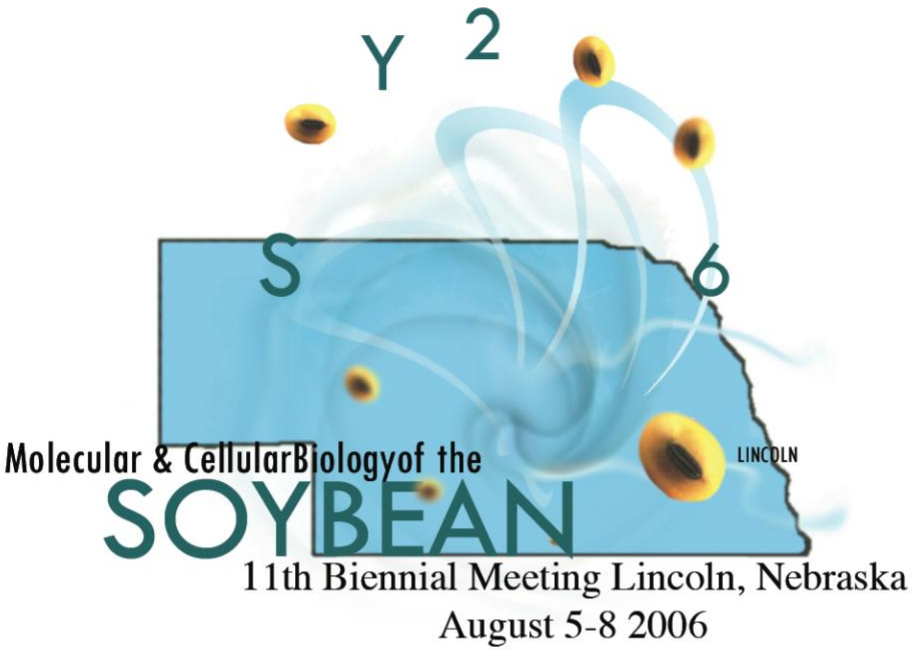
Soy/2006

The 11th Biennial Conference on the Molecular & Cellular Biology of the Soybean
5-8 August 2006
University of Nebraska, Lincoln NE

Conference Program

Abstracts of Oral & Poster Presentations



Sponsor and Donor Acknowledgements

As hosts of the Soy/2006 Conference, we are highly appreciative of the funds graciously provided by our corporate friends and soybean producer groups, whose logos are listed below. The funds provided by these Sponsors and Donors were used to partially support some Conference activities and functions, and supplemented the Conference Registration Fee income. We are mindful that this funding helped make this Conference an affordable one for the Attendees. We ask you all to appropriately applaud these Sponsors and Donors when they are recognized and thanked at the reception and in the program sessions.

Thank you all for your financial support! - Jim Specht and Tom Clemente, Local Hosts

WELCOME

We wish to extend a warm welcome to all Soy/2006 Conference attendees. The city of Lincoln is the home of Nebraska's flagship institution, the University of Nebraska-Lincoln and is the state's capitol. Nebraska, known as the "Cornhusker State", is the only state in the union with a unicameral legislature.

The Soy/2006 Conference program was assembled in large part by the session chairs and we feel you will agree that they put together a stellar group of presentations. We are truly grateful for the assistance provided by the University's Academic Conference Planning and Management group, specifically Virginia Uzendoski and Romeo Guerra, for web page maintenance and registration support. The site of the Soy/2006 Conference is the beautiful Embassy Suites situated in the heart of down town Lincoln. Local hotel arrangements and conference package were coordinated with assistance from Courtney Barr and Allan Tuttle. The conference banquet will be held at the University Champions Club and is being catered by Chances "R" of York, Nebraska. The Soy/2006 Conference hosts wish to recognize Jaime Johnson, director of building activities, for her help in organizing this activity. "The Executive Steel Band" a group originating out of New Orleans with a refreshing Caribbean sound provides the banquet's entertainment.

Finally we wish to thank each and every one of you for coming and helping make the 11th Biennial Conference of the Cellular and Molecular Biology of the Soybean a success.

Sincerely,

Tom Clemente & Jim Specht
Your Soy/2006 Hosts

Soy/2006 Conference Program

Saturday 5 August 2006

Conference Venue: Embassy Suites Hotel, Downtown, Lincoln, Nebraska

If you arrive in Lincoln by plane, take the no-cost hotel shuttle from the airport to the hotel
If you arrive in Lincoln by car, hotel parking is available (at a cost)

After arrival and hotel check-In:
If you have pre-registered, simply go to the Conference Registration Desk (near hotel lobby) to pick up your name tag, program, abstract book, and other materials.
If you did not pre-register, you will still go to the Conference Registration Desk to pay the registration on site.

Poster Presenters:
Please set up your posters (40Wx34H) soon after your arrival on Saturday, preferably before the guest lecture begins at the opening reception (but please not during the lecture – wait till after). The Poster Display Room will be posted.

Saturday Evening - Opening Reception
Hosts: Tom Clemente and Jim Specht
Embassy Suites Regents ABC

6:00pm: Welcome – Tom and Jim

6:15pm: Guest Lecture:

Random Fragment Sequencing of the Rice Genome: An Efficient Approach for Plant Gene Discovery. Stephen Goff, Syngenta Biotech, NC

Hors d'oeuvres and Refreshments immediately after the lecture

8:30pm: Reception Concludes – Dinner on your own (see Lincoln restaurant guide)

Sunday 6 August 2006

7-9am Breakfast (free for those staying at the Embassy Suites)

Morning Plenary Session I: Nutritional Genomics
Session Chair: Anthony Kinney, Pioneer/DuPont
Embassy Suites Regents AB

8:00am: Welcome and Announcements

8:05am: Nutritional Genomics: The Intersection of Dietary Molecules and Mammalian Genomics. Michael E. Fromm, UNL Center for Biotechnology, University of Nebraska, Lincoln, NE

8:45am: Nutrigenomic Approaches to Understanding the Health Benefits of *Omega*-3 Fatty Acids. Peter J. Gillies, E.I. DuPont de Nemours, Wilmington, DE

9:20am: Producing Nutritional Fatty Acids in Soybean Oil. Anthony J. Kinney, Pioneer Crop Genetics, DuPont Experimental Station, Wilmington, DE

10:00am: Refreshment Break

10:30am: Strategies to Enhance Metal Homeostasis in Soybean: Efforts to Enhance Seed Nutritional Value and Yield. Michael A. Grusak, USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX 77030

11:00am: Producing a Lower Allergen Content Conventional Soybean by Germplasm Search and Characterization. Eliot M. Herman, USDA/ARS, Danforth Plant Science Center, St. Louis, MO

11:30am: The Role of Systems-Biology in the Development of Soybean-Based Biorenewable Source of Fuels and Lubricants. Basil J. Nikolau, Plant Science Institute, The Biorenewables Initiative, Iowa State University, Ames, IA

12:00pm: Session Concludes

12:05pm: Lunch at the Hotel (provided to those with Name Badges)

Sunday 6 August 2006

Afternoon Concurrent Session I: Biotic Stress
Session Chair: Roger Innes, Indiana University
Embassy Suites Regents A

1:25pm: Welcome and Announcements

1:30pm: Gene Expression Profiling Soybean Response to Microbes. Steven J. Clough, USDA-ARS, Soybean/Maize Germplasm, Pathology and Genetics Research Unit, Urbana, IL

1:55pm: Gene Expression in Soybean Roots upon Invasion by the Soybean Cyst Nematode. Benjamin Matthews, USDA-ARS, Soybean Genomics & Improvement Laboratory, Beltsville, MD

2:20pm: A Genomic and Proteomic Analysis of the Resistance Response of Soybean to Cyst Nematode. David A. Lightfoot, Genomics Core Facility and Center of Excellence in Soybean Research, Teaching and Outreach, and Department of Plant, Soil and Agricultural Systems, Southern Illinois University at Carbondale, IL

2:45pm: Genomics of Disease Resistance in Soybean: QTL Mapping and Expression Profiling. M.A. Saghai Maroof, Dept. of Crop and Soil Environmental Sciences, Virginia Tech, Blacksburg, VA

3:10pm: Refreshment Break

3:30pm: New Sources of Resistance for *Phytophthora sojae*. Anne E. Dorrance, Dep. of Plant Pathology, The Ohio State University, OARDC, Wooster, OH

3:55pm: Cloning and Characterization of *Rps1-k*-interactors. Madan K. Bhattacharyya, Department of Agronomy, Iowa State University, Ames, IA

4:20pm: Comparison of Pathogen Recognition Mechanisms between Soybean and *Arabidopsis*. Roger W. Innes, Dep. of Biology, Indiana University, Bloomington,IN

4:45pm: Discussion

5:00pm: Session Concludes. Adjourn to Poster Viewing Session (Regents C**).**

5:00pm: Soybean Genomics Research Meeting (Embassy Suites Regency B**).**
6:00pm: Genomics Meeting Concludes. Adjourn to Poster Session.

7:00pm: Poster Session Closes

Dinner on your own. Consult Lincoln Restaurant Guide.

Sunday 6 August 2006

Afternoon Concurrent Session II: Molecular Breeding
Session Chair: Brian Diers, University of Illinois
Embassy Suites Regents B

1:25pm: Welcome and Announcements

1:30pm: A SNP-based Soybean Genome Map and Applications in Soybean Breeding and Genetics. Perry B. Cregan, [1]Soybean Genomics and Improvement Lab, USDA, ARS, Beltsville, MD

1:55pm: Marker-Assisted Selection and Its Contribution to Soybean Product Development . . . . Glancing Back, Looking Forward. Daria H. Schmidt, Pioneer Hi-Bred, International, A DuPont Company, Johnston, IA

2:20pm: Application of Molecular Markers to Improve Grain Yield Potential in Soybean. Warren M. Kruger, Monsanto Co, Ankeny, IA

2:45pm: Molecular Breeding for Quality Traits in Soybean. George L. Graef, Dep. of Agronomy & Horticulture, Univ. of Nebraska, Lincoln, NE

3:10pm: Refreshment Break

3:30pm: Confirmation of Associated Markers with Seed Protein Concentration in Soybean. Suk-Ha Lee, Dep. of Plant Science, Seoul National University, Seoul 151-921, Korea

3:55pm: Mapping and Confirmation of the 'Hyuuga' Red-Brown Lesion Resistance Gene for Asian Soybean Rust. Maria J. Monteros, Dep. of Crop and Soil Sciences, Univ. of Georgia, Athens, GA

4:20pm: Progress in Mapping QTL Controlling Yield in Soybean. Brian W. Diers, Dep. of Crop Sciences, Univ. of Illinois, Urbana, IL

4:45pm: Discussion

5:00pm: Session Concludes. Adjourn to Poster Viewing Session (Regents C**).**

5:00pm: Soybean Genomics Research Meeting (Embassy Suites Regency B**).**
6:00pm: Genomics Meeting Concludes. Adjourn to Poster Viewing Session.

6:30pm: Poster Session Closes

Dinner on your own. Consult Lincoln Restaurant Guide.

Sunday 6 August 2006 (evening)

5:00pm: Soybean Genomics Meeting (Embassy Suites Regents b**)**

The Soybean Genomics Executive Committee (Chair Specht, Brian Diers, Randy Nelson, & new member TBA) will host an informal meeting of all interested soybean scientists. The purpose of this meeting is provide a venue for the discussion of items of mutual interest relative to the Soybean Genomics Strategic Plan. Of interest is the Soybean Shotgun Sequencing Initiative, and Dan Rokhsar of DOE-JGI has been invited to provide an informal update on this activity. The Soybean Sequencing Steering Committee (Scott Jackson, Randy Shoemaker, Gary Stacey) will also provide an update on current activities related to a BAC Sequencing Effort. Other issues/concerns of Soybean Genomics Research Community can be raised and discussed at this time.

8:00pm: SoyCAP Meeting (Embassy Suites Regents B**) – This meeting is open to all scientists in the soybean genomics research community, and especially soybean breeders. A SoyCap proposal was submitted in 2005 in to the APGI-CAP program in the USDA CSREES National Research Initiative Competitive Grants Program, but the CAP award went to researchers in another crop. The purpose of this meeting is to engage the soybean research community in initial discussions about the possibility of submitting a revised or new SoyCap proposal relative to an anticipated USDA 2007 request for CAP proposals. Some detail about the prior SoyCap proposal effort can be found at this web site: http://digbio.missouri.edu/soycap/index.html A later meeting at this conference will be scheduled for those interested in going forward.**

Monday 7 August 2006

6 -9am Breakfast (free for those staying at the Embassy Suites)

Morning Plenary Session II: Structural and Functional Genomics
Session Chair: Scott Jackson, Purdue University
Embassy Suites Regents AB

8:00am: Welcome and Announcements

8:05am: The Structure of Soybean Genetic Diversity. David L. Hyten, Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD

8:35am: Sequencing Duplicated Regions of the Soybean Genome. Randy C. Shoemaker, USDA-ARS-CICGR, Ames, IA

9:05am: Evolution and Function of Chitin Signaling in Plants. Gary Stacey, National Center for Soybean Biotechnology, Divisions of Plant Science and Biochemistry, Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO

9:35am: Title TBA. Daniel S. Rokhsar, Dept. of Energy (DOE), Joint Genome Institute (JGI)

10:05am: Refreshment Break

10:30am: The Legume Information Network: A Component of the Virtual Plant Information Network. Greg D. May, National Center for Genome Resources, Santa Fe, NM

11:00am: Sequence-Based Comparisons of the Soy, *Medicago*, and *Lotus* Genomes. Steve B. Cannon, USDA-ARS and Dept. of Agronomy, Iowa State University, Ames, IA

11:30am: Organization of the Soybean Genome: Polyploidy and Genome Sequencing. Scott A. Jackson, Department of Agronomy, Purdue University, West Lafayette, IN

12:00pm: Session Concludes

12:05pm: Lunch at the Hotel (provided to those with Name Badges)

Monday 7 August 2006

Afternoon Concurrent Session III: Abiotic Stress
Session Chair: Henry Nguyen, University of Missouri
Embassy Suites Regents A

1:25pm: Welcome and Announcements

1:30pm: Everything You Can Do I Can Do Better - Functional Diversity in Abiotic Stress Responses. Hans J. Bohnert, Departments of Plant Biology and of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL

1:55pm: Identification and Confirmation of QTL Conditioning Drought Tolerance in Nepalese Soybean PI 471938. H. Roger Boerma, Center for Applied Genetic Technologies, University of Georgia, Athens GA

2:20pm: On-Farm QTL Mapping of Salt Tolerance in the Genetic Base of North American Soybean. Tommy E. Carter, Jr., USDA-ARS, Raleigh, NC 27607

2:45pm: QTL and Evaluation of Soybean for Tolerance to Soil Waterlogging. J. Grover Shannon, Division of Plant Sciences, University of Missouri-Columbia and Delta Center, Portageville, MO

3:10pm: Refreshment Break

3:30pm: Transgenic Approaches to Improving Drought Stress Tolerance in Maize. Jacqueline E. Heard, Monsanto Co., Mystic, CT

3:55pm: Genetic Engineering of Farnesylation for Crop Drought Tolerance and Yield Protection. Jiangxin Wan, Performance Plants, Inc., Bioscience Complex, Kingston, Ontario, Canada

4:20pm: Soybean Root Responses to Drought. H.T. Nguyen, National Center for Soybean Biotechnology and Division of Plant Sciences, University of Missouri-Columbia, MO

4:45pm: Discussion

5:00pm: Adjourn to Poster Viewing Session.

5:00pm: Informal meeting to discuss SNP Marker Genotyping as a Community Resource.

6:30pm: Poster Session Closes

7:30pm: Conference Banquet – University Champions Club
(Within easy walking distance of the hotel; includes cash bar, band, and dancing)
(If your spouse is not registered, he/she will need to purchase a banquet ticket)

Monday 7 August 2006

Afternoon Concurrent Session IV: Metabolic Engineering
Session Chair: Edgar B. Cahoon, Donald Danforth Plant Science Center
Embassy Suites Regents B

1:25pm: Welcome and Announcements

1:30pm: Genetic Modification of Oilseeds: Development of a High *Omega*-3 Vegetable Oil. Henry E. Valentin, Monsanto Co., Calgene Campus,

1:55pm: Elimination of Soybean Seed Phytate through Expression of Bacterial Phytase. Kristin D. Bilyeu, USDA-ARS, Plant Genetics Research Unit, Columbia, Missouri

2:20pm: Genetic Engineering of the Sulfur Assimilatory Pathway in Soybean. Hari B. Krishnan, Plant Genetics Research Unit, Agricultural Research Service-USDA, University of Missouri, Columbia, MO

2:45pm: Genetic Engineering of Tobacco, Tomato, *Arabidopsis* and Soybean Plants for Tolerance to Treatment with the Herbicide Dicamba. Donald P. Weeks, Department of Biochemistry, University of Nebraska-Lincoln, NE

3:10pm: Refreshment Break

3:30pm: Oxylipin Production in Soybeans. David F. Hildebrand, Department of Agronomy, University of Kentucky, Lexington, KY

3:55pm: Metabolic Flux Maps of Central Carbon Metabolism in Soybean Embryos. Jacqueline V. Shanks, Dept. of Chemical and Biological Engineering, Iowa State University, Ames, IA

4:20pm: Metabolic Redesign of Vitamin E Biosynthesis in Soybean for Enhanced Antioxidant Content. Edgar B. Cahoon, USDA-ARS Plant Genetics Research Unit, Donald Danforth Plant Science Center, St. Louis, MO

4:45pm: Discussion

5:00pm: Adjourn to Poster Viewing Session.

