branches and total length of branches/mainstem length ratio and 2 groups(a-open ; b-closed) for canopy width/length ratio. All soybean varieties for sauce and paste distributed in I-VI groups, those for bean sprout belonged to I(a), III(a) and VI(a), those for vegetable and summer type belonged to I and those for cooking with rice belonged to II(a), III(a).

9 SSR Analysis and Confirmation of Oleic Acid QTL in N00-3350 Soybean

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Soybean oil contains saturated (palmitic and stearic) and unsaturated fatty acids (oleic, linoleic, and linolenic). Increasing oleic acid content in soybean oil would decrease the total polyunsaturated fatty acids, reduce the need for hydrogenation and increase the oil quality for human consumption. The objective of this study was to map and confirm QTL conditioning increased oleic acid from N00-3350 (55 percent oleic acid) using simple sequence repeat (SSR) markers. A total of 316 F2:3 lines derived from the cross of G99-G725 (18 percent oleic acid) x N00-3350 were used for mapping and 231 F2:3 lines from the cross of G99-G3438 (16 percent oleic acid) x N00-3350 were used for confirmation. The results indicate that there are six QTL on four linkage groups (LG-A1, D2, G and L) that condition the oleic acid phenotype. In all identified QTL, the N00-3350 allele increased oleic acid content. To date, four of the six QTL have been confirmed.

11 Confirmation of Yield Enhancing QTL from Exotic Soybean Germplasm

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Putative QTL detected in mapping studies must be confirmed before they are used in breeding. The objective of our study was to confirm yield QTL previously mapped in a population developed from crossing BSR101 x LG82-8379. Five yield QTL were confirmed in a population of near isogenic lines (NILs) developed from a line from the original mapping population. The high yield allele traces to the PI parent for four of the five QTL. In all cases, this allele was also associated with later maturity, which was unexpected since there was not a strong association between these traits in the original population. Tests are continuing in 2004 to evaluate the yield QTL in different genetic backgrounds and provide us with more data to assess the effect of maturity on the QTL.

13 Molecular Identification of QTLs Associated With Resistance to Soybean Cyst Nematode Races 2, 3 and 5 in Soybean PI90763

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Soybean cyst nematode(SCN), *Heterodera glycines Ichinohe*, is a major soybean disease in the USA and world. PI90763 is one important resistance source in soybean breeding and one of the four differential lines used in the SCN race determination test and SCN HG type classification. Objective was to identify QTLs for resistance to SCN races 2, 3 and 5 in this line. MLGs G, J, and B1 were identified which is significantly associated with resistance to race 2. QTLs for resistance to race 3 were found on MLGs G, A2 and J and L. QTLs for resistance to race 5 were identified on MLGs G, B1 and E.

15 Fine Mapping a Seed Protein QTL on LG I in Soybean

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A quantitative trait locus QTL controlling seed protein concentration in soybean *Glycine max* L. Merr. was previously mapped to linkage group LG I. The objective of this research is to fine map the precise location of the QTL using substitution mapping techniques. Six populations were developed through five backcrosses of the LG I QTL using the high yielding *G. max* breeding line A81-356022 as a recurrent parent and the high protein wild soybean plant introduction PI 468916 as a donor parent. The six backcross populations each segregated for different regions from PI 468916 near the protein QTL. Field and marker testing of these populations resulted in the localization of the protein QTL to a 1.1 cM region flanked by the single sequence repeat SSR markers Satt127 and Satt496.

17 Construction of a BAC library towards positional cloning of some important QTL genes on molecular linkage group C2 of soybean

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We have constructed a soybean *Glycine max* L. Merrill bacterial artificial chromosome BAC library from green leaf protoplasts of the cultivar Misuzudaizu. The library contains 53,760 clones with an average insert size of 116 kb. PCR based screening of BAC library with a chloroplast-specific probe against BAC pools and colony hybridization revealed that the library contains about 2.93 chloroplast DNA origin. Apart from 2.8 clones having no insert this library represents 5.24 genome equivalents. With this genome coverage the probability of having any DNA sequence represented in this library is higher than 99.46% Three-dimensional pools of the BAC library in combination of HEGS high efficiency genome scanning electrophoresis system made it possible for a quick and efficient PCR-based screening. Based on the genetic linkage map constructed using an F_2 population derived from a cross between two varieties Misuzudaizu and Moshidou Gong 503 in previous study several important QTLs underlying flowering time FT1 maturity HAV1 reproductive period RP2 hard seededness HARD1 germination rate of seed GRS1 and water absorbability of seed WAS1 were localized within a 3-cM region between Satt365 and Satt100 on linkage group LG C2 although some of them may related. Towards successful positional cloning of these important QTL genes the contig development of this region is prerequisite. An average of 5.00 positive clones were identified after screening of BAC pools with SSR and RFLP converted PCR markers. A contig framework for the target region was established. Chromosome walking was preliminarily performed through BAC-end sequencing generated STS maker. This will facilitate full contig development of the target region towards positional cloning of these QTL genes.

19 Contributions and Locations of Two Genes Conditioning Low Phytic Acid in Soybean CX1834-1-2

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The mutant soybean line CX1834-1-2 produces seed with low levels of phytic acid (phytate), which is a highly desirable trait. Efforts to map the mutated gene(s) in a Boggs-RR x CX1834 F2:3 population revealed that more than one gene was involved in inheritance of the low phytate phenotype. Following the discovery of a phytate locus near Satt237 on LG N, a second locus was identified near Satt527 on LG L. The effects and locations of the two genes have now been confirmed in a Benning-RR x CX1834-1-2 F3:4 population that was fixed for the CX1834-1-2 allele on LG N, and segregating at markers linked to the LG L locus. It is not yet known whether both genes have mutations, but CX1834-1-2 alleles are essential at both loci to obtain phytate levels as low as those of CX1834-1-2.

21 Development of a SCAR marker for stem canker resistance in soybean

Eduardo Antonio Gavioli, Antonio Orlando Di Mauro, Sonia Marli Zingaretti Di Mauro, Sandra Helena Uneda-Trevisoli, UNESP-Campus of Jaboticabal

Development of a SCAR marker for stem canker resistance in soybean All new soybean cultivars released in Brazil must carry resistance to stem canker, which evaluation usually is performed by using the toothpick inoculation method. The development of reliable and less laborious method is highly desirable. Thus, crosses between resistant and susceptible parents were made and F_2 plants studied by BSA. A RAPD marker for resistant genotypes was found and converted into a SCAR marker. Enzymatic digestion (Hinc II) of the amplified fragments allowed the separation of homozygous, heterozygous and susceptible plants. Several tests carried out using resistant and susceptible cultivars and genotypes have shown the efficiency of this marker.