6:30pm: Poster Session Closes

5:00pm: Informal meeting to discuss SNP Marker Genotyping as a Community Resource.

7:30pm: Conference Banquet and Entertainment – University Champions Club
(Within easy walking distance of the hotel; includes cash bar, band, and dancing)
(If your spouse is not registered, he/she will need to purchase a banquet ticket)

Tuesday 8 August 2006

6-9am Breakfast (free for those staying at the Embassy Suites)

Morning Plenary Session III: Genomics, Micro-Arrays, Tilling, & More
Session Chairs: Jim Specht and Tom Clemente
Embassy Suites Regents AB

8:00am: Welcome and Final Announcements

8:05am: Agmagenomic Sequencing of Soybean. Matthew Hudson, Department of Crop Sciences, University of Illinois, Urbana, IL

8:35am: Gene Identification in Soybean by Microarray Analysis using Near Isogenic Lines. Lila O. Vodkin, Dept. of Crop Sciences, Univ. of Illinois, Urbana, IL

9:05am: Soybean TILLING: A Tool for Functional Genomics and Reverse Genetics. Khalid Meksem, Plants and Microbes Genomics and Genetics lab, Department of Plant Soil and Agricultural Systems, Southern Illinois University at Carbondale, IL

9:35am: Molecular Breeding - Evaluation of Chinese Germplasm with SSR Markers. Lijuan Qiu, National Key Facility of Crop Gene Resources and Genetic Improvement / Key Lab of Crop Germplasm & Biotechnology (MOA), Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, CHINA

10:05am: Refreshment Break

10:30am: Genetic Enhancement of Oleic Acid Concentration in Soybean Oil. Richard F. Wilson, USDA, Agricultural Research Service, Beltsville, MD

11:00am: Exhaust Emissions from a Diesel Engine Fueled with High Oleic Soybean Oil. Jon Van Gerpen, Biological and Agricultural Engineering, University of Idaho, Moscow, ID

11:30am: Why the United Soybean Board Is Still Interested in Genomics. Ed Ready, Production Program Manager, United Soybean Board, St. Louis, MO

11:45am: Closing Remarks – Tom Clemente and Jim Specht

11:55am: Brief Remarks - Host of the Soy/2008 (12th Biennial) Conference

12:00pm: Session and Conference Concludes

Soy/2006 Speaker Abstracts

Organized by Conference Program Session

Random Fragment Sequencing of the Rice Genome: An Efficient Approach for Plant Gene Discovery

Stephen Goff

Syngenta Biotech, NC

The genome of rice was sequenced to a depth of greater than 6-fold by a random genomic fragment draft sequencing approach, and assembled into approximately 42,000 sequence contigs. No finishing or manual sequence editing was used to generate this initial draft sequence and assembly. These assembled sequence contigs cover approximately 390 million base pairs (Megabase pairs or Mbp) of the estimated 430 Mbp genome with approximately 40 Mbp of repetitive DNA removed prior to assembly. The majority of the genome is covered with sequence contigs mapped to the physical and genetic maps. Approximately 42,000 genes or gene fragments longer than 500 base pairs were predicted using an integrated gene prediction, sequence homology, and protein domain/motif identification strategy. Approximately 75% of predicted genes with high or some supporting evidence appear to be duplicated in the rice genome. More than 85% of the predicted *Arabidopsis* genes display significant homology to genes predicted in rice, and approximately one-third appear to be plant-specific. The homologous proteins common to rice and *Arabidopsis* are approximately 30% identical on average. Synteny between the rice genome and other cereal genomes was found to be significant, whereas synteny between rice and *Arabidopsis* is restricted to short regions of the genome carrying 5 to 15% of the genes. This draft rice genome sequence represents the first crop genome to be sequenced to an extent to reveal the majority of genes, and the analysis reveals that the draft sequence of rice can serve as an excellent foundation for completion of the rice genome to high accuracy, as well as overlaying the genomes of the other major cereal crops maize, barley, and wheat.

Session: Opening Reception

Nutrigenomic Approaches to Understanding the Health Benefits of *Omega*-3 Fatty Acids

Peter J. Gillies[1], RA Velliquette[1], JP Vanden Heuvel[2] and PM Kris-Etherton[2]

[1]E.I. DuPont de Nemours, Wilmington DE;
[2]The Pennsylvania State University, University Park, PA

There is increasing awareness that long-chain polyunsaturated fatty acids (LC-PUFA) play a central role in the regulation of intermediary metabolism via modulation of gene expression. This regulation is often accomplished through a superfamily of lipid-activated nuclear transcription factors such as PPAR and SREBP. These proteins function as "sensors" of dietary lipids and in turn regulate lipid trafficking, storage, and metabolism. The vast number of genes and metabolic pathways regulated by these nutrient sensors has yet to be fully elucidated. In this regard, gene expression profiling offers a powerful tool to identify candidate genes and pathways regulated by LC-PUFA, and SNP analysis of these genes offers key insights into the highly variable response of humans to these dietary fatty acids. In general terms, the study of nutrient-gene interaction that focuses on the effects of nutrients on gene expression is called nutrigenomics, whereas a focus on the effect of structural variations in a gene on its response to various nutrients is called nutrigenetics. Using LC-PUFA as a case study, the effects of these fatty acids on PPARs, SREBP, SCD, and PPAR-SNPs will be discussed. The health benefit story that emerges from this case study underscores the importance of providing plant-based oils enriched in *omega*-3 fatty acids, such as EPA and DHA, to help individuals achieve dietary intakes consistent with contemporary recommendations (e.g., Gebauer et al, n-3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am J Clin Nutr 2006:83(Suppl):1526S-35S).

Session: Nutritional Genomics

Strategies to Enhance Metal Homeostasis in Soybean: Efforts to Enhance Seed Nutritional Value and Yield

Michael A. Grusak

USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX 77030

Iron is an essential nutrient that contributes to soybean growth and development. While this element is abundant in most soils, calcareous (high pH) conditions can lead to extremely low levels of available iron. Inadequate iron nutrition reduces photosynthetic capacity (chlorotic plants) and thus impacts yield. It also reduces the plant's pool of available iron for transport to and storage in seeds. We and others are interested in improving iron uptake capacity in soybean, but because iron is toxic in excess, any enhancements must be managed carefully. In this talk, I will present an overview of our knowledge of the existing physiological processes and homeostatic mechanisms in roots and leaves, which regulate iron acquisition and whole-plant partitioning of iron in soybean. I will discuss ongoing efforts to enhance root iron reductase capacity, which is believed to be the rate-limiting physiological process for root iron acquisition. The iron reductase is needed to enzymatically reduce rhizospheric ferric iron to ferrous iron; ferrous iron is the form transported into roots. We will discuss how this or other possible strategies can be used to successfully manipulate the endogenous iron homeostatic system, especially at the root level, in order to safely enhance iron nutrition. We will also discuss the potential impact of these changes on seed yield and seed nutritional quality.

Session: Nutritional Genomics

Nutritional Genomics: The Intersection of Dietary Molecules and Mammalian Genomics

Michael E. Fromm

UNL Center for Biotechnology, University of Nebraska, Lincoln, NE

The sequencing of the human genome has created new opportunities for understanding the molecular response to dietary molecules and how agricultural products can be used to increase human health and wellness. In particular, Affymetrix microarrays allow a whole genome response to be measured and the growing databases of the functions of individual genes connects changes in gene expression with possible functional and physiological implications. The specific example of the ability of dietary conjugated linoleic acid (CLA) to cause tremendous reductions in fat in mammals will be presented. Histological and microarray analyses of the fat loss in mice are helping to determine the mode of action of CLA to help guide its use in the diet. CLA occurs normally in the diet and can is being genetically engineered into soybeans for a low-cost, abundant source of the physiologically active isomer.

Session: Nutritional Genomics

The Role of Systems-Biology in the Development of Soybean-Based Biorenewable Source of Fuels and Lubricants

Basil J. Nikolau, Eve Syrkin Wurtele, Dan Nettleton, Jacqueline V. Shanks, Mark Westgate, Earl G. Hammond, Toni Wang, Sriram Sundararajan, Dermot Hayes

Plant Science Institute, The Biorenewables Initiative, Iowa State University, Ames, IA50011

Soybeans are a major source of high quality protein and edible oil. The increasing demand for alternative sources of bio-based energy and chemicals is providing new opportunities to develop novel soybean markets. This multidisciplinary team is developing a systems view of the soybean crop for the biorenewable economy that will simultaneously meet the increasing demands for biofuels and biolubricants, as well as the current needs for food and feed. To dissect the complex seed composition trait of soybean, we have developed near-isogenic soybean isolines, which differ markedly in their seed compositions. These isolines were derived from recombinant inbred populations, established between the parent lines, Evans, the high protein line PI153.296, and the low protein line PI438.472. The resulting isolines are being globally profiled for differences in gene expression at the level of transcriptomics, proteomics, metabolomics and metabolic flux. These datasets provide the basis for discovering molecular differences that ultimately express different seed composition. This understanding will provide insights into the processes that regulate seed composition, which we anticipate will reveal a rational strategy for manipulating soybean composition. In parallel, transgenic approaches are being undertaken to express novel genes that will improve the functionality of soybean oil for biolubrication and biofuel applications. Specifically, bacterial genes for the production of branched chain fatty acids are being expressed in soybean seeds -which should improve the lubrication properties of the oil. In addition, genes for the biosynthesis of monoacylesters (rather than the normal triacylesters) are being expressed in soybean seeds - which will make the oil more economical for biofuel applications. The oil-products of these genetic manipulations are being evaluated in for their physical, chemical, tribological and economic properties. This multidisciplinary approach is setting the stage for applying functional genomics tools to the issue of improving and developing new markets for soybeans.

Session: Nutritional Genomics

Producing a Lower Allergen Content Conventional Soybean by Germplasm Search and Characterization

Leina Joseph[1], Monica Schmidt[2], Theodore Hymowitz[1] and **Eliot M. Herman**[2]

[1]Dep. of Crop Sciences, Univ. of Illinois, Urbana, IL 61801;
[2]USDA/ARS, Danforth Plant Science Center, St. Louis, MO 63132

Soybean [*Glycine max* (L.) Merr.] seed contains an immunodominant human allergen P34 or Gly m Bd 30k (mentioned as P34) of the cysteine protease family. Of approximately, 16,266 accessions from USDA soybean germplasm screened, twelve P34 null lines were identified among soybean (*G. max*), wild annual (*Glycine soja* Sieb. and Zucc.) and wild perennial *Glycine* spp. *G. soja* were low P34 expressers while *G. max* and wild perennial spp. had non-detectable levels of the allergenic protein. Further investigation of *G. max* nulls by 2D-IEF/SDS PAGE showed all primary seed proteins present indicating the loss of P34 was not due to large scale restructuring of protein content. Southern/Northern analysis showed no large insertions or deletions to render the gene non-functional. The cDNA of both *G. max* nulls each showed the same 6 point mutations indicating the two nulls have a single origin. Of these six single nucleotide changes, four are predicted to result in an amino acid alteration. One such alteration results in a serine being replaced by a cysteine residue. The introduction of a cysteine residue might produce a mismatched disulfide bond formation producing an unstable P34 protein in the null soybean accessions. The isolation and introgression of soybean lines with low allergen levels will provide the basis for developing a low allergen line incorporated with other agronomically desirable traits in breeding program.

Session: Nutritional Genomics

Producing Nutritional Fatty Acids in Soybean Oil

Anthony J. Kinney

Pioneer Crop Genetics, DuPont Experimental Station, Wilmington, DE 19880-0353

Numerous studies have shown that inclusion of very long chain *omega*-3 polyunsaturated fatty acids (LCPUFAs), especially EPA (20:5, eicosopentaenoic acid) and DHA (22:6, docosohexaenoic acid), in the diet can have multiple positive health benefits including reduction of cardiovascular disease and improved cognitive function.. Fish oil can be a rich source of these fatty acids but is undesirable as a food ingredient because of the associated objectionable flavors that are difficult and cost-prohibitive to remove. Crop plants engineered to produce EPA and/or DHA offer a safe and cost-effective alternative to fish oils as a source of high quality *omega*-3 LCPUFAs for use as food ingredients. Towards this end, we have placed EPA and DHA biosynthetic pathways into soybeans with the goal of producing a commercially useful abundance of *omega*-3 LCPUFA in the seed oil. Soybean oil is typically rich in the *omega*-6 fatty acid linoleic acid (18:2) and, to a lesser extent, the *omega*-3 PUFA *alpha*-linolenic acid (18:3). Both 18:2 and 18:3 are potential precursors of 20:5 and 22:6 fatty acids. We have maximized the *omega*-3 LCPUFA content of soybean oil through the selection and expression of multiple EPA and DHA biosynthetic genes. By the isolation and characterization of a number of novel seed-specific promoters, by testing LCPUFA biosynthetic genes from multiple sources, by fine-tuning the activities of the expressed enzymes and by optimizing the spatial orientations of multiple transcriptional units, we have achieved optimal expression of the introduced pathways. Through this approach we have achieved target LCPUFA fatty acid contents up to 40 wt.% of the oil of homozygous soybean seeds. The LCPUFA traits are stable over multiple generations and prototype lines are planned for field-testing in the summer of this year.

Session: Nutritional Genomics

Gene Expression Profiling Soybean Response to Microbes

Steven J. Clough

USDA-ARS, Soybean/Maize Germplasm, Pathology and Genetics Research Unit, 1101 W. Peabody Dr., Urbana, IL 61801

We are using soybean microarrays to screen for gene expression changes in plants due to association with pathogenic and symbiotic microbes. In 2005 we published gene expression changes that occur during compatible and incompatible interactions with Pseudomonas syringae (Zou et al MPMI 18:1161-1174). Current projects underway are focusing on gene expression in response to the symbiont Bradyrhizobium japonicum as well as to additional pathogens where resistance does not involve the gene-for-gene hypersensitive response (HR). As our study of P. syringae supported the theory that photosystem centers may play a role in the HR, we are also examining gene expression profiles from soybean in response to herbicides that target photosystem centers. To assist with microarray analyses and to enhance cross project comparisons, we are developing a database of microarray gene information and expression results which will be made public once it is completed. In addition, PowerPoint presentations, wetlab protocols, PERL, R, and SAS code have been placed on our public websites (http://www.cropsci.uiuc.edu/faculty/clough/protocol.htm and http://www.cropsci.uiuc.edu/faculty/clough/project.htm) to assist others in the use of soybean cDNA and Affymetrix microarrays.

Session: Biotic Stress

Gene Expression in Soybean Roots upon Invasion by the Soybean Cyst Nematode

Benjamin Matthews[1,2], Vincent Klink[1], Margaret MacDonald[1], Hunter Beard[1] and Nadim Alkharouf[1,2]

[1]USDA-ARS, Soybean Genomics & Improvement Laboratory, Bldg 006, Beltsville, MD 20705; [2]School of Computational Sciences, George Mason University, Manassas, VA 20110 USA

The soybean cyst nematode (SCN), *Heterodera glycines*, is the major pest of soybean and causes an estimated one-half to one billion dollars in damage each year in the US. Genes expressed in soybean roots in response to SCN invasion were monitored using microarrays. Roots were trimmed and harvested from two independent biological samples of Peking inoculated with SCN race 3 (strain NL1-RHp; resistant reaction) and race 14 (strain TN8; susceptible reaction) at 0, 6 and 12 hr, 1, 2, 4, 6, and 8 days after infection by SCN and compared with uninfected controls and with the resistant cv. Peking at the same time points. Furthermore, laser capture microdissection (LCM) and EST (expressed sequence tag) analyses were used to study gene expression specifically in syncytial cells (feeding cells) formed by SCN. Roots of soybean, *Glycine max* cv. Kent L. Merr., plants susceptible to SCN, were inoculated and allowed to develop feeding sites (syncytia). Syncytial cells were isolated and collected using LCM. RNA was extracted from the isolated syncytia and used to make a cDNA library. ESTs were produced and analyzed. RT-PCR indicated enhanced expression of GmTubA1, GmTubB4, GmPIP2,2, aquaporin and several other genes including a pathogen resistance gene in syncytium-enriched samples as compared to samples extracted from whole roots. Gene and microarray databases were constructed and posted on-line with on-line analytical processing capabilities (OLAP), so scientists can mine the data without importing the data into third-party software. Analysis of these data identified a number of genes we are pursuing further to determine their role in the defense response and if they can be used to broaden resistance of soybean to SCN. See our web site at http://bldg6.arsusda.gov/benlab/ for further information.

Session: Biotic Stress

A Genomic and Proteomic Analysis of the Resistance Response of Soybean to Cyst Nematode

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The rhg1 gene or genes lie at a recessive or co-dominant locus, necessary for resistance to all Hg types of the soybean (*Glycine max* (L.) Merr.) cyst nematode (*Heterodera glycines*). Genomic research identified nucleotide changes within a candidate gene found at the rhg1 locus that were capable of altering resistance to Hg types 0 (race 3). A 1.5+0.25 cM region of chromosome 18 (linkage group G) was shown to encompass rhg1 using recombination events from four near isogenic line (NIL) populations and nine DNA markers. The DNA markers anchored two BAC clones 21D9 and 73P6. A single receptor like kinase (RLK; leucine rich repeat-transmembrane-protein kinase) candidate resistance gene was amplified from both BACs using redundant primers. DNA sequence showed 9 alleles of the RLK at Rhg1 in soybean germplasm. Markers designed to detect alleles showed perfect association between allele 1 and resistance to SCN Hg type 0 and 7 in three segregating populations, fifteen additional selected recombination events and twenty-two Plant Introductions. A quantitative trait nucleotide (QTN) in the RLK at rhg1 was inferred that alters A47 to V47 in the context of H297 rather than N297. The allele differences change the structure and activity of the RHG1 protein resulting in 53 proteins and 55 metabolites to significantly increase or decrease in abundance in seedling roots during SCN infection. Three nearly identical copies of the rhg1 gene were found at locations encompassed by QTL for resistance to SCN on L.G. A1 and E. A molecular basis for recessive and co-dominant resistance that involves interactions among paralagous disease resistance genes was inferred. Integrated –omic views of the cells responses to SCN will lead to discoveries that improve methods for developing new nematode resistant soybean cultivars.

Session: Biotic Stress

Genomics of Disease Resistance in Soybean: QTL Mapping and Expression Profiling

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Advancements in genome-wide expression profiling combined with molecular marker technology and map construction provide new opportunities to study complex traits including disease resistance. Little is known about the genetic and molecular basis of quantitative or partial resistance in crop plants. We are investigating the molecular genetic basis of partial resistance to *Phytophthora sojae* through expression profiling and QTL mapping. Eight soybean genotypes with partial resistance levels ranging from high to low were evaluated for gene expression in response to inoculation with *P. sojae* with Affymetrix arrays. Seven-day-old seedlings were inoculated below the root stem interface with either a mycelia slurry from a 7-day-old culture or an agar slurry for a control. Root pieces, 1.5 cm long, were collected surrounding the lesion margin at 3 and 5 days after inoculation (dai) from each plant, and were immediately frozen in liquid nitrogen. The lesion sizes were significantly different among the genotypes at 3, 5 and 7 days after inoculation. There were significantly higher levels of expression in 2201 genes at 3 and 5 dai between Conrad and Sloan and 152 genes in the resistant cultivars compared to the susceptible overall. There were 241 to 857 genes specifically up-regulated in individual resistant cultivars compared to the other resistant cultivars, suggesting that some mechanisms of resistance were specific to individual cultivars. Based on the disease reaction data, expression profiles and molecular marker diversity, two lines (V71-370 and PI407162) were selected for further analysis to dissect the genetic basis of partial resistance to *P. sojae*. In a preliminary study, an advanced generation RIL population of the two lines was screened with *P. sojae* resulting in the identification of several putative partial resistance QTLs. Chromosomal locations of these QTLs did not correspond to previously identified *Rps* genes or QTLs.

Session: Biotic Stress

New Sources of Resistance for *Phytophthora sojae*

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Phytophthora sojae, which causes Phytophthora root and stem rot of soybean, is managed primarily by deploying cultivars with single-gene mediated resistance. The objectives of this study were 1) to characterize the inheritance of resistance to *P. sojae* in PIs through a phenotypic analysis and 2) determine which *Rps* loci on (MLG) F, G, J or N were associated with resistance using SSRs. The PIs were crossed with the susceptible cultivar Williams and F2:3 and F2:4 populations were developed. The subsequent families were inoculated with *P. sojae* OH17 (vir 1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7), and OH25 (1a, 1b, 1c, 1k, 7). In two PIs, resistance was conferred by two genes to OH17 and three genes to OH25. Resistance to both isolates was conferred by a single gene in PI 398440 although the individual families were not resistant to the same isolates. Several populations have three *Rps* gene combinations while others may have either a novel *Rps* gene or a four-*Rps* gene combination. SSR markers were associated ($P<0.01$) with resistance to *P. sojae* isolate OH17 at the *Rps1* region in all populations. Two or more *Rps* genes conferred resistance to OH25 in all populations, but only one or no loci were identified with SSRs. Resistance conferred to OH17 and OH25 exist in different locations on the genome in some of these PIs, thus virulence already exists for some of the novel genes identified in this study. Based on this phenotypic and genetic analysis, both novel and uncharacterized *Rps* genes as well as of the combination of *Rps1c, 2, 3* (*a* or *b*) and *4* may be present. These PIs may serve as sources of novel *Rps* genes to more effectively manage Phytophthora root and stem rot.

Session: Biotic Stress

Cloning and Characterization of *Rps1-k*-interactors

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In the United States, the annual soybean yield loss suffered from the root and stem rot disease caused by *Phytophthora sojae* is valued over quarter of a billion dollars. Use of Phytophthora resistant cultivars has been the major method of controlling this pathogen. Phytophthora resistance is governed by a series of *Rps* genes. Unfortunately, *P. sojae* evolves rapidly to overcome resistance conferred by *Rps* genes. Therefore, incorporation of new *Rps* genes to soybean lines through backcrossing becomes a constant practice. Understanding the Phytophthora resistance mechanism could lead to development of transgenic soybean lines with stable and broad-spectrum resistance to most if not all *P. sojae* races. We have isolated the *Rps1-k-2* that encodes an intracellular receptor containing coiled-coil, nucleotide binding sites and leucine-rich repeats domains. The nucleotide binding sites (NBS) domain is comprised of two sub-domains, NB and ARC. ARC, is conserved in most plant and animal NBS-containing proteins such as apoptotic protease-activating factor 1 (Apaf-1), resistance (R) gene products and CED-4. Apapf1, the mammalian homologue of the nematode (*Caenorhabditis elegans*) CED-4, mediates caspase activation for apoptosis, a highly regulated cell death process. Recently we have isolated 12 candidate signaling factors that interact with *Rps1-k-2 in vivo* in yeast and *in vitro*. Candidate *Rps1-k-2*-interactors include a type II metacaspase. In order to determine the role of these candidate signaling proteins in Phytophthora resistance we have suppressed the expression of these factors through RNA interference in soybean cotyledons transformed by *Agrobacteriaum rhozogenes*. Silencing of several of the *Rps1-k-2*-interactor genes including the type II metacaspase, *GmMcII*, abolished the resistance against *P. sojae*. GmMcII is presumably the plant counterpart of mammalian caspases involved in apoptosis. Our data suggest that like Apaf1, *Rsp1-k-2* regulates GmMcII for initiating the programmed cell death, commonly known as hypersensitive cell death, associated with the expression of Phytophthora resistance.

Session: Biotic Stress

Comparison of Pathogen Recognition Mechanisms between Soybean and *Arabidopsis*

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This talk will focus on the molecular mechanisms by which plant disease resistance (R) proteins mediate pathogen recognition. In *Arabidopsis,* the RPM1 gene mediates resistance to Pseudomonas syringae strains that express either AvrB or AvrRpm1, while in soybean recognition of these two proteins is mediated by distinct, but closely linked R genes, Rpg1-b and Rpg1-r. With the help of several collaborators, we have cloned RPM1 and Rpg1-b and expect to soon clone Rpg1-r. Phylogenetic analyses revealed that RPM1 and Rpg1-b are not orthologous and almost certainly evolved the ability to detect AvrB independently. We were thus interested in determining whether the recognition mechanisms employed by RPM1 and Rpg1-b were the same or different. RPM1 has been shown to detect both AvrB and AvrRpm1 indirectly, via monitoring the status of a second *Arabidopsis* protein, RIN4. The current model proposes that AvrB and AvrRpm1 are injected into host cells by the bacterium, bind to RIN4 and thereby trigger phosphorylation of RIN4. This phosphorylation is then thought to cause a conformational change in RPM1, which then activates defense responses such as the hypersensitive response, a form of programmed cell death. Mutation of RIN4 blocks RPM1-mediated resistance. In addition, it has been shown that a third P. syringae protein AvrRpt2 can block RPM1-mediated resistance by proteolytically degrading RIN4. To test whether the soybean Rpg1-b protein employs a recognition mechanism similar to that of RPM1, we identified soybean orthologues of RIN4, tested their interaction with AvrB, determined whether they were substrates of AvrRpt2, and assessed whether Rpg1-b function was suppressed by AvrRpt2. Our data indicate that Rpg1-b likely detects AvrB by a mechanism very similar to RPM1, even though it evolved this ability independently. The implications of these findings relative to the identification and deployment of durable disease resistance genes will be discussed.

Session: Biotic Stress

A SNP-based Soybean Genome Map and Applications in Soybean Breeding and Genetics

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The discovery of single nucleotide polymorphisms (SNPs) in soybean would provide a source of markers that can be used in a diversity of applications including QTL mapping, marker assisted selection (MAS), positional cloning and association analysis. A total of 1,183 genes were positioned on the pre-existing SSR/RFLP-based soybean genome map by virtue of SNPs discovered and mapped in each gene. The SNPs were mapped using the Luminex flow cytometer as well as the Sequenom MassARRAY™ platforms. Functioning assays for 610 SNP loci are available for use on the Luminex flow cytometer. More than 1,000 assays are available on the Sequenom MassARRAY™ platform. The gene-based SNPs distributed evenly across the 20 soybean linkage groups (LG) as would be predicted based upon LG length although a significantly larger number of genes mapped to LGs E and J. Within LGs, the gene-based SNPs were clustered as would be anticipated from previous suggestions of gene-rich vs. gene-poor regions in soybean. An important question facing soybean breeders and geneticists is how best to use SNP markers in their research programs. Different research needs, from whole genome scans to MAS require different analysis systems. SNP analysis systems are now available that permit the assay of a few loci to thousands of loci in parallel. The availability of the integrated SSR/RFLP/SNP genetic map allows soybean researchers to choose the platform most efficient for their program. However, many of these loci with designed assays will not be polymorphic in a particular pair of parents. Thus, a system must be devised to determine which SNPs will be useful and if additional SNPs can be found within the sequence tagged sites for future assay development. We propose solutions to this and other problems related to the application of SNP markers by the soybean research community.

Session: Molecular Breeding

Marker-Assisted Selection and Its Contribution to Soybean Product Development . . . Glancing Back, Looking Forward

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Marker Assisted Selection (MAS) has held promise for impacting, perhaps revolutionizing, plant breeding disciplines. There are, however, few examples of commercial breeding programs making use of MAS in product development as a tool in applied breeding schemes for product development. Building the infrastructure of a high throughput MAS program is a costly and time consuming process, with vision and patience required. At Pioneer Hi-Bred, markers have proven to be a useful tool in the development of disease-resistant soybean (*Glycine max*) varieties. The efficiency MAS brings to the breeding process can be measured by the success in accumulating desired traits, particularly soybean cyst nematode resistance, in a larger percentage of candidate experimental lines throughout the breeding pipeline. This technology has been sufficiently streamlined to allow for high throughput data generation in a timely manner that supports traditional cultivar development. The improvements for sample turn-around and total data point capacity are continuing to make MAS a cost-effective tool in the breeding of high performing soybean varieties. Deployment of MAS for soybean product development at Pioneer Hi-Bred celebrated its 10th anniversary in 2005 and has significantly impacted early generation single plant selections, resulting in numerous successful commercial soybean varieties.

Session: Molecular Breeding

Application of Molecular Markers to Improve Grain Yield Potential in Soybean

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Plant breeding continues to improve on methodologies that sustain long-term improvement in developing new varieties. During the last 20 years, scientists have demonstrated that integrating DNA based molecular markers can improve plant breeding methodologies. Monsanto has integrated DNA based breeding tools into our global plant breeding programs. These tools improve selection efficiency in our plant breeding programs and enable development of new varieties. This presentation will outline some of the components to effectively use molecular marker information in a large scale breeding program.

Session: Molecular Breeding

Molecular Breeding for Quality Traits in Soybean

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Our breeding efforts at the University of Nebraska include both conventional and transgenic approaches to develop novel output traits in soybean. Two examples using transgenic approaches include: (1) soybeans with 85% oleic acid content in the seed oil and evaluation of the oil for biodiesel applications, and (2) soybeans with altered levels of both *omega*-6 and *omega*-3 fatty acids in the seed oil for improved human health benefits. Evaluation of agronomic and seed composition traits of a transgenic event designated 335-13 and its non-transformed parental line over multiple field environments and generations indicated stability of the high-oleic phenotype. Event 335-13 produced oil with 87% oleic acid to the current T10 generation evaluated in Nebraska and Puerto Rico. Monitoring of progeny derived from a cross with event 335-13 indicates the trait is inherited as a single dominant locus, with stability of the oleic acid phenotype maintained in different genetic backgrounds. Studies suggest that adjusting intake ratio of *omega*-6 to *omega*-3 fatty acids may enhance overall health. We produced a marker-free transgenic soybean event (420-5) carrying a *delta*-6 desaturase gene from the herb Borage. Transgenic and parental lines evaluated over environments showed *gamma*-linolenic acid (GLA) levels of 24% to 30% and stearidonic acid (STA) of 2% to 4% in the 420-5 event, vs. 0% in the parental control. Agronomic traits of the transgenic event were similar to the non-transgenic parent. Subsequent work to combine the borage *delta*-6 desaturase with the *Arabidopsis delta*-15 desaturase to increase STA levels was conducted. One event, 535-9, produced 6.4% GLA, 25.8% *alpha*-linolenic acid (ALA), and 38% STA in replicated field trials conducted during 2005 in Nebraska. Feeding trials in poultry and aquaculture applications are initiated to evaluate effects on quality of eggs and meat from animals fed with soybeans producing these novel *omega*-6 and *omega*-3 fatty acids.

Session: Molecular Breeding

Confirmation of Markers Associated with Seed Protein Concentration in Soybean

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An association map consisting of 159 markers was constructed on the basis of differences in allele frequency distributions between two subpopulations differing in protein content. Eleven putative QTLs were identified at P<0.0001, suggesting possible linkage to seed protein QTLs. Two of the markers (Satt431 on LG J, and Satt551 on LG M) may be linked to unreported seed protein QTLs. Two additional population sets with different protein content were used for confirmation of the QTLs detected by our analysis. Satt571 showed significant P-values at P < 0.05. Like the association study with the original population set, Satt551 and Satt431 newly identified QTL were confirmed again as the QTL for soybean seed protein content. The results suggest that our association analysis approach could be a viable alternative to linkage mapping for the identification of new QTLs in soybean. Using three simple sequence repeat (SSR) markers, 192 wild soybean (*G. soja*) and 160 cultivars populations were surveyed. These SSR markers were quantitative trait loci (QTL) detected as significant markers associated with soybean seed protein content by our previous association mapping study. In Satt384, 20 alleles were distributed without showing difference of allele frequencies, except one allele of 131bp in wild soybean. In contrast, two alleles of five alleles present in wild soybean showed very high frequency in cultivated soybean. Especially, the frequency of one allele in Satt182 was so high accounting for 76% of alleles in cultivated soybean. Some alleles were observed at a higher frequency in wild soybean than cultivated soybean. The frequency of the 131bp allele in Satt384 was high as above 50% of alleles in wild soybean, but cultivated soybean obtained this allele as only 2.9%. It could be thought these alleles present only in wild soybean with higher frequency would be unique alleles for soybean seed protein.

Session: Molecular Breeding

Mapping and Confirmation of the 'Hyuuga' Red-Brown Lesion Resistance Gene for Asian Soybean Rust

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Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi*, is a widespread disease of soybean [*Glycine max* (L.) Merr.], and has the potential to cause serious economic losses. The objective of this study was to genetically map red-brown lesion type resistance from the Japanese cultivar 'Hyuuga'. A population of 117 RILs from the cross of 'Dillon' (tan lesion type) × Hyuuga (red-brown lesion type, RB) was rated for ASR lesion type in the field at Attapulgus, GA, and fingerprinted using SSR markers. The RB resistance gene was mapped close to Satt460 on LG-C2. The Dillon × Hyuuga RILs were also inoculated with *P. pachyrhizi* in the greenhouse to confirm the RB-lesion phenotype. Using the greenhouse data, the resistance gene mapped between Satt460 and Satt307 on LG-C2. When field severity rating and lesion density in the greenhouse were mapped as quantitative traits, the *Rpp?*(Hyuuga) locus explained 22% and 15% of the variation, respectively ($P < 0.0001$). The RB lesion type was associated with fewer lesions and reduced sporulation when compared to the tan lesion type. A population of F5:6 lines from the cross of Benning × Hyuuga was screened with SSR markers in the 4 cM region on LG-C2 flanked by Satt134 and Satt460. Genotype at these markers was used to predict lesion type when the plants were exposed to *P. pachyrhizi*. All the lines selected for the Hyuuga markers in this interval had RB lesions and they averaged approximately 50% fewer lesions compared to F5:6 lines with tan lesions. Sporulation could only be detected in 6% of the RB lines compared with 100% in the tan lines. SSR markers associated with lesion type can be used by soybean breeders to develop cultivars with the *Rpp?*(Hyuuga) gene for resistance to ASR.

Session: Molecular Breeding

Progress in Mapping QTL Controlling Yield in Soybean

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Seed yield is the most important trait for both soybean breeders and producers and is controlled by a complex set of factors including the physiological efficiency of the plant, the environment, diseases, seed composition, and plant maturity. The economic importance of yield is the impetus to understand the genetic control of this trait and quantitative trait loci (QTL) mapping has made this possible. In the ten yield QTL mapping studies in the literature, 54 marker-yield associations have been reported. Many of these QTL also are associated with traits, such as maturity and disease resistance. For example, the region on linkage group C2 containing the maturity gene e1 was associated with yield in seven out of the ten studies in the literature. These associations may indicate the indirect effect on yield of these agronomic traits or they may identify a closely linked locus, but they do raise the question of how to define a yield QTL. We are interested in mapping yield QTL in order to identify alleles from exotic germplasm that could increase soybean yields in commercial cultivars. We have mapped yield QTL in seven populations that each has a parent with exotic ancestry. In these populations, we identified 18 genomic regions of exotic origin that are associated with yield increases. For 5 of these18 regions, there was no previous report in the literature of a yield QTL nor was there an association with an agronomic trait that may be indirectly increasing yield. We are attempting to confirm these yield QTL alleles in new populations and if successful, they will be useful in increasing the yield of North American commercial cultivars.