23 Genetic Mapping of Quantitative Trait Loci (QTLs) Controlling the Sugar Contents in Soybean Seeds

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Soybean contains several carbohydrates; glucose, fructose and sucrose are desirable, while raffinose and stachyose are undesirable. The contents of these sugars in soybean seeds are quantitative traits that are difficult to measure and select. Genetic mapping of quantitative trait loci (QTLs) controlling sugar content will facilitate the development of soybean cultivars by marker-assistant selection. A mapping population containing 156 F2:3 lines derived from a high sugar line MFS-591 and a low sugar cultivar Camp was used for QTL mapping. The sugar contents of these lines ranged from 6-15 as determined by High Performance Liquid Chromatography. Over 600 SSR primers were used to screen polymorphism between the two parents, and 120 SSR polymorphic primers were used for individual line genotyping. Results of QTL mapping for sugar content in this genetic population will be reported.

25 Inheritance of resistance to Phomopsis and Cercospora seed infection in PI 80837 and MO/PSD-0259 soybean and SSR mapping of resistance genes

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Inheritance of resistance to Phomopsis seed infection in PI 80837 and MO/PSD-0259, and of resistance to Cercospora seed infection in PI 80837 were characterized, and SSR markers were used to map resistance genes. Segregation in the F_2 nd in $F_{2:3}$ lines showed that Phomopsis resistance in both genotypes is conditioned by different single dominant genes. Resistance in PI 80837 was linked to Satt177 (4.3 cM) and Satt342 (15.8 cM) on the B2 linkage group (LG). In MO/PSD-0259 polymorphisms were found using Sat37 and Sat375 on the F LG, confirming pervious work by Berger and Minor (1999). Segregation ratios showed that resistance to Cercospora seed infection in PI 80837 is conditioned by a single dominant gene independent of Phomopsis resistance. Cercospora resistance was linked between Sat308 (6.6 cM) and Satt594 (11.6 cM) on the G LG. These SSR markers should be useful for selection of resistant genotypes in breeding programs.

27 Confirmation of QTL Associated with Resistance to Soybean Cyst Nematode Races 2 and 3 Using Pedigree Analysis in Soybean

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Pedigree tracking was used to confirm molecular markers used for marker-assisted selection (MAS) of soybean *Glycine max* (L.) Merr by examining the association between QTL for SCN resistance and flanking SSR marker alleles in U.S. cultivars. These cultivars are descended from PI 88788, PI 437654, PI 209332, PI 90763, and Peking through 60 years of breeding. It is assumed that presence of alleles from those ancestors at the marker loci conditioning resistance to races 2 and 3 was associated with SCN resistance in descendents. Based on pedigree, descendents from Fayette and Pickett seem to have resistant alleles from PI 88788 and Peking, respectively. Because some cultivars have two resistant origins (Bedford descendents from PI 88788, PI 90763, or Peking; Hartwig descendents from PI 437654 or Peking), the allelic information from flanking markers will be used to distinguish resistant sources for those descendents. Approach based on pedigree analysis using molecular markers will provide information on which flanking markers should be used for MAS to identify breeding lines resistant to an individual race or multiple races of SCN.

29 Mapping Large-insert Genomic DNA Clones of *Glycine soja* to the Physical Map of *G. max*

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Glycine soja is believed to be the ancestor species of soybean *G. max* (L) Merr. Comparative study of large-insert genomic DNA clones between *G. soja* and *G. max* will be useful for genetic diversity and domestication research of soybean. The objective of this research is to place BAC (bacterial artificial chromosome) clones of *G. soja* containing mapped simple sequence repeat (SSR) DNA markers and expressed sequence tags (ESTs) to the physical map of *G. max*. A BAC library of 39,936 clones from *G. soja* line PI 468916 and the physical map of *G. max* cultivar Forrest are used for this study. The *G. soja* library was screened to identify SSR- or EST-containing clones by a PCR-based approach using SSR primers or primers designed for the ESTs. One hundred and five clones for 65 mapped SSR markers and eight clones containing five ESTs were identified. These identified clones were fingerprinted and assembled into contigs. The fingerprints were then compared to the fingerprints of all clones in the *G. max* physical map to identify the matching regions on the physical map. Forty-five of the *G. soja* clones were mapped to the *G. max* physical map, whereas 56 were unable to be placed on the *G. max* physical map, suggesting that approximately 50 percent of the soybean genome has significantly diverged since it evolved and domesticated from *G. soja* at the nucleotide level.

31 Rps8 maps to a resistance gene rich region on soybean linkage group F.

Stuart G. Gordon, Dept. of Plant Pathology; Steven K. St. Martin, Dept. of Hort. and Crop Sci.; Anne E. Dorrance, Dept. of Plant Pathology, The Ohio State University

Phytophthora root and stem rot caused by *Phytophthora sojae* (M.J. Kaufmann & J.W. Gerdeman) is a serious disease of soybeans worldwide. Recently, a new locus for resistance to *P. sojae*, Rps8, was identified and mapped in two small soybean populations. The objective of this study was to verify the location of *Rps*8 in a much larger population and to identify molecular markers that are more tightly linked to *Rps*8 and suited for marker-assisted selection. One hundred forty F2:3 lines of Williams x PI399073 were genotyped using SSR and RFLP markers. The lines were inoculated using the hypocotyl inoculation technique with races 1, 17 and 25 of *P. sojae*. The segregation ratio of 26:71:43, RR:RS:SS, for *Rps*8 was consistent with that expected for a single, dominant gene. *Rps*8 maps to MLG F, 10 cM from the SSR marker Satt114. This region of the soybean genome contains numerous other resistance gene loci and pathogen and pest resistance QTL.

33 ADVANCED BACKCROSS QTL ANALYSIS IN A MATING BETWEEN *Glycine max* AND *Glycine soja*

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The advanced backcross (AB) method is a means of reducing the number of donor parent alleles present in any given backcross inbred line (BIL). With fewer donor alleles present, deleterious alleles can be readily exposed; conversely, favorable donor alleles at quantitative trait loci (QTLs) can be more easily recognized. To evaluate the AB method of QTL detection in soybean, a population of 296 BC2F4.6 BILs was generated from a mating between *Glycine max* (Dunbar) and donor parent *Glycine soja* (PI 326582A). The 296 BILs have now been genotyped at 200 simple sequence repeat (SSR) marker loci and four classical marker loci, creating a 1605.2 centimorgan (cM) genetic map of 44 linkage groups (LGs) that, on the basis of SSR map position, were aligned with the 20 published soybean LGs. There were some positive transgressive BIL segregants for seed yield, but none were significantly better than Dunbar. In the QTL analyses, a statistically significant seed yield QTL was detected on LG-A2, flanked by SSR marker Satt315 and classical marker I; however, the additive effect of the PI 326.582A allele at this QTL was a seed yield reduction of 226 kg ha^{-1}. No other significant yield QTLs were detected. In fact, all introgressed genomic segments of this *G. soja* parent had negative (though most were not statistically significant) effects on yield. However, some LG-C2 segments had nonsignificant positive additive effects. Two well-known QTLs on LG-I and LG-E that affect seed protein and oil content segregated in this population. The introgressed PI 326.582A alleles at these QTLs enhanced seed protein by a respective 1.4 and 0.7 percentage points, but also pleiotropically reduced oil by 0.5 and 0.4 percentage points, respectively. Two QTLs that affected seed weight were mapped near Sat332 (LG-D1a) and Satt306 (LG-M) at which the introgressed G. soja alleles reduced seed weight by 0.4 grams and 0.5 grams, respectively. Although a majority of the BILs were lower yielding than Dunbar, 27 BILs yielded about the same as the 24 Dunbar check entries. This suggested that the Dunbar genome can, without deleterious effect, tolerate introgression of some *G. soja* genome. However, many of these 27 BILs were later maturing and taller, which may have confounded the yield comparison.

35 Inheritance and Mapping of Two Disease Resistant Genes related to Bacterial Leaf Pustule in Soybean

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Responses of bacterial leaf pustule (BLP) in soybean (*Glycine max* (L.) Merr.) were characterized by small yellow lesions with a raised pustule in the leaf center and BLP was controlled by a single recessive gene, *rxp*. But, the response of PI 96188 was very different shown necrosis having pustule without yellowdish halos. Based on simple sequence repeat markers analysis, *rxp* gene was located at 9.4 cM apart from Satt372 on linkage group (LG) D2. However the gene that controls BLP in PI 96188 was linked to Sat108 on LG O instead of Satt372 on LG D2. F_1 progeny from two different populations using PI 96188 as a crossing parent showed different response with PI 96188, so novel symptom of PI96188 to BLP was inferred as a recessive trait. Mapping of two disease resistant genes related to BLP using more molecular markers and inheritance mode analysis of novel symptoms to BLP in PI 96188 using F_2 populations are going to be studied.

POSTER ABSTRACTS ON

PLANT STRESS

38 Type III secretion system (TTSS) of *Sinorhizobium fredii* USDA257, a soybean symbiont, is regulated by quorum-sensing

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Sinorhizobium fredii USDA257, a Gram-negative soil-bacterium, produces nitrogen-fixing nodules on soybean roots in a cultivar-specific manner. Nodule development involves the exchange of molecular signals between soybean and USDA257. Isoflavones released from soybean roots are potent inducers of several USDA257 nodulation (*nod*, *nol*, and *noe*) genes including *nol*XWBTUV. This plasmid-borne locus is involved in regulating soybean cultivar specificity and some of the genes in this locus code for components of a TTSS. Type III secretion system is employed by both symbiotic and pathogenic bacteria to deliver effector proteins into the host cells. NopX and NopB (formerly known as NolX and NolB), which regulate soybean cultivar-specific nodulation, have been identified as nodulation outer proteins (nops). Secretion of these proteins to the extracellular media is mediated by type III secretion machinery. An intact NodD1 and presence of inducer (isoflavone) are prerequisites for the secretion of NopX and NopB. In the present study, we demonstrate that the secretion of NopX and NopB is a population density-dependent event. Secretion of Nops including NopX and NopB is maximal at low cell densities and sharply decreases at higher densities. Addition of three-day-old culture filtrate to a fresh USDA257 culture, even at low cell densities, prevented the secretion of Nops. However, this effect was reversible since USDA257 is able to secrete proteins when cultured in fresh medium. Consistent with these observations, the transcription of a *nop*B-*lac*Z and a *nop*X-*lac*Z reporter was also controlled in a density dependent manner suggesting the presence of a quorum sensing mechanism.

40 Free radicals: Potential candidates for development of soybean sudden death syndrome caused by *Fusarium solani* f. sp. *glycines*

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Fusarium solani f. sp. *glycines* (*Fsg*) have been reported to produce at least two phytotoxins. Cell-free Fsg-culture filtrates containing phytotoxins have been shown to develop sudden death syndrome (SDS) in soybean leaves. We investigated the disease leaves developed by feeding soybean seedlings with cell-free *Fsg*-culture filtrates. We have shown that a highly abundant protein was consistently degraded in diseased leaves. The mass fingerprint of this protein was determined by MALDI-TOF MS. A protein sequence database (NCBInr 2003) search using the mass fingerprint revealed that the abundant protein was the ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) large subunit. This result was confirmed by western blotting experiments. We have shown that light is essential for development of foliar symptoms and degradation of the Rubisco large subunit. Degradation of the Rubisco large subunit interrupts the carbohydrate assimilation process and led to rapid accumulation of reactive oxygen species, which are presumably involved in foliar symptom development. TUNEL staining data suggested that the programmed cell death process was initiated in leaves fed with cell-free *Fsg* culture-filtrates. Rubisco large subunit degradation, free radical accumulation, and initiation of the programmed cell death process were also observed in leaves fed with partially purified *Fsg* phytotoxin. These data support the notion that phytotoxins elaborated by *Fsg* accelerate SDS development by generating free radicals through interruption in the carbon assimilation process.

42 Comparison of Gene Expression Profiles among Soybean Cultivars with Different Degrees of Tolerance to Fsg Toxin

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Soybean Sudden Death Syndrome (SDS) has become a major disease in many soybean-growing states. It can cause severe yield losses. The soilborne fungus, *Fusarium solani* f.sp. *glycines* (*Fsg*) is the causal organism of soybean SDS. The fungus releases a toxin that translocates to leaves where it causes distinctive chlorotic and necrotic interveinal patterns. These characteristic foliar symptoms can be reproduced by placing plant cuttings in solution containing sterile *Fsg* filtrate. The different response levels (susceptible or resistant) to Fsg toxin were characterized among several different soybean cultivars. Three cultivars, Essex (susceptible), Williams82(less susceptible) and PI567374 (partially resistant) were selected for this study. Based on the assumption that different gene expression patterns will be found within these cultivars in response to Fsg toxin, we used 9,216 element soybean cDNA microarrays to monitor genes expression patterns. Plants were grown for 2 weeks under controlled conditions, cut just above the root transition, and placed into *Fsg* toxin diluted 1:30. Leaf samples were collected at 8hr post inoculation. cDNA microarray analysis identified significantly different genes among these 3 cultivars based on R MAANOVA analysis. Gene expression differences were noted in response to toxin treatment as well as without treatment suggesting that tolerance may or may not be an inducible trait. qRT-PCR will be performed for a few physiologically interesting genes to verify the array results and to allow more in-depth studies of this disease.

44 Expression of defense genes in root tissues of two soybean cultivars with different levels of partial resistance to *Phytophthora sojae*.