Session: Molecular Breeding

The Structure of Soybean Genetic Diversity

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Soybean has undergone several genetic bottlenecks. These include domestication in Asia to produce numerous Asian Landraces, introduction of relatively few Landraces to North America, and then selective plant breeding over the past 75 years. These genetic bottlenecks can decrease genetic diversity, change allele frequencies, increase linkage disequilibrium (LD), and eliminate rare alleles in the resulting population. Our goal was to evaluate the effect of these bottlenecks on the soybean genome. We sequenced 111 fragments from 102 genes and multiple fragments throughout three 300+kb regions in four distinct soybean populations: *Glycine soja*, the wild soybean; Asian *G. max*; N. Am. cultivar ancestors; and N. Am. public cultivars released in the 1980s. We show that the domestication bottleneck had the most impact, when the low sequence diversity present in the wild species was halved, 60% of the genes exhibited evidence of significant allele frequency changes, many rare sequence variants were lost and LD was increased from 60 kilobases (kb) up to 100 kb to >600 kb. Although soybean genetic diversity has been eroded by human selection after domestication, it is notable that modern cultivars have retained 72% of the sequence diversity present in the Asian Landraces and LD has been minimally affected. Despite the lack of extreme genetic erosion as a result of the introduction bottleneck and modern breeding, the genetic diversity of N. Am. elite cultivars is extremely low and LD is quite extensive. While the extensive level of LD may be useful for mapping QTL via association analysis, it is unknown what the impact of low diversity in modern soybean will have on future genetic improvement of this important crop.

Session: Structural and Functional Genomics

Sequencing Duplicated Regions of the Soybean Genome

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Most flowering plants are now thought to have polyploidy in their evolutionary past. Gene and genome duplication provide raw material for genetic diversity and have played a major role in shaping plant evolution. Genetic mapping data and several recent studies using soybean ESTs have confirmed that soybean has probably undergone at least two rounds of large-scale genome duplication. Analyses of gene transcripts show that gene family size and complexity reflects gene loss as well as gene family expansion. It is now clear that gene and genome duplications are prevalent in soybean. Depending upon the degree of conservation among duplicates, those duplicated regions may become a problem as 6 - 8 X genome equivalents of whole-genome shotgun sequences are being consolidated into contigs. In this study we identified and sequenced several duplicated regions within the genome. Data from these duplicated regions suggests that soybean is a mosaic of structures. We observed examples of extensive conservation of gene order and structure (cotton model) as well as regions showing extensive fractionation (maize model). In some instances conservation of sequence was maintained even within intergenic regions. In other instances, only the gene sequence used to identify BACs from putatively duplicated regions was in common between BACs. The analysis of the sequence data permits us to better understand the organization of the soybean genome and to gauge the potential difficulty in reconstructing a whole genome sequence.

Session: Structural and Functional Genomics

Evolution and Function of Chitin Signaling in Plants

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Chitin, a polymer of N-acetylglucosamine, is found in the cell walls of plant pathogenic fungi, as well as in insect exoskeletons. Plants have evolved to recognize chitin as an elicitor, responding with potent defenses to ward off the invading pathogen or pest. Lipo-chitin is produced by symbiotic rhizobia where it plays a crucial role in the initiation of the nodulation process on leguminous plants, such as soybean. In the first case, chitin triggers the plant to defend against the pathogen while, in the second, lipo-chitin triggers a response to welcome the invading symbiont. Our laboratory is interested in how plants can distinguish between these two chitin signals. We have used *Arabidopsis thaliana* as a model system to define the mechanism of chitin recognition and response. In the case of the lipo-chitin nodulation signal, recent data suggest that LysM receptor-like kinases (LysM RLKs) are the likely receptor. The LysM domain was first characterized from bacterial enzymes that modify the peptidoglycan cell wall. Peptidoglycan, a polymer of β 1-4 linked N-acetylglucosamine and N-acetylmuramic acid, is structurally related to chitin. Hence, it is hypothesized that the LysM domain recognizes chitin and chitin-like molecules and transduces these signals through the intracellular kinase domain. We identified 27 LysM domain-encoding genes in soybean, of which 12 encode LysM RLK proteins. These genes were mapped to contigs generated by fingerprinting soybean bacterial artificial chromosome (BAC) clones. The corresponding BACs were sequenced. In some cases, the regions were also genetically mapped. These results allow us to reconstruct the relationship of the various LysM RLKs to corresponding regions in other legumes and non-legumes. Surprisingly, a high degree of microsynteny was found to non-legumes, especially in those regions known to encode the putative Nod signal receptor. Sequence comparisons allow the reconstruction of the evolutionary history of the LysM motif found within the LysM RLKs. The data suggest a very ancient origin, consistent with the hypothesis that chitin recognition may be an ancient trait in plants.

Session: Structural and Functional Genomics

Title:

Author: Daniel S. Rokhsar

Author Affiliation: Dept. of Energy (DOE), Joint Genome Institute (JGI)

Abstract:

Session: Structural and Functional Genomics

The Legume Information Network: A Component of the Virtual Plant Information Network

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Soybean research today involves transcending genomic, transcript, proteomic, metabolic, genetic and phenotypic data often relying upon integrative and comparative analyses using model and reference species. Researchers frequently search multiple, often unlinked web sites to find data, information, and analysis tools to support their efforts. The Legume Information Network (LIN; http://lin.ncgr.org) is an extensible consortium of legume information resources using emerging semantic web services technology supplied by the Virtual Plant Information Network (VPIN) (http://vpin.ncgr.org/). LIN virtually integrates heterogeneous data and services from disparate legume information resources that are independently evolving. Currently, LIN links data and services provided by the Legume Information System (LIS) (http://www.comparative-legumes.org/) and *Medicago truncatula* DataBase (MtDB) (http://www.medicago.org/MtDB/). LIN allows legume researchers to dynamically discover data and execute services to build a user-defined research pipeline. Services can be one of three types: data provider (e.g. precomputed sequence annotation) services, analysis (e.g. BLAST) services, and visualization (e.g. synteny browser) services. New information resources and services can be registered using semantic web technologies, effectively growing the LIN over time.

Session: Structural and Functional Genomics

Sequence-Based Comparisons of the Soy, *Medicago*, and *Lotus* Genomes

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The legumes will soon be the first plant family to include three sequenced genomes. The majority of the euchromatic genomes of the model legumes *Medicago* truncatula and *Lotus* japonicus are now sequenced, and the sequencing of the soybean genome is underway. Genome-wide sequence-based comparisons between three genomes with common ancestry at less than ~50 million years will enable us to infer many features of the ancestral genome, to trace evolutionary differences such as rates of particular gene- or transposon-family expansions or losses, and to better understand processes of genome remodeling that follow polyploidy. It is already possible to make sequence-based comparisons of the *Medicago* and *Lotus* genomes, and genetic marker-to-sequence comparisons between soybean and *Medicago* or *Lotus*. These comparisons show a lack of large-scale genome duplications within the *Lotus* or *Medicago* genomes following separation of those lineages approximately 40 Mya, evidence of an older shared polyploidy event, and clear evidence of a more recent duplication in soybean (also observed by other researchers using other methods) following the separation from the *Medicago* and *Lotus* common ancestor at approximately 50 Mya. In contrast to the extensive rearrangements observed in the *Arabidopsis* genome, the *Lotus* and *Medicago* genomes have retained substantial gene collinearity, at the scale of whole chromosomes or chromosome arms. Interestingly, comparisons between soybean and *Lotus* or *Medicago* also show many instances of collinearity at the scale of chromosome arms -- which is good news for translational genomics across a broad spectrum of crop legumes.

Session: Structural and Functional Genomics

Organization of the Soybean Genome: Polyploidy and Genome Sequencing

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The soybean genome is being sequenced by genome shotgun sequencing in conjunction with an integrated physical map. Underlying all this is a 1.1 Gb genome with multiple rounds of polyploidization in its evolutionary past. We have found centromeric repeats that indicate that the most recent polyploid event may have been allopolyploid. In situ mapping of genetically anchored BACs to chromosomes has also revealed chromosome-level homeology in the soybean genome--presumably, a relic of the most recent polyploid event as well. We will present a progress report of the physical mapping of the Williams82 cultivar genome as well as our most recent progress in dissecting the evolutionary history of the soybean genome. This will be presented in the framework of the ongoing genome sequencing.

Session: Structural and Functional Genomics

Everything You Can Do I Can Do Better - Functional Diversity in Abiotic Stress Responses

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On an evolutionary time scale, the basal set of some 30,000 plant genes has adapted to various environments, such that with only a few exceptions every climate zone carries plant life. This has been accomplished through evolution by genome and gene duplications, the action of transposition events, domain shuffling, and nucleotide changes in control as well as protein coding regions. The availability of genome and transcript sequences from an increasing number of plants now provides tools for probing the basis of functional diversity of wild species, ecotypes, and lines of crop plants, and finding the mechanisms that have evolved in, for example, preparing plant tolerance to "stress".

Comparing abiotic stress responses between *Arabidopsis thaliana* and its close, extremely stress-tolerant relative *Thellungiella halophila*, physiological, phenotypic and molecular analyses indicated that *Thellungiella*, apparently using the same set of genes as *Arabidopsis*, represents a "stress anticipating" species. *Thellungiella* shows high pre-stress expression of genes and pathways that require induction in its stress-sensitive relative. This statement applies to primary (C/N) metabolic reactions, classical defense reactions, as well as to hormonally induced pathways that operate in diverse abiotic stress conditions.

As well, within one species, *A. thaliana* ecotypes show distinct functional responses to external conditions, which we have analyzed by growing plants in the field. For example, ecotypes Columbia-0 and Cape Verde Island-0 show different sensitivity to elevated levels of [CO_2] in a FACE facility, largely by different regulation of pathways that respond to excess carbon that is perceived as nitrogen deficiency. Visibly distinct phenotypes can clearly be recognized in the transcript and metabolite profiles.

Finally, bioinformatics tools are increasingly useful to probe for critical pathways that confer stress resistance. Whole genome expression profiling coupled with advanced clustering methods effectively reveals cross-talks and difference between different stresses responses in a single species, or between different species reacting to the same stress.

*Supported by NSF, DOE and UIUC institutional funds. I wish to thank the people presently in the lab, Qingqiu Gong, Tamara Lee, Pinghua Li, Shisong Ma, Dong-Ha Oh, Valeriy Poroyko and Indu Rupassara, former lab members, and acknowledge the valuable input by colleagues at U. Missouri, Purdue U., UC Riverside, Virginia Tech U. & UIUC.

Session: Abiotic Stress

Identification and Confirmation of QTL Conditioning Drought Tolerance in Nepalese Soybean PI 471938

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Drought is a major abiotic stress that limits soybean yield. It is generally accepted that significant improvement in drought tolerance will require a combination of several plant traits. The present research was undertaken to identify and confirm quantitative trait loci (QTL) associated with seed yield in rainfed environments and associate these QTL with plant traits related to drought tolerance. Plant introduction PI 471938 was initially collected in an arid region in Nepal and has been previously shown to possess drought tolerance. In our study we initially mapped three yield QTL in a population of 140 F4-derived lines from the cross Hutcheson x PI 471938. One of these QTL was mapped to linkage group (LG) D2 near Satt226 and two were identified on LG F (LG F-1 near Sat_375 and LG F-2 near Sat_074). The alleles for increased seed yield in rainfed environments were inherited from PI 471938 for the LG D2 and for LG F-1 QTL. These QTL were also associated with slow wilting in a separate series of field experiments. A second population of 889 F5-derived lines from the same cross was created and eight lines homozygous for the eight combinations of the Hutcheson and PI 471938 alleles at the three QTL were selected. These lines were evaluated in replicated yield tests at two locations in 2004 and five locations in 2005. Lines with combinations of PI 471938 alleles at LG D2 and LG F-1 QTL and Hutcheson alleles at LG F-2 QTL were among the highest yielding across environments. The alleles derived from PI471938 at Satt226 were associated with the lowest values in carbon isotope discrimination.

Session: Abiotic Stress

On-Farm QTL Mapping of Salt Tolerance in the Genetic Base of North American Soybean

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Salt damage is common in soybean in low–lying flood-prone fields near the Atlantic coast and in the rice lands of Arkansas. Most cultivars are susceptible. However, the landmark cultivar 'Lee', its parent and soybean ancestor 'S-100', and many of Lee's progeny (e.g. 'Forrest') are tolerant. The tolerance of Lee is conditioned by a single dominant allele (Ncl). Surprisingly, little else is known about the genetics of salt tolerance in soybean. We undertook two approaches to fill this void: a) QTL mapping of salt tolerance in S-100, and b) screening the genetic base of North American soybean for salt tolerance. In experiment I, 106 F2-derived lines from the cross of tolerant S-100 and sensitive ancestor 'Tokyo' were evaluated in a saline coastal field and in a greenhouse in NC. The visual salt tolerance ratings ranged from 0 (complete death) to 5 (normal appearance). Agreement between field and greenhouse results was good. Marker analysis revealed that a single QTL, flanked by SSR markers Sat_091 and Satt237 on linkage group N, accounted for ~80% of the genetic variation in salt tolerance. This QTL is presumed to be the Ncl locus. In experiment II, 37 ancestors were compared for salt reaction in a series of greenhouse tests in NC and MO. Agreement between MO and NC results was good. 8 ancestors, in addition to S-100, were not significantly different from the tolerant checks Lee and Forrest: AK (Harrow), Illini, Adams, Fiskeby III, Fiskeby_840_7_3, Capital, Flambeau, Bilomi #3. The PI 88788, Peking and Dunfield appeared to have intermediate response (i.e. less damage than susceptible check Essex). Discovery of salt tolerance in very early maturity ancestors (e.g. Fiskeby III) was surprising, indicating that salt tolerance may be more common among modern Midwestern cultivars than previously believed.

Session: Abiotic Stress

QTL and Evaluation of Soybean for Tolerance to Soil Water-Logging

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Excess rain and over-irrigation on poorly drained fields or low-lying areas can result in flooding and soil water-logging. Soil water-logging for as little as two days can reduce yields by 25%. Soybean may never fully recover from flooding injury. Genetic variability for flooding tolerance exists among soybean varieties. A quantitative trait locus (QTL) associated with improved soybean growth (18%) and grain yields (180%) in water-logged environments was identified in two recombinant inbred populations after being subjected to two weeks of water-logging. This QTL linked to marker Sat_064 from the parent Archer was identified at two locations in two years and may be near a locus for phytophthora root rot (PRR) resistance. However, a field test of near isogenic lines under water-logging conditions in Arkansas and Missouri showed no significant benefit of the Sat_064 QTL in two populations with Archer as a parent. However, one marker each on LG A1 and N and three markers on LG F were significant ($p<.0001$) for water-logging tolerance in both populations. Some of these markers for water-logging tolerance were linked to *Rps* genes conferring resistance to PRR.

In a field screening of soybean cultivars for tolerance to severe soil water-logging, most tolerant cultivars had yield reductions of 40% versus an 80% reduction for most susceptible cultivars. Still the 40 % yield loss to severe flooding for tolerant cultivars would severely impact a farmer's profits. Thus, varieties that limit yield losses by 10% or less are needed to protect soybean yields under severe field flooding. Because current U.S. soybean cultivars are derived from a narrow genetic base, efforts to develop varieties with better water-logging tolerance have been directed to exotic germplasm and plant introductions. Soybean plant introductions (PIs) from the USDA collection could offer higher levels of soil water-logging tolerance than in current soybean cultivars. In 2005, 262 maturity group III PIs collected from both wet and dry areas of the world were planted in three replicate hills near New Franklin, MO in specially constructed channels where the duration and amount of flooding can be precisely controlled by pumping water on and off of plots as necessary. A water-logging tolerant soybean, PI 408105A, identified in earlier studies was also planted among these PIs for comparative purposes. Plots were flooded about 5cm deep at flowering until plants began to yellow, wilt and die (about 14 d). Plots were then drained, soil allowed to dry and PIs were rated for injury on a 1 (no injury) to 5 (all plants dead) scale after a two week recovery period. Twenty (20) soybean PIs had flood tolerance scores of 1.0 to 2.0 and showed little injury to severe flooding compared to a score of 2.6 or moderate injury for PI 408105A. Seventy-eight (78) PI lines had scores of 2.3 to 3.0 and were moderately tolerant – moderately sensitive to excess water; and 164 PIs were severely injured from severe soil water-logging with scores of 3.3 – 5.0. Data combined from New Franklin in 2005 and in 2006 from New Franklin and Portageville, MO will identify PIs that show the best flooding tolerance over years and locations. Mapping populations have been developed to determine QTL for high tolerance to soil water-logging not found in current cultivars. Session: Abiotic Stress

Transgenic Approaches to Improving Drought Stress Tolerance in Maize

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Efficient use of water in agricultural production will be one of the great challenges during the 21st Century, with agriculture currently being responsible for ~70% of freshwater withdrawal. As such, yield improvement through tolerance to water deficits that occur routinely in the Central Corn Belt and frequently in western states are an important challenge in the coming decade. Benefits of improving water utilization efficiency, in addition to higher yield, are expected to include reduced water consumption and environmental sustainability. Genetic approaches using model systems are adding to our understanding of plant pathways that are important to water stress tolerance. Transgenic approaches in model systems such as *Arabidopsis* have identified genes that effectively confer drought tolerance in both *Arabidopsis* and crops such as soybean and corn. This presentation will illustrate our ability to uncover novel drought protection mechanisms using genomics data and will highlight data from *Arabidopsis*, corn and soy transgenics that demonstrate the enormous opportunity that exists for the application of genomics to product development in crops.