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Phytophthora sojae is the causal agent of root and stem rot of soybean, and is considered a major constraint to the production of this crop worldwide. In partially resistant soybeans, the damage caused by the pathogen is restricted to the tap root and lower stem. An earlier study suggested that the interface between the expanding lesion and healthy tissue is the site where active lesion-limiting mechanisms and defense responses are important. To confirm this initial finding, we used Northern blot analysis to determine the expression level of nine defense-related genes including pathogenesis-related (PR) proteins and enzymes of the phenylpropanoid pathway during the course of infection. Following inoculation of soybean cultivars Conrad (partially resistant) and OX20-8 (susceptible) with *P. sojae*, 1.5 cm long root sections were collected at 0, 6, 12, 24, 48, 72 and 120 h after inoculation (h.a.i.). Analogous root tissue samples were collected from non-inoculated and mock inoculated control plants. Interestingly, the expression of genes involved in the phenylpropanoid pathway together with PR-1a accumulated at a higher level in OX20-8 than Conrad between 48 and 72 h.a.i. In contrast, levels of beta-1,3-endoglucanase (EGL) at 6 h.a.i., basic peroxidase (IPER) at 72 h.a.i. and matrix metalloproteinase (MMP) at both 6 and 48 h.a.i., were significantly greater in Conrad. These results suggest a possible involvement of EGL, IPER and MMP as factors involved in the expression of partial resistance to *P. sojae* in soybean.

46 Root response to *Fusarium solani* f. sp. *glycines*: Changes in transcript abundance in partially resistant and susceptible soybean

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Complete and partial resistance depends upon pathways induced after pathogen strain recognition. Temporal rate reducing resistance to sudden death syndrome (SDS) of soybean is conferred by 6 quantitative trait loci (QTL) in progeny derived from cultivars Essex x Forrest. In a recombinant inbred line 23 (RIL 23), beneficial alleles of all 6 QTL were stacked. Roots of RIL23 and its susceptible parent Essex were infested with the SDS pathogen, *Fusarium solani* f. sp. *glycines*. Transcript abundance (TA) of 191 ESTs was studied at five time points after infestation. The initial response of the roots was not different among genotypes. By day 7 and 10 the infested roots of Essex failed to accumulate transcripts of sufficient defense related genes in response to infection, resulting in the onset of disease symptoms by day 14 after inoculation. However, in RIL23, the abundance of 35 transcripts was increased by more than two fold at day 3, 7 and 10 after inoculation. Four trend groups were detected among clusters of genes with similar patterns of transcript accumulation. Each cluster encompassed some of the early (81), middle (88) and late (129) accumulating transcripts. Gene functions included resistance (analogs), signal transduction, plant defense, cell wall synthesis and transport of metabolites. Pathways that responded included the protein phosphorylation cascade, the phospholipase cascade and the phenolic natural products pathways, including isoflavone and cell wall synthesis. The number of genes increased in TA and the number of pathways where these genes belong is an indicative of the complexity of partial resistance of soybean roots to *Fsg* infestation.This research is funded in part by a grant from United Soybean Board to MJI.

48 ISOLATION OF DEFENSE SIGNALING GENES THAT ARE INDUCED IN THE SOYBEAN-*PHYTOPTHORA SOJAE* INTERACTION

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Stem and root rot disease caused by the oomycete pathogen *Phytopthora sojae* is a serious soybean disease. We are interested in isolating soybean genes that are transcriptionally regulated immediately following infection with the pathogen. A cDNA library (Gm-c1084) was constructed from an equal amount of poly (A) RNA isolated from infected etiolated hypocotyl tissues, harvested two and four hours following inoculation with *P. sojae* race 1. Over six thousand cDNA clones were sequenced and sequences of 4,737 were deposited in the GenBank. In silico subtraction of these 4,737 sequences from 152,000 expressed sequence tags (ESTs) originating from unstressed soybean cDNA libraries resulted in identification of 225 genes that are unique to the Gm-c1084 library. Of these 225 genes only eight were identified to be *P. sojae* genes. Blasting of remaining 217 soybean genes individually to the GenBank database resulted in putative annotation of 90 genes. Among these 56 percent encode metabolism-related proteins, 13 percent receptors, 9 percent transcription factors, 7 percent cell structure-related proteins, 4 percent signal transduction proteins, 4 percent stress-related proteins, 3 percent plant defense-related proteins, 3 percent transporters, and 1 percent retrotransposons. Macro array analyses of all 225 selected genes led to identification of 67 soybean genes that showed two-fold induction in *P. sojae* infected tissues as compared to the water control. Northern blot analyses showed that of the 11 candidate transcription factors or signal transduction pathway genes six were induced following infection with *P. sojae*. Identification of these defense signaling genes should facilitate the creation of novel soybean germplasms with enhanced disease resistance against *P. sojae* and other soybean pathogens.

50 Genetic Engineering of Soybean Plants for Resistance to the Herbicide, Dicamba

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Dicamba is a cost-effective herbicide that is widely used for the control of broad-leaf weeds in the production of corn and wheat (with usual application rates of 0.25 to 0.5 lb/acre). Because of its specificity for killing dicot plants, dicamba cannot be used in the production of dicot crops such as soybeans, canola and cotton. We have utilized the oxygenase gene from Pseudomonas maltophilia, strain DI-6, a bacterium that metabolizes dicamba, to genetically engineer tobacco, tomato, Arabidopsis and soybean plants for resistance to treatment with dicamba. Transgenic soybean plants sprayed in the field at 2.5 lb/acre should no signs of damage (compared with nonsprayed, nontransgenic plants of the same variety), while dicamba-treated nontransgenic plants were dead one week after spraying.

53 Identification of QTL for Soybean Resistance to Sclerotinia Stem Rot *Sclerotinia sclerotiorum* in the Merit x PI194639 Population

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Sclerotinia stem rot has become a major disease in soybean production areas of the Midwestern United States. Although thousands of soybean plant introductions have been evaluated for resistance to the disease no soybean varieties or PIs have been identified as completely resistant to this pathogen. Genetic studies have shown that this resistance is quantitatively inherited. The objective of this study was to map quantitative trait loci for the resistance in a population of recombinant inbred lines derived from a cross between Merit and PI194639. A total of 500 simple sequence repeat markers were screened. Of these 261 markers were found to be polymorphic and were used to genotype 153 F45 RILs. The disease response of these lines was evaluated in a greenhouse under controlled environmental conditions using the cut stem inoculation method. Lesion length at 14 days after inoculation was measured for each tested plant. A linkage map was made with JoinMap 3.0 and QTL analysis was performed using single factor ANOVA with SAS 9.0 and composite interval mapping with MapQTL 4.0. Single factor analysis showed that 20 markers on eight molecular linkage groups were significantly $P<0.05$ associated with lesion length at 14 DAI. The analysis showed these markers mapped four putative resistant QTLs to MLG A2 D1b J and K. Although no major QTLs were detected from PI194639 associations between lesion length and the markers on MLG D1b and J showed potential benefits for soybean improvement programs. It was particularly noted that one QTL on MLG J was in the same genetic region as genes conferring resistance to brown stem rot in soybean.