Session: Abiotic Stress

Genetic Engineering of Farnesylation for Crop Drought Tolerance and Yield Protection

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Protecting crop yield under drought stress is a major challenge for modern agriculture. One biotechnological target for improving plant drought tolerance is the genetic manipulation of the stress response to the hormone abscisic acid (ABA). Previous genetic studies have implicated the involvement of the β-subunit of the *Arabidopsis* farnesyltransferase (*ERA1*) in the regulation of ABA sensing and drought tolerance. Here we show that molecular manipulation of protein farnesylation in *Arabidopsis*, through down-regulation of either the α or β subunit of farnesyltransferase, enhances the plant's response to ABA and drought tolerance. To test the effectiveness of tailoring farnesylation in a crop plant, transgenic *Brassica napus* carrying an *ERA1* antisense construct driven by a drought inducible *rd29A* promoter was examined. In comparison with the non-transgenic control, the transgenic canola showed enhanced ABA sensitivity, as well as significant reduction of stomatal conductance and water transpiration under drought stress conditions. The antisense down-regulation of canola farnesyltransferase for drought tolerance is a conditional and reversible process, which depends on the amount of available water in the soil. Furthermore, the transgenic plants were more resistant to water deficit-induced seed abortion during flowering. Results from three consecutive years of field trial studies suggest that with adequate water, the transgenic canola plants produced the same amount of seed as the parental control. However, under moderate drought stress conditions at flowering, the seed yields of the transgenic canola were significantly higher than the control. These results represent a successful demonstration of engineered drought tolerance and yield protection in a crop plant under laboratory and field conditions. Using protein farnesyltransferase as an effective target, we have made significant progresses on engineering drought tolerance in other important crop species. Other genetics approaches for enhanced growth under water stress conditions will be presented.

Session: Abiotic Stress

Soybean Root Responses to Drought

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Drought is the major abiotic stress factor limiting crop productivity worldwide. Water is an increasingly limited resource, and water availability limits crop productivity in many parts of the US. Major parts of the soybean producing areas in the mid-west have experienced severe drought conditions over the past few years. Repetitive occurrences of severe drought continued to affect the soybean yield significantly. The genetic basis of drought tolerance is not well understood, and understanding how plant growth responses to drought are regulated is vital for efforts to modify the impact of water supply on soybean plants. Much less is known about root biology than about the above ground parts of the plant. Also, root architecture is a critical factor in plant survival, water and nutrient uptake and can be very important in plant productivity. A better understanding of drought tolerance mechanisms at gene, protein and metabolite levels are pre-requisite for the gene discovery and further crop improvement. We have screened and identified soybean lines which exhibit genetic diversity in root system developmental plasticity in response to soil drying, in order to enable physiological and genetic analyses of the regulatory mechanisms involved. We have studied the responses of soybean transcriptome under water stress conditions in root tissues at seedling and vegetative stage. This study help identify novel drought responsive genes and these candidates will be used for enhanced drought tolerance in soybean through genetic/metabolic engineering. Ongoing research approaches of our research group to understand the root biology and stress responses in soybean will be presented.

Session: Abiotic Stress

Genetic Modification of Oilseeds: Development of a High *Omega*-3 Vegetable Oil

Henry E. Valentin

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The average Western diet favors the consumption of *omega*-6 fatty acids over *omega*-3 fatty acids. High ratios of *omega*-6 to *omega*-3 fatty acids in the diet have been associated with increased cardiovascular disease risk. Soybeans represent the predominant source for edible oils in the US. Soybean oil contains roughly 55% *omega*-6 (linoleic acid, LA) and about 7% *omega*-3 fatty acids (*alpha*-linolenic acid, ALA). LA and ALA are essential fatty acids for humans and serve as precursors for the biosynthesis of arachidonic acid and eicosapentaenoic acid (EPA) which aid pro-, and anti-inflammatory processes in the human body. Stearidonic acid (SDA) is an intermediate in the conversion of ALA to EPA with substantially improved conversion rates compared to ALA. Compared with EPA, SDA is less susceptible to oxidative decay, and therefore represents an interesting alternative to the most common *omega*-3 fatty acid sources as it combines improved bioactivity compared to vegetable oils with improved stability compared to fish oils. This presentation introduces ongoing activities for the development of an *omega*-3 vegetable oil at Monsanto.

Session: Metabolic Engineering

Elimination of Soybean Seed Phytate through Expression of Bacterial Phytase

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Phytate serves as a phosphorus and mineral storage depot in mature grain seeds. In soybean seeds, up to two percent of the seed mass is phytate. Because humans and other monogastric animals lack significant digestive phytase activity, the phosphorus and minerals present in seed meal are sequestered in phytate and not nutritionally available. The objective of this work was to alter soybean phytate accumulation by expressing a bacterial phytase enzyme during soybean seed development. Transgenic plants were produced containing the soybean lectin promoter driving expression of the E. coli appA gene without the native periplasmic signal peptide. Several transgenic soybean lines were recovered with significant increases in available phosphate and reductions in phytate in the seeds. These lines also contained high levels of phytase activity in mature seeds. A transgenic line with no measurable seed phytate was chosen for further characterization. The results indicate that when prevented from accumulating seed phytate, normal plant and seed development occurs. Additionally, the active phytase enzyme present in mature soybean seeds can act to reduce phytate in mixed meal formulations.

Session: Metabolic Engineering

Genetic Engineering of the Sulfur Assimilatory Pathway in Soybean

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Although soybeans are an excellent source of protein for humans and animals, the quality of the protein could be enhanced by increasing the sulfur amino content. Toward this goal, we have generated transgenic soybean plants that express the maize 11 kDa *delta* zein, a protein rich in methionine. Expression of the zein however did not increase the methionine content of the seed. Transmission electron microscopy revealed that zein accumulation occurred only in specific cotyledonary cells which were proximal to the vascular bundles. When the transgenic soybeans were grown in presence of supplemental sulfur, accumulation of the zein was enhanced. This observation suggests that the availability of sulfur is not adequate to meet the demand created by the introduction of methionine-rich protein. To increase sulfur availability to developing seeds, we are currently manipulating key enzymes involved in the sulfur assimilatory pathway. Serine acetyltransferase (SAT) and O-acetylserine (thiol) lyase (OASTL), two enzymes in the sulfur assimilatory pathway, are subject to multiple levels of regulation. SAT contains catalytic, protein-protein interaction and allosteric domains. The interaction domain facilitates complex formation with OAS-TL. Feedback inhibition by cysteine is facilitated through the allosteric domain. Currently, we are producing transgenic soybean plants expressing a modified SAT that is insensitive to feedback inhibition. Transgenic soybeans over-expressing OAS-TL are also being generated to facilitate increased cysteine synthesis. Improving the methionine content and thus nutritional quality of soybean seed protein will likely require alterations in more than one aspect of the enzymatic processes involved in sulfur transport and assimilation.

Session: Metabolic Engineering

Genetic Engineering of Tobacco, Tomato, *Arabidopsis* and Soybean Plants for Tolerance to Treatment with the Herbicide Dicamba

Donald P. Weeks, Mark R. Behrens, Razvan Dumitru, Wen Zhi Jiang, Brad LaVallee, Nedim Mutlu, and Tom Clemente

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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. Because of its specificity for killing dicot plants, dicamba cannot be used in the production of dicot crops such as soybeans, canola and most vegetables. In order to make dicamba useful for agricultural production of dicot crops, development of dicamba-tolerant plants has been pursued using genetic engineering techniques. A three-component enzyme system found to be involved in the degradation of dicamba has been isolated and purified from *Pseudomonas maltophilia*. The three components are oxygenase, reductase and ferredoxin. We have shown in transgenic tobacco, tomato and *Arabidopsis* plants that only the oxygenase gene is needed to convey resistance to dicamba. Stable Mendelian inheritance of the dicamba-resistance gene was observed. The oxygenase gene has been incorporated directly into the genome of tobacco chloroplasts by particle bombardment. Plants containing the dicamba-resistance gene in the chloroplast genome showed high levels of resistance to dicamba. More recently, transgenic soybean plants have been shown to be resistant to dicamba applied at 2.5 lb/acre (compared with the usual application rate in monocot crops of 0.25 lb/acre) and are being evaluated for commercialization.

Session: Metabolic Engineering

Oxylipin Production in Soybeans

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The term “oxylipin” refers to a diverse group of oxygenated fatty derivatives. This presentation is an update on oxylipin production from both lipoxygenases and epoxygenases in soybean seeds. Lipoxygenase is a ubiquitous enzyme of plants and animals that catalyzes the peroxidation of polyunsaturated fatty acids with soybean seeds the most abundant natural source. Soybean seeds normally contain three lipoxygenase isozymes designated LOX 1, 2 and 3. Single, double and triple nulls for soybean LOX are available. We have developed a system for production of the valuable “green-note” flavor and aroma compounds, E-2-hexenal, Z-3-hexenal and Z-3-hexenol, at levels 10-fold greater than prior reports. This system utilizes free linolenic acid, soybean LOX 1+2, and a watermelon hydroperoxide lyase. LOX2 is most active in catalyzing green-note formation followed by LOX1 and LOX1 has greater stability.

The aim of the work with epoxygenases is to develop soybeans as a commercial source of epoxy fatty acids. Expression of single epoxygenases in soybean seeds generally leads to 10% or less of epoxy fatty acids in oil and for effective commercialization a level of 50% or greater would be desired. Interestingly soybean lines expressing an epoxygenase frequently have large reductions in linoleic acid levels with corresponding increases in oleic acid similar to expression of such epoxygenases in *Arabidopsis*. One line shows a large increase in linolenic acid levels. Studies with plants that naturally accumulate 60-80% of an epoxy fatty acid in oil indicate the presence of diacylglycerol acyltransferase(s) DGAT with high specificity with epoxy substrates. We have evidence that soybean oil is synthesized by two DGAT1 enzymes, *Glycine max* (Gm)-DGAT1a and GmDGAT1b, that are highly homologous. GmDGAT1a and GmDGAT1b have poor activity with epoxy containing substrates. High epoxy and other oxylipin accumulating seeds have additional DGAT(s) with specificity for oxylipin substrates not present in developing soybean seeds.

Session: Metabolic Engineering

Metabolic Flux Maps of Central Carbon Metabolism in Soybean Embryos

Jacqueline V. Shanks

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Metabolic flux analysis (MFA) in plants is instrumental in the detailed understanding of metabolism, but is difficult to perform on a systemic level. Toward this aim, we have developed a computer-aided metabolic flux analysis tool that enables the concurrent evaluation of fluxes in several primary metabolic pathways. Labeling experiments were performed by feeding a mixture of U 13C sucrose, naturally abundant sucrose, and glutamine to developing soybean (*Glycine max*) embryos. 2-D [13C, 1H] NMR spectra of seed storage protein and starch hydrolysates were acquired, and yielded a labeling data set consisting of 155 13C isotopomer abundances. We developed a computer program to automatically calculate fluxes from this data. This program accepts a user-defined metabolic network model, and incorporates recent mathematical advances toward accurate and efficient flux evaluation. Fluxes were calculated and statistical analysis was performed to obtain standard deviations. A high flux was found through the oxidative pentose phosphate pathway (104.2 carbon mol ± 23.0 carbon mol per 100 carbon mol of sucrose uptake). Separate transketolase and transaldolase fluxes could be distinguished in the plastid and the cytosol, and those in the plastid were found to be at least 6-fold higher. The backflux from triose to hexose phosphate was also found to be substantial in the plastid (113.2 carbon mol ± 26.0 carbon mol per 100 carbon mol of sucrose uptake). Forward and backward directions of anaplerotic fluxes could be distinguished. The glyoxylate shunt flux was found to be negligible. Such a generic flux analysis tool can serve as a quantitative tool for metabolic studies and phenotype comparisons, and can be extended to other plant systems.

Session: Metabolic Engineering

Metabolic Redesign of Vitamin E Biosynthesis in Soybean for Enhanced Antioxidant Content

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The oxidative stability of soybean oil is determined by its fatty acid composition and antioxidant content. Oxidative stability is a critical factor for the use of vegetable oils in food processing and industrial lubricant applications. The primary antioxidants in soybean oil are tocopherols. Tocopherols, together with tocotrienols, comprise the vitamin E family of antioxidants in plants. While tocopherols are found in nearly all plant organs, the occurrence of tocotrienols is limited primarily to monocot seeds. Both forms of vitamin E are potent antioxidants that scavenge free radicals and protect unsaturated fatty acids of vegetable oils from oxidative breakdown. The committed step in tocopherol biosynthesis is the condensation of phytol diphosphate and homogentisate, which is catalyzed by homogentisate phytyltransferase (HPT). We have recently identified an enzyme designated "homogentisate geranylgeranyl transferase" (HGGT) from monocot seeds that shares approximately 60% identity with known HPTs. In contrast to HPT, recombinant HGGT from barley was approximately five-fold more active with geranylgeranyl diphosphate than with phytol diphosphate in condensation reactions with homogentisate. In addition, expression of barley HGGT under control of the CamV35S promoter in *Arabidopsis* was sufficient to confer tocotrienol biosynthetic ability and to increase the vitamin E antioxidant content of leaves by >10-fold. To test the efficacy of HGGT in soybean, a cDNA for the barley enzyme was expressed under control of the strong seed-specific promoter for the alpha'-subunit of *beta*-conglycinin gene. Seeds from the resulting transgenic events displayed a three- to five-fold increase in the total content of vitamin E antioxidants. These increases were due largely to the production of *delta*- and *gamma*-tocotrienols, which are normally found in only trace amounts in soybean seeds. Seeds from these lines will be used to assess the oxidative stability of the oil and to test its performance in food processing and lubricant applications.

Session: Metabolic Engineering

Agmagenomic Sequencing of Soybean

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A microbead-based sequencing technology was used to generate 717,383 genomic survey sequences from soybean (*Glycine max*) cv. Williams. These sequences together represent over 80Mb of sequence, or an estimated 7% of the genome of soybean. The randomly spaced reads are an average of 112 base pairs in size, and represent genomic sequence fragments dispersed at average 2Kb intervals in single copy regions of the soybean genome. 10,464 of the reads could be identified as derived from likely genomic protein coding regions. 41% of the conceptual translations of these regions are not known soybean protein sequences, giving over 4,000 novel proteins or protein fragments. These reads represent a comprehensive survey of soybean and are being used to provide potential genetic markers at an unparalleled density. They also represent a dataset - free of cloning bias - that can be used to reconstruct repetitive sequences on a whole-genome scale. New repeat assembly and detection tools have been developed to reconstruct repetitive sequences from these short reads in silico. Over 30,000 multi-copy sequences, of which 4213 are present in 100 or more copies per genome, were detected using these tools. These sequences include transposons, satellites, and structural repeats from centromeres and telomeres. We have annotated these repeats and developed a website and database where these sequences can be accessed and searched. Using this database, we estimate that 41% of the soybean genome is present in more than 14 copies per haploid set, in agreement with Cot measurements.

Session: Structural and Functional Genomics

Gene Identification in Soybean by Microarray Analysis using Near Isogenic Lines

Lila O. Vodkin, G. Zabala, M. Hunt, A. M. Boone, D. O. Gonzalez, and J. Tuteja

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We demonstrate that soybean microarrays can be used successfully to examine isogenic lines differing with respect to a mutant phenotype and thereby to define a small list of candidate sequences encoded or modulated by the mutant gene. The microarrays contain 36,000 cDNAs representing soybean ESTs (Vodkin, et al., BMC Genomics 5:73, 2004). We have also developed a set of 70-mer oligo arrays that represent 38,000 unigenes (see poster by Gonzalez). We used the soybean cDNA microarrays to identify candidate genes for a stable mutation at the *Wp* locus in soybean, which changes purple flowers to pink, and found that flavanone 3-hydroxylase cDNAs were over-expressed in purple flower buds relative to the pink flowers. RFLP analysis and RNA blots of purple and pink flower isolines, as well as the presence of a 5.7 kb transposon insertion in the *wp* mutant allele, have unequivocally shown that flavanone 3-hydroxylase gene 1 (F3H1) is the Wp locus (see Zabala and Vodkin, Plant Cell, 17, 2005; and poster, this meeting). Other experiments with a pair of isogenic lines at the *I* locus that controls pigmentation show clear differences between chalcone synthase mRNA levels that are downregulated by naturally occurring CHS siRNAs (Tuteja et al., Plant Cell 16, 2004). We have recently examined several other isolines affecting morphological traits as seed coat integrity (Boone et al., in preparation) and the glabrous phenotype that results in lack of trichome hairs on plant leaves and stems (see poster by M. Hunt). Candidate cDNAs that vary between the standard and mutant phenotypes have been verified by RNA blots. We are currently investigating whether any of these candidate cDNAs are the molecular basis of the morphological traits or whether they represent downstream genes affected in the near isogenic lines containing the variant alleles. Supported by USB, NSF, USDA, and ISA.

Session: Structural and Functional Genomics

Soybean TILLING: A Tool for Functional Genomics and Reverse Genetics

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TILLING (Targeting Induced Local Lesions IN Genomes) is a reverse genetics tool used for the identification of gene function using chemical mutagenesis. With the soybean genome being fully sequenced, a central Tilling facility is needed to speed gene discovery in soybean. The need to provide a robust link between DNA sequences and phenotype is becoming increasingly urgent, as more economically important genes are identified in soybean. Therefore, cost effective and time saving technologies are needed to validate gene function of economically important genes. The majority of the available and committed soybean genomic tools are being developed primary from two cultivars "Forrest" and "Williams 82". Therefore, we used TILLING as a reverse genetics tool for functional analysis of soybean genes using two platforms, one from Forrest and the other from Williams 82. Since the project is community oriented, our main goals are: I. To improve soybean TILLING to allow for an efficient non-transgenic *in vivo* system gene functional analysis and alternative alleles discovery in soybean. II. To establish a central facility for soybean TILLING that will archive, curate, distribute and store the soybean mutagenized population's lines and provide TILLING services to the soybean community. An update about our effort will be presented at this conference.