55 Loci Underlying Resistance to Manganese Toxicity Mapped in a Recombinant Inbred Line Population of Essex x Forrest.

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Resistance to Mn toxicity is associated with some soybean cultivars grown on acidic soils or in hydroponics. Our objective was to identify SSRs linked to QTL for resistance to Mn toxicity. A RIL population from Essex x Forrest cross and 240 SSRs were used. The response of five plants per genotype to Mn was measured by leaf chlorosis (scored from 0-5) and root necrosis (scored from 0-5) from 7-28 days after treatment with 125 M of Mn in hydroponics. ANOVA and MapMaker/QTL were used to identify regions underlying the responses. Three genomic regions on different linkage groups were found to contain QTL for resistance to necrosis during Mn toxicity. The regions located on linkage groups C2 (BARCSatt291), I (BARCSatt239) and G (OPOEO2) were each significantly associated ($P<0.005$, R220) with root necrosis at 7 days after treatment. The regions all derived the beneficial allele from Essex. One of the previously identified RAPD associated root necrosis QTL was identified in this new study. However, no QTL for leaf chlorosis were detected ($P<0.005$) and none of the RAPD identified leaf chlorosis QTL could be identified. We conclude that root and leaf resistance to manganese toxicity are environmentally sensitive quantitative traits determined by separate loci of different number and magnitude of effect.

57 Lignin synthesis and degradation and soybean root resistance to the soilborne pathogen *Fusarium solani* f. sp. *glycines*

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The changes in lignification of root tissues induced by the soilborne fungal pathogen *Fusarium solani* f. sp. *glycines* (*FSG*) infection were studied using the hairy root cultures of two soybean genotypes with different sensitivity to FSG: partially resistant PI567.374, and susceptible Spencer. TGA thioglycolic acid lignin levels were higher in non-inoculated roots of PI567.374 than in Spencer. FSG inoculation of hairy roots activated lignin synthesis as measured by ^{14}C-phenylalanine (Phe) incorporation after a 2h exposure. The ability of *FSG* to degrade lignin was shown by (i) a decrease in the amount of ^{14}C-Phe incorporated into lignin over time after FSG inoculation of hairy roots, (ii) the *FSG* fungus catalyzed the release of $^{14}CO_2$ from purified ^{14}C-labeled Klason lignin, (iii) *FSG* degraded polymeric aromatic dyes in culture and (iv) the FSG ability to produce laccase and lignin peroxidase, the major fungal lignin degrading enzymes. The *FSG* laccase and lignin peroxidase activities and ability to decolorize polymeric dyes were similar to the levels found with the known powerful lignin-degrading fungi *Polyporus tulipifera* and *Schizophyllum commune*. This ability to degrade lignin by *FSG* may play an important role in entry and colonization of soybean roots.

POSTER ABSTRACTS ON

COMPARATIVE AND FUNCTIONAL

GENOMICS

60 Analysis of soybean responses to *Xanthomonas axonopodis* with oligo microarray containing genes related to disease resistance and metabolism

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BLP caused by *Xanthomonas axonopodis* pv. *glycines* is one of the most prevalent bacterial diseases in soybean . Responses to *X. axonopodis* were different depending genotypes. The purpose of this study is comparisons of gene expression profiles before and 24 hr after *X. axonopodis* inoculation with two soybean genotypes, PI 96188 showing novel response to *X. axonopodis* and SS2-2, BLP-resistant. After leaves from each genotype were harvested before and 24 hr after inoculation, cDNAs from before and 24 hr after inoculation were applied to oligo microarray containing only 100 genes related to disease resistance and metabolism with three replicates and dye swamping. After several steps of normalization were proceeded, several genes differentially expressed by double were identified. 15 different genes were up-/down-regulated in PI 96188 showing novel response to BLP, whereas only 5 genes were observed in BLP resistant SS2-2.

62 Analyses of Expressed Sequence Tags of *Fusarium solani* f. sp. *glycines* and Soybean During Infection

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Fusarium solani f. sp. *glycines* (*Fsg*) causes soybean sudden death syndrome. A cDNA library was constructed from cultured *Fsg* and *Fsg*-infected soybean roots. Over 2,000 randomly selected cDNA clones were sequenced and 1,940 expressed sequence tags (ESTs) corresponding to 1,659 unique sequences were identified. Sixty-nine percent of the 1,940 ESTs significantly matched entries in the NCBI nr protein database. A comparison of the predicted amino acid sequences of ESTs with translated versions of available fungal genome sequences identified 633, 904, 459, 692, 707, and 441 matches to the *Aspergillus nidulans*, *F. graminearum*, *Magnaporthe grisea*, *Neurospora crassa*, *Saccharomycetes cerevisiae*, and *Ustilago maydis* genome sequences, respectively. Comparison of EST nucleotide sequences with the soybean UniGene Set identified 905 EST matches, of which, 14 were similar to disease/stress related genes. The majority of classified ESTs were related to metabolism (38&#37), cell growth and/or maintenance (18&#37), and protein biosynthesis (16&#37).

64 Global expression analyses using microarrays of a soybean cDNA unigene set

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As part of the NSF-sponsored Soybean Functional Genomics Program, we have accumulated a set of unique genes from a larger collection of soybean 5' ESTs. The current unigene collection (or tentatively unique sequences) represents 36,864 low redundancy cDNA clones. These include reracked libraries Gm-r1070 (a set of 9216 cDNA clones from various stages of immature cotyledons, flowers, pods, and seed coats); Gm-r1021 plus Gm-r1083 (a set of approximately 9216 cDNA clones from 8-day old seedling roots, seedling roots inoculated with B. japonicum, whole seedlings, and 2 month old roots); Gm-r1088 and Gm-r1089 (a collection of 9216 cDNA clones each from a number of libraries made from cotyledons and hypocotyls of germinating seedlings and leaves and other plant parts subjected to various pathogens or environmental stress conditions). Functional assignments of clones were inferred by matching the BLASTX hits of the 5' and 3' sequences to the non-redundant databases. The inserts were amplified from each clone by PCRand were spotted onto glass slides for microarray analysis. Initially three arrays were produced, each with approximately 9,216 cDNAs, for a total of 27,648 genes. Currently we are producing two arrays with approximately 18,432 cDNAs each, for a total of 36,864 genes. We are currently using these arrays to study gene expression during soybean seed development (see poster by Jones, et. al.); during reprogramming of cotyledon cells associated with induction of somatic embryos in soybeantissue culture (Thibaud-Nissen, et al., Plant Physiol, 132:118-136, 2003),and to analyze isogenic lines containing mutations in the flavonoid pathway.