Session: Structural and Functional Genomics

Molecular Breeding Basis-Evaluation of Chinese Germplasm with SSR Markers

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It has become urgent task to discover and utilize useful genes from abundant Chinese soybean germplasm. In order to improve the efficiency of study and use the huge germplasm, the core collection concept was introduced into germplasm evaluation. By analyzing phenotypic variation of more than 20000 accessions for Chinese soybean accessions (Qiu et al., 2003), a candidate primary core collection was selected and on the basis of a core SSR marker evaluation (Xie et al., 2003). The genetic structure of landraces (Li et al., 2005), the distribution of genetic diversity (Wang et al., 2004; Piao et al., 2005, Li et al., 2005; Xie et al., 2005), and the genetic diversity center/sub-centers (Wang et al. 2005) of the primary core collection were clarified at DNA levels. Then the core collection was established (Wang et al., 2006a) with the proper sampling strategy (Wang et al., 2006b). Some working collections relative to important traits such as soybean mosaic virus resistance (Miet al., 2004) and soybean cyst nematode resistance (Ma et al., 2006) with the biggest genetic diversities were also established. Meanwhile, the core collection were genotyped with some marker associated with genes or QTLs of important traits such as salt tolerance (Guo et al., 2000), SMV resistance (Zheng et al., 2003), Gly m Bd 28K allergic protein null (Guan et al., 2004), protein content, oil content and certain yield components (plant height, number of branches, 100 seed weight, etc.) (Yang et al., 2005). Other markers we developed such as SNPs and EST-SSRs that were associated with agronomic traits (e.g., soybean cyst nematodes resistance) were also used for genotyping. The core collections has been used for screening novel genes and finding the germplasm with new traits such as β (*beta*) unit; or A3 subunit low content so far. The core collection should provide a basic study platform for phenomics, genomics and breeding.

Session: Molecular Breeding

Genetic Enhancement of Oleic Acid Concentration in Soybean Oil

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Enhancing the level of oleic acid in soybean oil meets several consumer needs. Higher-oleic improves oil stability to oxidation, may improve nutrition, helps reduce dietary trans-fat, and may improve the quality of bio-fuels. Research to genetically modify oil composition has focused on genes that control five enzymes in fatty acid and glycerolipid synthesis: KAS-II, D-9 18:0-ACP desaturase, FAT B, FAD2 and FAD3. Natural and induced changes or polymorphisms in these genes mediate phenotypes with high or low palmitic, stearic, linoleic, linolenic, or oleic acid concentration. Although transgenic approaches have shown that oleic acid levels may be raised from about 20% to 80% in soybean, genomics has revealed the complexity of genetic regulation of this trait. Recently, scientists have reported two isoforms of FAD2-1, two isoforms of FAD2-2, and three isoforms of the FAD3 in soybean. The revelation that soybean has multiple forms of FAD genes increases the number of potential mutations or alleles a plant breeder will have to deal with in developing high-oleic cultivars. As an example, there are at least five different polymorphisms among the FAD3 isoforms in soybean. CAPS markers that distinguish these alleles will significantly facilitate the development, and protection, of elite varieties with higher oleic oil. As an example, soybean germplasm with debilitating mutations in both isoforms of FAD2-1 enable oil with about 70% oleic acid. Future research will focus on the combination of traits such as higher stearic and oleic acid concentration. Without doubt, the genetic system that mediates this phenotype will prove to be extremely interesting, and quite useful in developing soybeans exhibiting a stable non-hydrogenated oil with elevated solid-fat content.

Session: Nutritional Genomics

Exhaust Emissions from a Diesel Engine Fueled with High Oleic Soybean Oil

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Biodiesel is an alternative fuel for diesel engines that is produced from vegetable oils and animals fats. High petroleum prices and federal government incentives have caused a large expansion of interest in this fuel. This paper describes the production of biodiesel from a novel soybean oil with high oleic fatty acid content (>85%) and with low saturates (<6%). The fuel was produced using an alkali-catalyzed process with a 6:1 methanol to oil molar ratio using sodium methylate as the catalyst. The fuel produced meets the ASTM D6751-03a specifications. The exhaust emissions from this novel biodiesel are compared to biodiesel from soybean oil, canola oil, and petroleum diesel. Particular attention is directed to the effect of the high oleic soybean-based biodiesel on the emissions of oxides of nitrogen. Other measurements performed include smoke and fuel consumption.

Session: Industrial Uses

Why the United Soybean Board Is Still Interested in Genomics

Ed Ready

Production Program Manager, United Soybean Board

The mission of the United Soybean Board is to help make the US soybean farmer more competitive and profitable in a global market. One way to accomplish this is to improve the soybean, both yields and composition. Yields can be improved by protecting existing yield potential from biotic and abiotic stresses and by increasing genetic potential. Compositional improvements include modifying fatty acids to avoid the need for partial hydrogenation, increasing protein, improving amino acid balance, increasing digestible sugars, and reducing phytate phosphorous. All of these improvements involve modification of the soybean's genetics. USB is pleased to have contributed to the advancement of soybean genomics through funding projects to develop a physical map, make microarrays available to researchers, increase the understanding of the genome's structure and study the function of genes of interest. USB will continue to work with the research community to maximize the usefulness of data developed by the Department of Energy's Joint Genomic Institute as they sequence the soybean genome.

Session: Wrap-up

Soy/2006 Poster Abstracts

Poster 1

The *wp* Mutation of *Glycine max* Carries a Gene-Fragment-Rich Transposon of the CACTA Superfamily

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We used soybean cDNA microarrays to identify candidate genes for a stable mutation at the *Wp* locus in soybean, which changed a purple-flowered phenotype to pink, and found that flavanone 3-hydroxylase cDNAs were over-expressed in purple flower buds relative to the pink. RFLP analysis and RNA blots of purple and pink flower isolines, as well as the presence of a 5.7 kb transposon insertion in the *wp* mutant allele, have unequivocally shown that flavanone 3-hydroxylase gene 1 (*F3H1*) is the *Wp* locus. The genomic sequences of the *Wp* gene (*F3H1*), the mutant allele *wp*, and that of a related gene (*F3H2*) were determined, compared and their expression examined. *F3H1* is expressed strongly in seed coats and more weakly in young flower buds of *Wp* plants but aberrantly expressed in homozygous *wp*. The 5.7 kb insertion in *wp* represents a novel type of transposable element (termed *Tgm-Express1*) with inverted repeats closely related to those of other *Tgms* (transposable-like elements, *Glycine max*) but distinct from them in several characteristics including the lack of subterminal inverted repeats. More significantly, we show that *Tgm-Express1* contains four truncated cellular genes from the soybean genome (The Plant Cell, 17, 2005). In this respect, it resembles the Pack-MULEs (Mutator-like transposable elements) found in maize, rice and *Arabidopsis* and the *Helitrons* of maize. The finding of the *Tgm-Express1* element causing the *wp* mutation and a second, *Tgm-Express2* element in another location in the soybean genome, reinforces that the ability to acquire and transport host DNA segments is extended to the CACTA family of elements to which both *Tgm* and the prototypical maize *Spm/En* elements belong.

Session:Genomics/Functional Genomics

Poster 2

Functional Transition of the Cotyledon from Storage to Photosynthetic Activity during Soybean Germination Assessed by Transcript Profiling with an Oligo Array

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The soybean cotyledons contain the nutrients and food reserves that supply the needs of the young plant during germination and for about 7-10 days after emergence. In our study, we defined several time points starting when the dry seed begins to take up water (imbibition) until the expansion and unfolding of the unifoliolate. Following imbibition, cell metabolism resumes rapidly by initially using the enzymes and reserves synthesized during development and conserved in the dry seed. Eventually cell division starts, DNA and protein synthesis take place and new enzymes and cellular components are made. Shortly after emergence the hooked shaped hypocotyl straightens out and the cotyledons transition from being mainly a nutrient and food reserve tissue to an active photosynthetic tissue. To better understand at the genetic level the natural transition in the cotyledon from storage to photosynthetic activity, we studied the transcript abundance profile at different time points during germination using a new soybean chip containing 19,200 oligonucleotide probes (70 mer long). After normalization and statistical analysis, we determined that 3,590 genes presented a significantly altered expression in at least one of the time points defined during this study. Cluster analysis by k-means allowed us to create 12 different groups of genes with similar expression profiles along the development of soybean germination. We also defined lists of over and under expressed genes involved during the cotyledonary functional transition from nutrient and food reserve (yellow tissues) to photosynthetic activity (green tissues). The highest number of over-expressed genes during this time study was found in the stages that cover the natural functional transition (combination of yellow and green tissues), while the number of under-expressed genes seems to constantly increase from nutrient and food reserve (yellow tissue) to photosynthetic activity (green tissue). In depth analysis of these lists has allowed us to identify known and unknown genes that may have relevant biological importance during germination and emergence. Several of these genes have been confirmed by Northern blot analysis and qRT-PCR. These results give us a closer insight to the genetic control mechanism that modulates and regulates the functional transition in the cotyledons during this developmental process.

Session:Genomics/Functional Genomics

Poster 3

Oligonucleotide Macroarray Analysis of Differential Gene Expression against *Xanthomonas axonopodis* pv. glycines in Soybean [*Glycine max* (L.) Merr.]

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Xanthomonas axonopodis pv. glycines (*Xag*) is a pathogen that causes bacterial leaf pustule (BLP) disease in soybean grown in Korea and the southern United States. Typical and early symptoms of the disease are small yellow to brown lesions with raised pustules, which develop into large necrotic lesions that can lead to a substantial loss in yield by premature defoliation. The intensity of symptoms in response to *Xag* can differ depending on the inoculum concentration, growth conditions and soybean genotype. After PI 96188 was infected by *Xag*, only pustules without chlorotic haloes were observed. This novel symptom was determined to be a resistant response instead of a hypersensitive response. To identify differentially expressed genes before and 24 hr after *Xag* inoculation in PI 96188, which demonstrated this novel symptom to BLP, and BLP-resistant SS2-2, an oligonucleotide macroarray was constructed with 100 genes related to disease resistance and metabolism from soybean and *Arabidopsis*. After cDNAs from PI 96188 and SS2-2 were applied onto oligonucleotide macroarrays with three replicates and dye swamping, 50 and 85 genes showed significant differences in expression between 0 hr and 24 hr in PI 96188 and SS2-2, respectively. Quantitative real-time RT-PCR was performed on ten selected genes that showed altered gene expression in both PI 96188 and SS2-2, using tubulin and Jangyeobkong (BLP-susceptible) as controls, in order to validate the macroarray results. In most cases, the oligonucleotide macroarray data concurred with the quantitative real-time RT-PCR results, supporting the accuracy of the oligonucleotide macroarray experiments.

Session:Genomics/Functional Genomics

Poster 4

The Soybean Breeder's Toolbox: A New Resource for Soybean Genetic and Genomic Information

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In order to make soybean genetic data more accessible and retrievable, data contained in the current SoyBase AceDB database was converted to a relational database schema. This data is now available through an internet interface titled "The Soybean Breeder's Toolbox". The interface was designed to use software developed by the Generic Model Organism (GMOD.org) project and extended by us to deliver functionality that was not readily available through AceDB. Genetic map displays are created using the comparative map viewer, CMap. CMap allows the display and comparison of single or multiple linkage groups. This feature will allow the visualization of homeologous segments shared between linkage groups. Genetic markers and QTL visualized on the various genetic maps are in turn linked back to data in the Toolbox. The Toolbox will allow ad hoc searching as well as pre-defined searches. It is designed to accept specific requests from users accustomed to working with soy bean genetic data types such as QTL, molecular markers, germplasms, diseases and gene loci or general requests that will present the user with a list of data types for further exploration. Automated requests will be accepted by direct URL based queries. Facilities will be provided for direct SQL querying of the database. The database will be expanded to include links to external data such as corresponding markers on other legume species' genetic maps or to the soybean physical map when they become available. A description of the database and representations of the interface will be presented.

Session:Genomics/Functional Genomics

Poster 5

Transcriptomic Analysis of Soybean Seed Development and Regulation of Seed Composition

C.C. Periappuram, L. Li, F. Qiu, D. Wang, D. Nettleton, M.E. Westgate, E.S. Wurtele, and B.J. Nikolau

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Due to the complex genetic and environmental determinants of seed composition, this trait has eluded molecular understanding. To aid the dissection of this complex trait we have developed near-isogenic soybean isolines that differ markedly in their seed composition. These isolines were developed from two independent recombinant inbred populations that were established between three parent lines, Evans, the high protein line PI 153.296, and the low protein line PI 438.472. The resulting isolines are being used to profile differences in gene expression at the level of mRNAs using two independent platforms: 1) an expressed sequence tag-based microarray containing about 9,000 unique cDNAs; and 2) the Affymetrix The GeneChip® Soybean Genome Array. Using these platforms we have profiled mRNAs at 5 time points (from 25 to 50 days after flowering) during the development of soybean seeds. A mixed linear model analysis of cDNA array data revealed 388 genes that were differentially expressed. Clustering by k-medoids was used to group these 388 genes into 11 clusters according to the similarity of their estimated expression profiles during seed development. While a total of 6148 genes were differentially expressed on Affymetrix The GeneChip® Soybean Genome Array. These genes were grouped into 9 clusters based on the similarity of their estimated expression profiles. These RNA-profiling data provide the basis for comparing the molecular differences between Evans and the isogenic lines that express different seed composition traits. This understanding will provide valuable insights into the processes that regulate soybean seed composition.

Session:Genomics/Functional Genomics

Poster 6

Metabololite Profiling of Developing Soybean Seeds

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Metabololite profiling provides a "snapshot" of chemical composition of tissue at a given development stage. Comparison of absolute amount and the relative ratio of metabolites among samples will allow us to evaluate the accumulation related to the changes in gene expression, protein function and regulation of enzyme activities. We are conducting GC-EI-MS based metabolomic analysis of developing soy seeds collected at various time points after flowering (DAF), at between 25 to 50 DAF. The metabolomics analysis includes: 1. targeted metabolite analysis including profiling of fatty acids, sterols and free amino acids; and 2. non-targeted total metabolomic analyses. In these analyses, the extracts are partitioned into non-polar and polar phases followed by trimethylsilyl (TMS) and tert-butyldimethylsilyl (TBS) derivatization and GC-MS analysis. The metabolites from targeted analyses are identified by comparing their mass spectra to that of authentic standards from our lab or NIST mass spectrum library. GC-MS data collected from non-targeted analyses are normalized and deconvoluted using AMDIS (NIST) program. Over 300 metabolites can be readily identified in each sample; about 100 structures are positively deconvoluted among those metabolites using library constructed by our lab, The Golm Metabolome Database and NIST mass spectrum library. The statistic analysis indicates dynamic changes of metabolites in different biological pathways along the developing time line.

Session:Genomics/Functional Genomics

Poster 7

Identifying Possible Mutant Genes in Glabrous Soybeans by Transcript Analysis

M.R. Hunt and L.O. Vodkin

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Trichome development has been well characterized in the *Arabidopsis* model system. Many of the transcription factors involved in the initiation and morphogenesis of trichomes have been described in *Arabidopsis*. Trichome development, however, has not been as well characterized in agronomically important species such as soybean and cotton. Glabrous (trichomeless) varieties of soybean are available in the USDA germplasm collection. These varieties have the potential to be used to identify key developmental genes involved in trichome formation outside of *Arabidopsis*. These varieties could also be used to determine the similarities and differences between the *Arabidopsis* and soybean trichome development systems. In order to identify candidate genes contributing to the mutant glabrous phenotype in soybean, a microarray study was initiated to compare standard Clark and glabrous Clark soybean isolines. The comparison was carried out on both shoot tips and older leaf tissues with two complete biological replicates of the experiments. This comparison was carried out using the soybean ~27,000 unigene cDNA microarray library set derived from various soybean tissues, developmental stages, and stress conditions (Vodkin et al. 2004). The data has been used to develop gene lists of candidate genes that may be involved in the glabrous developmental phenotype. The candidate genes' expression levels have been confirmed by northern blot analysis comparing standard and glabrous Clark isolines.

Session:Genomics/Functional Genomics

Poster 8

The FAD2 Gene Family of Soybean: Insights into the Structural and Functional Divergence of a Paleopolyploid Genome

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The w-6 fatty acid desaturase (FAD2) gene family in soybean consists of at least five members in four regions of the genome and are responsible for the conversion of oleic acid to linoleic acid. It is thought that duplicate genetic factors may result from genomic duplication events. Bacterial artificial chromosomes (BACs) corresponding to these FAD2-containing regions were sequenced to investigate structural and functional conservation between duplicate loci. Sequence comparisons show that the soybean genome is a mosaic with some duplicate regions retaining high sequence conservation in both genic and intergenic regions while others have only the FAD2 genes in common. Genetic mapping using SSRs from within the BAC sequences showed that two BACs with high sequence homeology mapped to linkage groups I and O; linkage groups with syntenic markers between them. Another BAC mapped to linkage group L. The last BAC could not be mapped. Reverse transcriptase-PCR analysis of the five FAD2 genes showed that the FAD2-2B and FAD2-2C copies were the best candidates for temperature dependent expression changes in developing pod tissue. Semi-quantitative RT-PCR confirmed these results with FAD2-2C showing upwards of an eight-fold increase in expression in developing pods grown in cooler conditions relative to those grown in warm conditions. The implications of these results suggest a candidate gene for controlling the levels of linoleic acid in developing pods grown in cooler climates.