66 Proteomic Analysis of Soybean Root Hairs after Infection by *Bradyrhizobium japonicum*

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Infection of soybean root hairs by the compatible symbiont *Bradyrhizobium japonicum* is the first of several complex events, eventually leading to the formation of nodules on roots. In the current proteomic study, root hairs of soybean *Glycine max* (L.) Merr. after inoculation with *B. japonicum* were separated from roots by gentle physical shearing and filtering in liquid nitrogen and total proteins were analyzed by two-dimensional (2-D) polyacrylamide gel electrophoresis. In one experiment, ninety-six protein spots were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) to compare the protein profiles between uninoculated roots and root hairs. Another 37 spots derived from the root hair samples treated with *B. japonicum* over different time points were also analyzed by tandem MS. As expected, some proteins were shown to be differentially expressed in root hairs compared with roots (e.g., a chitinase and phosphoenolpyruvate carboxylase). Out of 37 spots analyzed by tandem MS, 30 candidate proteins were identified by database comparisons. These included several proteins that have previously been shown to respond to rhizobial inoculation (e.g., peroxidase and phenylalanine-ammonia lyase). However, novel proteins were also identified (e.g., phospholipase D and phosphoglucomutase). This research will lay a strong foundation for investigations into the earliest events of legume infection by rhizobia. In a more general sense, this work is also significant since it represents a rare case where functional genomic methods can be applied to a single, plant cell type.

68 Transcriptome and functional analysis of *Medicago truncatula* under water stress

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Drought is the major environmental stress affecting productivity of the economically important crops, including soybean. Although the molecular basis of drought tolerance has been well studied in *Arabidopsis* and cereal grass, limited information is available for legumes. Unique biological features like small genome, simple diploid, self-pollinated, fast generation time and the ability to be genetically transformed valued *Medicago truncatula* a good model species for legumes. The overall goal of this research is to gain a better molecular understanding of drought response in *Medicago* and seek to translate the information into soybean. In this study, we gave a gradual drought stress to *Medicago truncatula* by withholding water under controlled conditions and performed the transcriptome profiling of the leaves using the 16K 70mer oligoarray. Leaf tissues at different water deficit levels were collected during a gradual water stress after monitoring leaf water potential and other stress-related physiological parameters. This system helps discover genes induced by drought and the genetic network for stress adaptation. Moreover, re-hydration experiment helps find out the interesting molecular genetic mechanisms related to most of the leaf survival traits. Microarray hybridization was completed and the data analysis is in progress. From the transcriptome profiling data, we will select differentially expressed genes and perform quantitative RT-PCR to validate the data from expression profile. This will lead to the identification of the key signaling pathways involved in drought tolerance. This study could be beneficial for legume drought tolerance improvement especially for soybean.

70 Microarray analysis of differential gene expression between resistant and susceptible soybean cultivars in response to *Aphis glycines*

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Soybean aphid, *Aphis glycines*, is a relatively new pest to soybean in the U.S. and may cause yield losses of up to 50 percent. Sources of aphid resistance were discovered in seven soybean accessions, but the molecular basis of the resistance is unknown. The objective of this study was to identify genes induced or repressed in response to aphid feeding and to identify candidate genes associated with aphid resistance. Two sets of soybean cDNA microarrays representing over 18,000 soybean ESTs were used. Induced defense responses presumably occurred within 6 hours after initial probing since the differences in probing time varied when aphids were compared on resistant and susceptible plants. Therefore, to monitor transcriptional responses to aphids by microarray analysis, leaf samples were collected at 6 and 12 h after aphid application for RNA extraction. Based on an ANOVA statistical test and fold-change thresholds, differentially expressed genes between resistant and susceptible cultivars were selected. Approximately 96 and 64 genes changed or were at a higher level in the resistant line compared to the susceptible line at 6 and 12 h after initial aphid probing. qRT-PCR of a few specifically induced genes were assayed to verify the microarray results and to monitor gene expression at additional time points.

72 Novel identification, using microarrays, of the *Wp* flower color locus as flavanone 3-hydroxylase, an enzyme that plays a key role in modulating the flavonoid pathway during pathogen challenge.

Gracia Zabala, Lila Vodkin, University of Illinois

A stable mutation at the *Wp* locus in soybean (*Glycine max*) that changed the phenotype of purple colored flowers to pink and has an epistatic effect on seed size and composition, was derived from a flower color chimeric plant that arose spontaneously in the field (Stephens and Nickell, 1992; Johnson et. al 1998). Using purified RNAs isolated from young flower buds of mutant, pink and purple isolines in hybridizations to one of the soybean cDNA microarray element (re-rack Gm-r1070), two flavanone 3-hydroxylase cDNAs were found to be highly over-expressed in the purple flower buds. RFLP analysis and tissue specific expression of this gene in purple and pink flower isolines have unequivocally shown that this flavanone 3-hydroxylase gene (*f3h*) is the *Wp* locus. A pivotal role for the *f3h* gene expression in the metabolism of flavonoid synthesis is becoming apparent. This is supported by our results from the analysis of steady state RNA levels of the *f3h* gene compared to those of several other key genes of the phenylpropanoid pathway after inoculation of soybean plants with a pathogen.Stephens, P.A. and Nickell, C.D. (1992) Inheritance of pink flower color in soybean. Crop. Sci. 32, 1131-1132.Johnson, E.O.C., Stephens, P.A., Fasoula, D.A., Nickell, C.D. and Vodkin, L.O. (1998) Instability of a novel multicolored flower trait in inbred and out-crossed soybean lines. J. Hered. 89 (6), 508-515.

74 Cellular Function Prediction and Biological Pathway Discovery in *Arabidopsis thaliana* Using Microarray Data

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Determination of protein functions and biological pathways at the genome scale is one of the most important and challenging tasks in the post-genomic era. Towards this, we have developed an integrated probabilistic method for cellular function prediction with a probability assessment, using microarray gene expression profiles, in conjunction with predicted protein-protein interactions and annotations of known proteins. We have applied our method to the *Arabidopsis thaliana* proteome and assigned function to 4451 out of the 19717 unannotated proteins. Towards biological pathway discovery, we have extended our computational method using *Dijkstras* algorithm to identify the components and topology of a pathway, and applied it for predicting the signaling pathway of phosphatidic acid as a second messenger in Arabidopsis.

75 Construction and Expression of Phenylalanine-free Gamma Zein Protein in Soybean Seeds

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Phenylketonuria (PKU) is an inherited genetic disorder in which individuals cannot break down the amino acid phenylalanine (PHE). A build-up of PHE in the bloodstream results in seizures, brain damage, and mental disorder. Individuals suffering from this disease must maintain a strict life-long diet low in PHE. This low protein diet is supplemented by PHE-free crystalline amino acids, which cannot be cooked. The long-term goal of our project is to provide PKU patients with a more nutritious, palatable source of dietary protein. Our strategy is to produce a PHE-free protein in transgenic soybean as a value-added (nutraceutical) trait, which then can be separated from the native soy proteins and used as a protein supplement. Phase I of this project reported here is to construct a synthetic version of a gene encoding the 27kD gamma zein gene lacking PHE, express this gene in soybean, and biochemically purify the protein from the transgenic seed.