Session:Genomics/Functional Genomics

Poster 9

Expression Profiling of Early Stage Developing Pods on Soybeans

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To better understand the genetic changes occurring in developing pods and seeds expression profiles were compared at three distinct stages of pod development. RNA was isolated from developing pods and seeds of cultivar Williams (PI548631) collected at four, eight, and 25 days after flowering (DAF). Utilizing the Affymetrix Soybean GeneChip®, expression profiles of seeds and pods were compared across the three time-points. Approximately the same number of genes were shown to be differentially expressed between tissue collected 8 and 25 DAF (290) and between 4 and 25 DAF (315). Thirty-nine percent of the genes differentially expressed beween 4 and 25 DAF, were also differentially expressed in the comparison of 4 and 8 DAF tissue while 65% were differentially expressed in the comparison of 8 to 25 DAF tissue. Annotation and analysis of these differentially expressed genes should facilitate a better understanding of the changes in gene expression that occur during early stages of pod and seed development in soybean.

Session:Genomics/Functional Genomics

Poster 10

Genomic Analysis of a Seed Protein QTL Region on Soybean LG-I: Candidate Gene Identification and Analysis of Homoeologous Regions

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A seed protein QTL mapped to an interval between Satt239 and Satt496 on LG-I (Chung et al, 2003) increases the seed protein content up to 2 percent when homozygous for the high protein alleles. Identification of the candidate gene(s) for this QTL would help us understand the genetic basis of seed protein variation in soybean. Physical mapping of the QTL region resulted in a minimal tiling path (MTP) of eleven BACs spanning the region. Several BACs from the MTP were sequenced. Sequencing of a contiguous region of approximately 400Kb is close to completion. Among the putative genes in the region, glycerol 3-phosphate acyl transferase (GPAT) could be a likely candidate gene for the QTL. GPAT is an enzyme involved in seed oil synthesis. It catalyzes the transfer of acyl chain to the Sn-1 position of glycerol 3- phosphate, the first step in the formation of triacylglycerol (TAG). Considering the negative correlation between the protein and oil contents in soybean seeds, GPAT could be a candidate gene for the QTL. A SNP developed from soybean Nodulin 35 coding sequences also is positioned on the physical map of the region. Nodulin 35 encodes uricaseII, an enzyme involved in biosynthesis of ureide, the major form of transport of fixed nitrogen from nodules to the shoots. Therefore nodulin 35 gene also is a likely candidate for the QTL. Analysis of the candidate genes for allelic differences between high potein and low protein soybean lines is underway. The genes identified from the QTL region were also used as probes to screen the Wms82 BAC libraries (GmW1 and GmW2) by hybridization. This screening identified BACs from LG-I as well as BACs from the QTL homoeologous regions. The results shows LG-O and LG-D2 have genes from LG-I QTL region duplicated on them.

Session:Genomics/Functional Genomics

Poster 11

SNP Discovery in Soybean Genes and BAC-end Sequence

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Soybean genes including ESTs and full-length cDNAs, gene sequence from genomic clones, and BAC-end sequence (BES) are sources of sequence for the discovery of SNPs. Discovering SNPs in these sequences will provide genetic markers and will also permit the positioning of the corresponding gene or BAC-clone on the genetic map. A total of 9636 and 2546 PCR primer sets were designed to genes and BES, respectively. A total of 7055 (73.22%) and 2186 (85.86%) of the primers designed to genic sequence and BES (screened to eliminate genic sequence and repetitive elements) produced a single discrete band on an agarose gel, respectively. Single amplicons were verified via sequence analysis of the PCR product. A total of 4735 (49%) of the fragments amplified from genes (2.926Mbp) and 1055 (41%) of those amplified from BES (0.493Mbp) were verified to be robust sequence tagged sties (STS). SNPs were discovered via the alignment of sequence traces obtained from the sequence analysis of each STS amplified from a set of six diverse genotypes. At least one SNP was found in 2346 (24.35%) of the genes and 606 (23.80%) of the BES to which primers were designed. In the 2.926 Mbp of gene sequence, the mean nucleotide diversity (theta) was 0.00095. In the 0.493 Mbp of sequence derived from BES theta was 1.5 fold higher (0.00176) than in the genic sequence. These results support the suggestion of lower levels of sequence variation in genic sequence where sequence conservation is required to maintain gene function. One surprising result was the relatively low proportion of robust STS that were developed from BAC-end sequence vs. genic sequence (41% vs. 49% of primers designed). Apparently there is a high degree of sequence conservation between duplicated non-coding regions of the genome.

Session:Genomics/Functional Genomics

Poster 12

A Cytogenetic Approach to Assign Linkage Groups to Chromsomes

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Previous studies have attempted to assign gene linkage groups (both classical and molecular) to soybean chromosomes, but it has proven to be difficult. Chromosomal mapping of BAC clones was performed on soybean chromosome preparations using fluorescent in situ hybridization (FISH) with BACs that have previously been anchored to each molecular linkage group. The BACs chosen were labeled with two fluorophores to allow for orientation of the linkage groups and not just assignment. By utilizing the elongated pachytene chromosomes from anthers various characteristics can be measured, including: the length of the entire chromosome in order to identify it numerically and distances between BACs and chromosomal characteristics, such as telomeres or centromeres, to localize the BAC. So far, forty-two BACs, nineteen of which are single locus, and represent fourteen MLGs, have been mapped to soybean chromosomes.

Session:Genomics/Functional Genomics

Poster 13

Establishing of High-throughput SNP Survey and Screening of Soybean Mutants

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Targeted induced local lesion in genomes (TILLING) allows high-throughput detection of single nucleotide mutation as essential target for reverse genetics in soybean. The alkylating agent, ethyl methane sulfonate (EMS) is used in TILLING for inducing a lot of mutations. Many practical applications of screening single nucleotide polymorphism (SNP) have been developed. Among them, denaturing high performance liquid chromatography (DHPLC) is known as precise, economical and suitable to high-throughput mutation screening. To optimize the conditions, more than 100 amplicons obtaining SNPs in soybean expressed sequence tag (EST), which previously identified between two soybean cultivars, Pureunkong and Jinpumkong 2 were screened by DHPLC. After all the amplicon sizes were checked on the agarose gel to determine buffer compositions, the whole sequences included primer sequence were used for set up the oven temperature of DHPLC. The various amplicons in the lengths and in the number of SNP were used. The amplicons over 1kb that produced blunt peaks were not suitable to DHPLC detection, because it would not distinguishable if mutation ratio is low in pooled genomes. Since each template has to be amplified evenly in a pool in order to heteroduplex, different annealing temperatures were used depending on primers among cultivars after with amplification of a certain template. Usually DHPLC could not identify the number of SNPs, but an amplicon with two SNP sites showed two kinds of heteroduplex peaks in one graph. With these selected primers, M_2 EMS mutant lines from Sinpaldalkong 2 and Jack will be screened.

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Poster 14

Legume Genomics as Part of Plant Genomics at the National Center for Biotechnology Information

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Plant genomics is a simple expansion of the scope of genomics at the National Center for Biotechnology Information (NCBI). In addition to the tools for storage of and analysis of nucleotide sequence such as, respectively, GenBank and BLAST, genomics at NCBI includes databases that enable 1) monitoring the progress of genome sequencing projects (Genome Projects), 2) datamining of probes (Entrez Probe), 3) datamining of primer sequences (UniSTS), and 4) viewing of genome units (MapViewer). These resources have been populated with data from *Glycine max*, *Medicago sativa*, *Medicago truncatula*, *Phaseolus vulgaris*, *Lotus corniculatus*, and *Vigna radiata*. The use of these resources will be described.

Session:Genomics/Functional Genomics

Poster 15

Centromeres as Relics of Allopolyploidy in *Glycine max*.

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Centromeres are a key component of chromosomes in almost all eukaryotic species. They serve as a site for kinetochore formation and sister chromatid cohesion during cell division. The functional centromeres have been associated with two key components: satellite repeats and the centromeric retrotransposons (CRRs). SB92 is a previously described satellite repeat in *Glycine max*. Our Fluorescence insitu Hybridization (FISH) experiments have shown this tandem repeat to be localized on only 12 soybean chromosomes. In an attempt to investigate what the other eight centromeres look like at the sequence level, we screened the soybean Genome Survey Sequences (GSS) for satellite repeats and found SB91 sharing ~80% similarity with SB92. Both these sequences have been found to be conserved in G.max and G.soja using Southern Hybridizations. FISH experiments have shown the localization of these two centromeric repeats to different sets of chromosomes. In order to examine how the centromeric histone 3 (CENH3) proteins have co-evolved, we used a degenerate primer approach using RT-PCR to clone the CENH3s from soybean. Soybean anti-CENH3 antibody will be raised and used in Chromatin Immunoprecipitation (ChiP) experiments in order to clone the centromeric sequences derived from chromatin and containing the CENH3s.

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Poster 16

Characterization of the LysM Gene Family in Soybean

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LysM is a ubiquitous and ancient domain that binds peptidoglycan. Although LysM genes have been identified in various plant species such as *Arabidopsis*, *Medicago*, *Lotus*, rice and poplar, little is known about the evolution and biological function of this gene family. A few members of this family have been shown to be essential for the establishment of the nitrogen fixing symbiosis between legumes and rhizobia. The goal of our work is to understand the evolution and various biological functions of LysM genes in the agriculturally important soybean plant. Twenty-three soybean EST sequences encoding LysM domains were identified in sequence databases. Sequence alignment showed that soybean LysM domains, as well as LysM domains from other plant species, are highly diversified. Soybean LysM genes (GmLysMs) fall into some 6 ancient clades in the phylogenetic tree of plant LysM genes. Generally, 1-3 distinct LysM motifs are identified in individual LysM genes. Our preliminary analysis suggests that intragenic expansion of LysM motifs occurred before the divergence of monocot and dicot plants. To gain more information about the genomic contexts surrounding GmLysM genes, the majority of GmLysMs were physically mapped onto BACs and nine BACs were sequenced. Development of SNP markers and genetic mapping of the GmLysMs are in progress. Interestingly, some syntenic regions around LysM genes are conserved not only in legume species, but also in *Arabidopsis*, rice and poplar plants. We hope to use this information to reconstruct the evolution of this important gene family and to investigate its biological functions.

Session:Genomics/Functional Genomics

Poster 17

Sequence Analysis of Duplicated Genes in Homeologous Regions of Soybean and Legume Species

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Polyploidy is a common theme in plant evolution that is thought to contribute to genetic diversity and species evolution. Soybean is a paleo-polyploid having undergone 2-3 rounds of polyploidization (~45, 14 and 4 MYA) and thus presents a model for exploring the structural and functional effects of polyploidy. Homoeologous BACs centered around the RFLP probe pA711 were sequenced. Sequence conservation, duplication and inversion between the two homeologous regions were found. In order to further understand the mosaic phenomenon derived from genome duplication in soybean, the flanking regions of these two BACs are being extended and studied. Inter-species relationships, including intra-Tribe comparison and inter-Tribe comparison were evaluated. BACs centered around the RFLP probe pA711 from different legume species were extracted from database, or were screened from BAC libraries and sequenced. For intra-Tribe comparisons, another important crop legume, Phaseolus vulgaris (common bean), which is a diploid, 2x, was compared to soybean, which is an ancient tetraploid, 4x. For inter-Tribe comparisons, *Lotus japanicus* from Tribe Lotea and *Medicago truncatul*a form Tribe Trifolieae were compared to soybean. Our results demonstrate the role of genome duplication in legume genome evolution.

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Poster 18

Preliminary Identification and Characterization of Several CAD Gene Family Members in Soybean

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The cinnamyl alcohol dehydrogenase (CAD) gene family encodes a class of NADPH-dependent enzymes that catalyze the reduction of several phenylpropanoid aldehydes in the biosynthesis of lignin. One member from *Arabidopsis* thaliana with the greatest homology to CADs from other plant species was used as query in a tblastn search of *Glycine max* dbEST. 250 hits with $E < 0.01$ assembled (minimum overlap of 20 with 100% identity) into 17 contigs ranging from 611 to 1355 bases and 2 to 42 ESTs each. Nine contigs that exhibited nearly full length ORFs were analyzed further by aligning ORFs and recording differences with regard to codon position. Six contigs exhibited differences indicative of evolutionary changes constrained by purifying selection. Further analysis of library genotypes for the contig ESTs was used to suggest whether contigs represented alleles or actual paralogs.

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Poster 19

Anchoring the Soybean Physical Map to the Genetic Map

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Enhanced genome research of soybean will largely rely on genome-wide physical mapping of its genome using large-insert DNA clones, such as BACs. Such a platform will be useful for enhanced, effective and high-throughput gene and QTL cloning, EST mapping, marker development, genome sequencing and comparative genomics research. To accelerate soybean genomics research, the soybean research community fingerprinted using restriction enzymes over 100,000 soybean Williams 82 BAC clones (J. Dvorak and M-C. Luo, unpubl). The generation of a final physical map from this effort will be aided by mapping of markers onto the physical contigs and then anchoring these markers to the genetic map; thereby, creating an integrated physical and genetic map. We have anchored over 1,300 genetically-mapped molecular markers to the BAC-based physical contigs using a PCR based approach. In addition, mapping of genes related to SCN resistance, abiotic stress, seed composition, and transcription factors is underway. This integrated physical/genetic map will facilitate cross-referencing for positional cloning and fine mapping of genes or QTLs of interest. In order to integrate the physical map and genetic map on a fine scale, we are constructing a high resolution genetic map using 760 F2 lines derived from a Forrest × Williams 82 cross using SSR and SNP markers. This "gold standard" genetic map will also provide a scaffold for integration of two soybean physical maps generated from these two cultivars. Research supported by a grant from the National Science Foundation, Plant Genome Program.

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Poster 20

Comparing Gene Expression Profiles from Herbicide- and Pathogen-Treated Soybean

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Previous studies implicated a role for photosynthetic components in disease resistance (Allen et al 1999, Seo et al 2000, Zou et al 2005). Several papers suggested more specifically that inhibition of photosystem II (PSII) was involved in the hypersensitive response (Allen et al 1999, and Seo et al 2000). To learn more on how soybean defense against pathogens might involve PSII, we examined soybean transcriptional response to PSII interfering herbicides. Atrazine inhibits photosynthetic electron transport by competitively binding to the plastoquinone (QB) binding site of the D1 protein of PS II (Steinback et al 1981, Hess 2000). It's generally believed that bentazon interacts at the same site of PSII but there is evidence that suggests the actual binding site of bentazon is different from that of atrazine (Nimbal et al 1996). In our current study, soybean (*Glycine max* cv. Williams 82) plants were sprayed with atrazine, bentazon, or water mist when the first trifoliate leaves were fully expanded. First trifoliate leaflets were sampled at 1, 2, 4, 8 hours after treatment. Microarrays of PCR products amplified from soybean cDNA were carried out to monitor the gene expression patterns to the herbicide treatments. Microarray data from herbicide-treated plants are being compared to expression data from pathogen-challenged plants. Preliminary data about similarities and differences between herbicide and pathogen treatments will be presented.

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Poster 21

Development of Soybean Expression Database (SED)

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Difficulties in handling large volumes of microarray expression data is an obstacle for comparison among different microarray projects. To address this problem we are developing a web-based database, tentatively called Soybean Expression Database (SED), using PERL/CGI, C and an ORACLE database management system. SED will include three components. Data Mining will allow to compare normalized gene expression data derived from the public soybean cDNA microarray studies, including experimental design and array information.The Gene Information component will serve as an information warehouse pertaining to all spots on the cDNA slides produced in the lab of Lila Vodkin at the University of Illinois as well as Affymetrix soybean expression chips. Information will include microarray gene identifiers, annotations from NCBI and TIGR, as well as ability to identify which cDNA spots best match a given gene probe set on the Affymetrix soybean gene chip to allow comparing soybean cDNA array data to that of Affymetrix soybean chip data. Web interfaces have been built for information retrieval. Users may cut and paste or upload gene lists and search for a variety of annotation options such as original clone ID, TIGR TC, GO terms and pathway information. The database is also searchable by keyword which can be restricted to searches of individual print sets. Thirdly, SED will contain raw and normalized digital expression (EST abundance) data derived from analysis of the complete public soybean EST collection obtained from 120 different non-normalized EST libraries. The database will be hosted by the University of Illinois National Center for Supercomputing Applications with the goal of public access Fall 2006.

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Poster 22

Gene Expression Profiling Soybean Challenged with *Sclerotinia sclerotiorum*

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Sclerotinia sclerotiorum is a necrotrophic fungal pathogen that infects soybean causing the disease called white mold or Sclerotinia stem rot. Oxalic acid is known to be a major pathogenicity factor of *S. sclerotiorum*. Fifteen-days-old soybean plants of a partially resistant variety (PI-194639) and a susceptible variety (Williams 82) were inoculated with actively growing mycelia utilizing the cut-stem technique and 1 inch sections were sampled at 8 hpi and 14 hpi for gene expression analysis. In a second experiment an OxO transgenic plant (line 80(30)-1) that showed resistance to the pathogen and its susceptible parent AC Colibri were inoculated using infected flower buds. Samples of the inoculated leaflets were taken at two early stages of disease development. For each experiment, the samples obtained were frozen in liquid nitrogen within 30 seconds of collection. RNA was isolated from the samples, reverse transcribed using amino-allyl incorporation for indirect labelling. Cy3 and Cy5 were assigned as labels according to loop designs for the experiments. Samples were hybridized on soybean cDNA microarrays and images were obtained for each of the hybridized slides. A response measurement in log2 ratio intensity was obtained. Preliminary analyses on biological replicates were conducted for both experiments and will be presented. Significance of these results and identification of differentially expressed genes are being assessed using various statistical tools such as R/MAANOVA and SAS.