79 Characterization of a soybean with oil low in saturated fatty acids, low in polyunsaturated fatty acids and high in oleic acid

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Oils high in oleic acid possess increased oxidative stability. Such oil circumvents the need for hydrogenation thereby negating production of trans-fatty acids. Moreover, enhanced oxidative stability is also desirable for industrial applications, such as biodiesl. Employing a genetic engineering approach we have simultaneously down-regulated two soybean genes in a seed-specific fashion, FAD2-1 and FatB. This resulted in the recovery of transgenic soybeans with enhanced oleic acid and a concomitant reduction in polyunsaturated fatty acids along with low palmitic acid in the seed storage lipids. Field evaluations from a transgenic event designated 335-13 have been conducted over the last few seasons across two environmental conditions in Nebraska and Puerto Rico. The average fatty acid profile of this event at the T_7 generation harvested in Puerto Rico in 2004 among 219 samples representing approximately 182 bushels was 4.10.5 palmitic acid, 2.30.2 stearic acid, 86.42.5 oleic acid, 2.71,7 linoleic acid and 3.50.4 linolenic acid. In collaboration with Iowa States Biomass Energy Conversion Center this novel soybean oil is currently being evaluated as a biodiesel fuel.

81 Using an allergen-suppressed soybean line as a platform for altering protein content

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Soybean (*Glycine max* L.) seeds with 40 protein are an ideal vehicle for the production of exogenous proteins. To date, the level of foreign protein expression in plants has been limited by such constraints as allocation of nutrient resources and cellular compartmentalization. In an effort to produce large quantities of an introduced protein in soybean seeds we are investigating the use of RNA interference (RNAi) technology to reduce endogenous protein levels and to free resources for foreign protein synthesis. Since 80 of seed protein in soybean is composed of the two storage proteins, 7S conglycinin and 11 S glycinin, our strategy is to down-regulate these exogenous proteins by RNAi. Some lines were futhered engineered to simultaneously suppress the production of oil in the soybean seed by using RNAi targeted towards oleosin (the major constituent of oil body membranes) in an attempt to suppress oil accumulation. For proof-of-principle, the introduced model foreign protein used was phaseolin from green bean (*Phaseolus vulgaris*). Phaseolin was chosen because it has been successfully expressed in plant systems so it was anticipated it would be correctly transcribed, translated and accumulated in the storage vacuoles. Previous work by other researchers has isolated variants from germplasm collections that were individual nulls for each of the two storage proteins. When these naturally occurring nulls were crossed the resultant plants were null for both storage proteins but had greatly increased levels of other seed proteins, in particular the immunodominant seed allergen Gly m Bd 30 k (P34) indicating that down-regulating one or both storage proteins could increase endogenous allergen content. In order to preclude increasing allergenicity in our transformants we used a previously engineered sense-suppressed P34 line as a source of starting material. Lines putative for RNAi11S and RNAi7S were produced by biolistics. Differential two-dimensional gel analysis (DIGE) with fluorescent dyes showed collateral changes in protein content in the transgenic lines compared to non-transformed controls. Western blot analysis showed the down-regulation of the storage proteins in these lines and the continued suppression of the P34 allergen. This work demonstrates the potential of down-regulating the two endogenous soybean seed proteins while maintaining the suppression of the immunodominant soybean allergen. By using the P34 suppressed line as source material for further genetic modification it will be feasible to introduce new genes into soybeans while minimizing the potential regulatory concerns of altered allergenicity of genetically engineered crops.

83 Characterisation of a 24 kDa Protein from Soybean Seed Coat

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Seed coat serves as a multifunctional organ with its role in protection as well as supply of nutrients to embryo sac during development. The composition of legume seed coat differs from embryo in many ways including its protein composition. We have identified an abundant 24 kDa protein (SC24) from soybean (*Glycine max* L. Merr) seed hulls by gel filtration chromatography. The corresponding cDNA and genomic DNA clones for SC24 were isolated and characterised, and expression patterns were determined. The deduced protein sequence of 219 amino acids included an N-terminal signal peptide and a region with similarity to the fibronectin type III domain. Complete sequencing of 15.8 kb EcoRI genomic fragment containing the 1.76 kb SC24 gene revealed the presence of a single intron within the gene. Analysis by DNA blot hybridisation showed that SC24 is a single or low copy gene in soybean genome. SC24 gene transcripts were present in seed coat from 30 days after pollination (DAP) till maturity. However, the protein did not accumulate in seed coats until 50 DAP. The expression of SC24 was also induced by pathogen infection. Furthermore, SC24 from the crude seed hull extract exhibited citrate binding activity. This study demonstrates the diversity in protein composition of seed coat compared to other tissues and complements our previous findings that defence-related proteins are abundant in the seed coat tissues.

85 Two-hybrid gene switch: A chemical inducible ecdysone receptor (EcR)-based gene regulation system for plants

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Chemical inducible gene regulation systems that activate or inactivate transgene expression have many potential applications in the basic understanding of gene function in plants. We have developed a two-hybrid EcR-based gene switch that regulates the expression of a reporter gene (luciferase) placed under the control of a minimal promoter and 5X GAL4 response elements. The two receptor constructs along with the reporter construct were electroporated into protoplasts isolated from cell suspension cultures of soybean (W82), as well as corn (BMS) and tobacco (Xanthi). The electroporated protoplasts were exposed to varying concentrations of methoxyfenozide and luciferase activity was measured after 24 h. Based on transient expression studies, the two-hybrid gene switch was found to be more sensitive with lower background and higher induction and expression of the reporter gene compared to the single format gene switch.

87 Engineering a feedback-inhibition insensitive serine acetyltransferase from soybean.

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Nutritional quality of soybean seed protein is compromised by paucity of the sulfur containing amino acids, cysteine and methionine. Efforts to ameliorate this condition have included plant breeding, expression of heterologous proteins with high sulfur content, and genetic manipulation of enzymes involved in the sulfur biosynthetic pathway. Recently, we isolated and partially characterized serine acetyltransferase (SSAT1), a key enzyme in sulfur metabolism, from soybean (Chronis and Krishnan, 2004, Planta 218:417-426). In plants, two types of SATase have been described. One is allosterically inhibited by L-cysteine and the other shows no inhibition. Mutational analysis of SATase has shown critical C-terminal amino acid residues which are essential for this inhibition. Drawing from this data, we generated L-cysteine insensitive soybean SSAT1 by site-directed mutagenesis. Enzyme activity studies indicated that residues Gly-277 or His-282 are critical in SSAT1 for L-cysteine inhibition. Complete elimination of the allosteric site abolished the feedback effect of L-cysteine, but did not affect the activity of SSAT1. The Km values for the mutated SSAT1 were comparable with the endogenous SATase. Currently, *Arabidopsis thaliana* and soybeans are being transformed with the native SSAT1 or feedback-inhibition-insensitive SSAT1. It remains to be determined whether preventing the allosteric inhibition of SSAT1 by L-cysteine could enhance accumulation of sulfur containing amino acids.

89 Isolation and Partial Characterization of a Soybean HSP90-like Promoter

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A soybean promoter was isolated using primers designed against an EST from soybean embryo development microarray data. The EST showed homology to a Hsp90 heat shock protein gene expressed during early embryogenesis. The promoter was recovered using PCR amplification of fragments from GenomeWalker libraries. Two fragments, extending to different upstream points in the promoter region, were placed upstream of *gfp*. These two constructions were introduced into lima bean cotyledons and soybean embryogenic tissue for evaluation of transient expression. Transient GFP tracking indicated delayed expression in lima bean cotyledons relative to the CaMV35S and Gmubi promoters. Efforts are underway to recover soybean tissues and plants, stably transformed with the HSP90-like promoter fused to *gfp*.

91 Characterization of Transgenic Soybean Containing an Inositol Methyl Transferase Gene from Ice Plant

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In an attempt to generate soybean with increased drought tolerance, transgenic soybean was generated following particle bombardment of embryogenic suspension culture tissue with an inositol methyl transferase (*imt*) gene from ice plant. IMT converts myo-isositol to ononitol, which is the first of two steps in the production of pinitol. Pinitol has been linked to drought tolerance in plants. From the 11 transgenic embryogenic lines, one line showed altered carbohydrate levels *in vitro*. This line accumulated around 30 mg ononitol per gfw, which is higher than previously observed for any soybean tissues, both transformed and wild-type. As embryos developed and plants were regenerated from this transgenic line, ononitol levels declined and pinitol levels increased. Pinitol levels in regenerated transgenic plant tissues were the same as control tissues, suggesting that the *imt* gene was either turned off or that the inositol to ononitol conversion is not rate limiting for pinitol production in soybean plants.

93 Metabolic Engineering of Vitamin E Biosynthesis in Oil Seeds and Photosynthetic Bacteria

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Tocopherols are synthesized by certain photosynthetic bacteria and plants and are micronutrients with antioxidant properties that play key roles in animal and human nutrition. Biosynthesis of tocopherol can be enhanced through increasing the supply of the tocopherol precursors homogentisic acid-HGA and phytyldiphosphate-PPP. Increased HGA production was detected in *Synechocystis* through a dark coloration of culture supernatant when the *p*-hydroxyphenylpyruvate dioxygenase (HPPD) and bifunctional prephenate dehydrogenase (*tyr*A) were expressed. In transgenic Arabidopsis seed-specific expression of the same two genes produced dark colored seeds. Alteration of HGA-levels in such seeds was confirmed by LC-MS and oxidative byproducts of HGA are known to produce a dark coloration. Coordinated expression of HPPD, *tyr*A, homogentisate phytyltransferase (VTE2) and geranylgeranyldiphosphate reductase in *Synechocystis* led to a total tocopherol increase of 16.5-fold compared to control cultures. Similarly when HPPD, *tyr*A, and VTE2 were expressed in transgenic Arabidopsis soybean and Canola seeds the tocopherol levels increased up to 5- 3.65- & 4.12-folds respectively with the majority of tocopherols being accumulated as tocotrienols. Tocotrienols represent another class of tocopherol compounds with their own health benefits. This work and previous studies now provide the tools for precise tailoring of oil seeds for tocopherol content and composition to enhance oil stability and nutritional value.

95 Definition of Soybean Genomic Regions that Control Seed Phytoestrogen Amounts.

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Soybean seeds contain large amounts of isoflavones such as genistein, daidzein, and glycitein that display biological effects when ingested by humans and animals. In seeds, the total amount, and amount of each type, of isoflavone varies by 5 fold between cultivars and locations. Previously we had identified 6 QTL controlling isoflavone content using 150 SSR markers. This study aimed to identify and delimit loci underlying heritable variation in isoflavone content with additional SSR markers. We used a RIL population (n100) derived from the cross of Essex by Forrest, two cultivars that contrast for isoflavone content. Seed isoflavone content of each RIL was determined by HPLC and compared against 240 polymorphic SSR markers by one-way analysis of variance. Two QTL that underlie seed isoflavone content were newly discovered. The additional markers confirmed and refined the positions of the six QTL already reported. The first new region anchored by the marker BARC-Satt063 was significantly associated with genistein (P0.009, R229.5) and daidzein (P0.007, R217.0). The region is located on linkage group B2 and derived the beneficial allele from Essex. The second new region defined by the marker BARC-Satt129 was significantly associated with total glycitein (P0.0005, R232.0). The region is located on linkage group D1aQ and also derived the beneficial allele from Essex. Jointly the eight loci can explain the heritable variation in isoflavone content. The loci may be used to stabilize seed isoflavone content by selection and to isolate the underlying genes.

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1. Addison's - Variety
2. Bambino's - Italian
3. Bagkok Gardens - Thai
4. Booche's - Cheeseburgers
5. Boone Tavern - Variety
6. Brady Commons - Fast Food
7. CC's City Broiler - Fine Dining
8. Cherry Street Wine Cellar - Variety
9. Cherry Street Artisan - Cafe/Breakfast
10. Chipotle - Mexican Style Fast Food
11. CJ's - Wings and Variety
12. Coffee Zone - Variety
13. Colosseum Bistro - Variety
14. Cucina Sorella - Breakfast Only
15. Das Kaffehaus - Middle Eastern
16. El Rancho - Mexican
17. Ernie's Steak House - Diner/Breakfast
18. Felinis - Variety
19. Flat Branch Pub & Brewing - Variety
20. Formosa - Chinese
21. Harpo's - Variety Bar Food
22. International Cafe - Middle Eastern/Greek
23. Jimmy John's Gourmet Subs - Sandwiches
24. Lakota Coffee Company - Pastry/Coffee
25. Main Squeeze - Vegetarian/Smoothies
26. Memorial Union - Sandwich/Pasta
27. Ninth Street Deli - Sandwiches/Salad
28. Osama's - Middle Eastern
29. Otto's Corner Bar and Grill - Variety
30. Panera Bread - Sandwiches/ Salad
31. Pasta Factory - Italian
32. Quinton's - Sandwich/Salad
33. Shakespeare's - Locals Favorite Pizza
34. Sake - Sushi
35. Shilo Bar and Grill - Variety Bar Food
36. Sub Shop - Sandwich/Soup
37. Subway - Sandwiches/Salad
38. Tellers - Sandwich/Soup/Salad
39. Trattoria Strada Nova - Fine Dining
40. Uprise Bakery
41. Village Wine and Cheese - Variety
42. Willie's - Variety Bar Food
43. Wing Zone - Chicken Wings