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Poster 23

Comparative Analysis of Legume Genome Evolution in the Vicinity of the *Rpg1-b* Disease Resistance Gene

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We are using comparative genomics to investigate genome evolution in the legumes. Our goal is to determine the sequence of a ~1Mb interval in soybean (*Glycine max*) and the orthologous and homoeologous regions in several related legumes positioned at varying phylogenetic distances. The region being characterized contains a cluster of disease resistance (R) genes in soybean that are effective against a diverse array of pathogens. This analysis will provide important insights into genome evolution in the legumes, particularly with regard to the role of polyploidization. We have assembled BACs derived from the soybean *cv.* Williams82 into 2 tightly linked contigs that cover over 1Mb. These BACs are being sequenced and over 755kb is available for analysis. The region contains at least 12 NBS-LRR type R genes, one of which encodes *Rpg1-b* (a gene conferring resistance to bacterial blight). These NBS-LRR genes are grouped into several clusters separated by stretches of genes unrelated to disease resistance. The NBS-LRR genes belong to at least 4 small gene families, two of which are interspersed, suggesting a complex evolutionary history. Soybean is an ancient tetraploid and we are also characterizing the homoeologous region corresponding to the R gene cluster described above. Preliminary analysis indicates dramatic expansion of the homoeologous region due to the insertion of retroelements. In parallel with this work, the Maroof, Young, and Doyle laboratories have assembled BAC contigs for the orthologous regions in a second *G. max* cultivar (PI96983), *Glycine tomentella*, and *Teramnus labialis*, respectively. Sequencing of these BACs is also well advanced, and comparisons between the homoeologous / orthologous regions will be presented. Further details are available from: www.bio.indiana.edu/~nsflegume. This work is funded by the NSF Plant Genome Research Program.

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Poster 24

Protein Characterization of Soybean Seed Coats

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The soybean seed coat is an agricultural by-product that is removed during the processing of the seed and has little value to soybean seed processors. Even so, within the dried seed coat, different specialized tissues are the target for accumulation of proteins, including the enzyme soybean peroxidase (SBP). SBP is expressed mainly in the hourglass cells of the seed coat. Hourglass cells are sclereids that provide a contained environment for SBP storage, which remains stable even after several years of storage. As part of this study, the different layers of mature seed coats; epidermis, hourglass cells, parenchyma and aleurone layers were separated and their protein content was analyzed by SDS-PAGE. Several differences were observed in the protein makeup of the different tissues. Western blot analysis of mature seed coat extracts of high (Harosoy 63) and low peroxidase (Jack) expressing cultivars showed that SBP represents 10% of the soluble proteins in high-peroxidase expressing cultivars, and that SBP was not detectable in seed coat extracts of low-peroxidase cultivar. Proteome analysis of the seed coats could lead to the discovery of proteins expressed in a tissue-specific fashion, generating valuable information for future biotechnological efforts to enhance soybean market value.

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Poster 25

Characterization of Soybean Promoters through Evaluation of GFP Expression in Transgenic Soybean

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Four different promoters from soybean (*Glycine max* Merrill. cv Jack) were generated using either genomic DNA for amplification of known sequences (elicitin, ubiquitin), or Genome Walker libraries for amplification of the 5' regions of known ESTs (HSP90, actin). Full length and truncated promoters/promoter fragments were ligated to the gfp gene and introduced into either imbibed cotyledons of lima bean (*Phaseolus lunatus* cv 'Henderson') for transient expression studies or proliferative embryogenic tissue of soybean for analysis of expression in stably transformed cultures and plants. Analysis of the elicitin promoter in stably transformed plant tissues indicated very low levels of expression in embryogenic tissues and root tissues of regenerated plants. Analysis of the ubiquitin promoter and an "intron-less" version of the ubiquitin promoter revealed very high levels of expression in both the lima bean transient expression system and in stably transformed soybean tissues. Expression was slightly lower with the intron-less version of the ubiquitin promoter but surprisingly, both versions of this promoter showed higher expression than a standard 35S promoter. Use of the full-length ubiquitin promoter gave expression in most plant tissues. Stepwise truncation of the HSP90 promoter revealed that a 450 bp segment was required for high expression using the transient expression assay system. Expression in stably-transformed soybean tissue of the full length HSP90 promoter was restricted to young proliferative somatic embryos and roots. The actin promoter also showed GFP expression in proliferative embryos and roots, with additional expression in young leaf and petiole tissues. Overall, GFP expression using these 4 different promoters was most easily observed in root and proliferative embryogenic tissues. GFP expression in leaf tissue was more difficult to observe, possibly from interference from chlorophyll autofluorescence. These 4 promoters offer additional flexibility to use soybean regulatory elements for control of transgene expression in soybean.

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Poster 26

Comparative Analysis of *Rpg1-b* Region In Soybean Molecular Linkage Group F

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Soybean (*Glycine max*) accession 'PI96983' carries several disease resistance genes located on the molecular linkage group F (MLG F), including the *Rpg1-B* gene conferring resistance to bacterial blight. The objective of this study was comparative analysis of one megabasepair (Mbp) sequence encompassing the region containing the RFLP probes R45 and php2265. We have assembled BACs derived from the soybean cv. PI 96983 into tightly linked contigs. BAC contigs from the homoeologous region were also assembled. BAC-end sequence (BES) genetic mapping approach has revealed that a big portion of the homoeologous region is located in MLG E. A total of 23 BACs were identified from MLGs E and F. These BACs are being sequenced, and over 900 kb is available for analysis. Comparative analysis identified several conserved gene families and loci in these regions. Preliminary analysis suggested the possibility of a complex evolutionary history for these loci. Phylogenetic analysis points to the highly dynamic evolution of these regions and gene families. In this project, a total of 2 MB sequence from PI96983 will be compared with the sequences from the soybean cultivar 'Williams 82' and several legume species for comparative genomics studies

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Poster 27

Characterization of Gene Expression Involved in Dark-induced Photoperiod Response

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The adaptation of a soybean cultivar is usually restricted into a narrow region due to its short-day (SD) photoperiod characteristics. Identifying genes involved in soybean photoperiodic control of flowering is vital for breaking this restriction in soybean production. A cDNA subtractive library enriched for dark-induced up-regulated ESTs was constructed from leave tissues of a photoperiod-sensitive cultivar DongNong L13 by SSH methodology. A total of 148 clones with about 250-600bp cDNA inserts were sequenced, obtaining 43 ESTs with putative functions and 27 ESTs with unknown functions. Six cDNAs (GAMYB binding protein, DNA binding protein RAV, Transcription factor-related, Zinc finger protein, NAC and Light receptor) in transcript abundance exhibited about 4-9 fold of increment by real-time RT-PCR. The results of genetic functional analysis demonstrated that genes putatively encoding proteins were widely related to diverse aspects during organism development including biological regulation pathways such as transcription, signal transduction and programmed cell death, protein, nucleic acid and carbohydrate macromolecule degradation, the cell wall modification, primary and secondary metabolism and stress response. Furthermore, full-length genes of soybean GAMYB binding protein (DQ112540) and DNA binding protein RAV (DQ147914) that may be involved in SD soybean photoperiod response were cloned by RACE. GAMYB binding protein mediated GA signaling in growth and photoperiodic flowering responses. RAV was identified as a DNA binding protein possessing an N-terminal AP2/ERF (or EREBP)-type DBD and a C-terminal B3 domain, which showed a significant increment in short-day condition. The significant increase in the level of soybean RAV expression probably implies that it may have an important role for photoperiodic control of flowering in soybean. More detail functions of the two genes are under the investigation using transgenic tobacco plants and RNAi technology in soybean.

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Poster 28

Development of a Transposon Tagging System for Soybean

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The long-term goal of this project is to build a repository of transposon-tagged soybean events as a resource for soybean functional genomics programs. We are using the maize *Ac/Ds* transposon system as a reverse genetics tool for soybean. Transgenic soybean events have been generated that carry gene trap and enhancer trap elements, with the respective trap elements delineated by *Ds* termini. Monitoring for activation of the visual marker gene GUS was conducted on progeny derived from 57 transgenic events, by examining various organs using a histochemical assay. Nineteen events displayed GUS activity in at least one tissue type. Sequence of junction fragments has been obtained for a subset of the events with GUS activity. In one of these events (456-1), the soybean locus HPS1.5 has been interrupted, which encodes for a precursor peptide deposited in endocarp tissue of the pod and influences seed luster. This location has been confirmed by Southern blot and PCR across the junction. Analysis of HPS RNA accumulation is ongoing. In another event (457-13), T-DNA interrupts the soybean EST Gm-c1065-9605. The corresponding RNA is undetectable by RT-PCR in this event. An event designated 453-56 in which junction sequence displays homology to a *M. truncatula* EST has been mapped to the top of LG B2. Preliminary experiments confirmed that the non-autonomous *Ds* element is immobile in the absence of *Ac* transposase and that *Ds* movement is observed when combined with *Ac* transposase. To fully utilize this system, a series of crosses has been made to pyramid the *Ds* delineated elements with *Ac* transposase-expressing soybean lines. F2 and F3-derived populations from these crosses are currently being characterized at the molecular level to monitor the frequency of *Ds* transposition in the soybean genome. Several somatic transpositions and 1-2 germinal transpositions have been observed among 20 crosses analyzed to date.

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Poster 29

Molecular Characterization of a Soybean β-amylase Mutant

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ß-amylase hydrolyzes a-1,4-glucosidic linkages from the reducing ends of starch to generate maltose. Even though ß-amylase has been implicated in starch degradation, the precise in vivo function remains unclear. ß-amylase is expressed abundantly in soybean seeds. Two soybean near-isogenic lines, one containing normal ß-amylase activity (Altona *Sp1b*) and the other with undetectable ß-amylase activity (Altona *sp1*) have been earlier characterized. However, the molecular basis for the absence of ß-amylase activity in Altona *sp1* is not known. In this study, we report the molecular characterization of this mutant. Southern blot analysis indicated that there are two copies of this gene in the soybean genome. A 3.8 kB XbaI fragment, which showed hybridization with ß-amylase gene, was absent in Altona *sp1* instead a 2.6 kB fragment was detected. The ß-amylase gene from the wild-type and Altona *sp1* were PCR amplified from genomic DNA based on the published ß-amylase cDNA sequence. Analysis of the nucleotide sequence of the ß-amylase gene from the wild-type revealed a complete open reading frame that was interrupted by six introns. In contrast, the ß-amylase gene from Altona *sp1* had a 1207 bp deletion near the 5' region that included the second and third exon regions. SDS-PAGE gel analysis revealed that the ß-amylase mutant, in contrast to the wild-type, accumulated trace amounts of a 52 kD protein. Northern blot analysis revealed the accumulation of 1.6 kB RNA transcript in developing seeds of the wild-type but not in Altona *sp1*. Interestingly, a less abundant truncated RNA transcript was detected in Altona *sp1*. Our results demonstrate that the deletion in the 5' region of the ß-amylase gene is responsible for the lack of ß-amylase activity in soybean Altona *sp1* ß-amylase mutant.

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Poster 30

Phosphoproteomics Approaches for Investigating the Soybean-*Phytophthora sojae* Interaction

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Phosphorylation plays a regulatory role in the activation of many proteins for carrying out biological functions. Many of these proteins could be less abundant; and therefore, their isolation becomes arduous. We have applied the immobilized metal affinity chromatography (IMAC) to isolate soybean phosphopeptides. It was observed that over 75% of the peptides bound to zirconium ions of IMAC contain phosphorylated residues. However, IMAC strategy may fail to isolate the phosphopeptides of many less abundant phosphoproteins. Here we describe a comparative phosphoproteomics method for identifying putative phosphorylated or dephosphorylated proteins. The strategy has been used in isolating soybean proteins that are either phosphorylated or dephosphorylated following infection with the oomycete pathogen, *Phytophthora sojae*. We have shown that soybean proteins, not identified by the IMAC-based phosphopeptide isolation strategy, were isolated by this comparative phosphoproteomics approach. In this method we enrich the less abundant phosphorylated proteins by applying IMAC and then separate them in two-dimensional (2D) polyacrilamide gels. Comparison of protein profiles in 2D gels allowed us to identify proteins that were putatively either phosphorylated or dephosphorylated following *P. sojae* infection. In order to reduce the artifacts arisen from binding of acidic proteins to the column, protein samples were esterified prior to IMAC. Esterification step enhanced the efficacy of IMAC presumably by reducing the non-specific binding of acidic proteins to the zirconium ions. The phosphoproteomic approaches described here should be applicable to the isolation of phosphoproteins from other biological systems.

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Poster 31

Comparison of Light-Responsive Gene Expression in *Arabidopsis* and Soybean

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Photomorphogenesis is relatively well understood in model systems like *Arabidopsis*. Despite its importance in crop morphology, physiology and yield, photomorphogenic effects in crop plants are under-researched. In our study, we focused on the transcriptional regulation of photomorphogenesis in soybean using microarrays. We used the soybean cDNA microarray developed by the NSF Soybean Functional Genomics project to find genes whose expression changes rapidly in etiolated soybean seedlings in response to a pulse of far red light. We have used published *Arabidopsis* whole-genome Affymetrix microarray data (Majid Ghassemian, et al, Arch Biochem Biophys. 448(2006), 45-59) in an attempt to translate existing knowledge of photomorphogenesis into a better understanding of the corresponding mechanism in soybean. This comparison also gives some insight into the extent of the variation in gene-level light responses between soybean and *Arabidopsis*. We computationally determined the orthologous relationships between the genes represented on the NSF soybean cDNA microarray and the *Arabidopsis* whole-genome oligonucleotide array. We then compared the phytochrome regulated gene networks in soybean predicted by our experiments, to those published in *Arabidopsis*. Our results show that although some of the light regulated genes in the soybean microarray have orthologs in *Arabidopsis*, many are unique to soybean, suggesting overlapping and yet distinct transcriptional networks controlling photomorphogenesis in the two plant species.

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Poster 32

Analysis of T-DNA lines for Ds and Tnt1 Tagging of Soybean Genome

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Our project focuses on transposon tagging of soybean genes using two transposons systems, the maize transposable element Ds and the tobacco retrotransposable element Tnt1. Twelve, activation-tagging Ds vectors were constructed, each containing various combinations of either the CaMV 35s promoter or individual seed-specific promoters (e.g., phaseolin, soybean seed lectin, or glycinin). Several hundred transgenic soybean lines harboring the different Tnt1 and Ds element constructs were made using Agrobacterium-mediated transformation. Analysis of these transformants by Southern blot revealed that 50% of the lines were single copy lines, 30% were double copy and 20% carried more than 2 copies. The average T-DNA insertion number was 1.5 insertions per line. At present, we have analyzed the DNA sequences adjacent to the transposon insertion in approximately 100 of these lines. A Soybean Transposon Insertion Mutant Database was developed to serve as a resource to the community. When completed, this database will allow easy access to all available information (flanking sequencing, genetic and physical map location, associated phenotypes, etc.) for each transposon insertion. At present, we are characterizing the ability of both the Ds and Tnt1 elements to transpose in soybean. The goal of the project is the development of sufficient transposon insertions to target any soybean gene.

This project is funded by a grant from the United Soybean Board.

Key words : Reverse genetics, Soybean, PCR walking, Database, Flanking Sequences, Transposons

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Poster 33

A Transposon Tagged Male Fertility Mutant of Soybean

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Genetic male sterility can result from a mutation in one of many genes involved in microsporogenesis. These genes are collectively referred to as male fertility genes. In soybean, over 10 genetic loci have been identified as being essential for male fertility. Cytological studies of sterile mutants for many of these loci indicate that microspore breakdown is diverse in timing, as well as its effect, on microspore morphology. This suggests that microsporogenesis is a complex process, controlled by more than one mechanism. Despite the number of male sterile mutants described in soybean, little progress has been made in characterizing genes responsible for male fertility. Most male sterility research has been descriptive. Recently, using the transposable element Ds (Dissociator), we tagged a gene responsible for male fertility in soybean. This Ds induced mutation inhibits the formation of mature pollen. The mutant lacks pollen, preventing normal self-fertilization, a characteristic important for production of hybrid seed in many crop plants. Data will be presented relating to the the genetics and the cytology (microsporogenesis and microgametogenesis) of that mutant.

This research is funded by a grant from the United Soybean Board.

Key words : male sterility, soybean, microsporogenesis

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Poster 34

Extending CMap for Displaying the Soybean Physical and Genetic Maps

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CMap, a part of the GMOD Project, is a powerful tool originally conceived as a way to display and compare multiple genetic maps. We have extended this functionality to display contigs and their constituent BACs in the Williams physical map aligned with the most recent soybean genetic map. Features in common between the maps are identified using both color and connecting lines between the features. This allows the user to see the relationships between the physical and genetic maps, and gives an immediate genetic context to features on the physical map. In addition, we have modified CMap to include several exploratory data analysis tools including 1) popup contextual menus that show additional information about map features by querying the underlying database and 2) the ability to temporarily color-code individual map features, or sets of features. A web based tutorial demonstrating these tools is available at: http://soybase.org/tutorials/physmap/physmap_tutorial.html. The source code for these modifications and the schema for the underlying MySQL database needed to implement the new features are freely available upon request.

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Poster 35

Filter Markup: A Program for Collection and Analysis of Overgo Data

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Pooled overgo hybridizations are a useful method to collect data on the sequence content of very large numbers of BACs in a relatively short time. This is typically accomplished by using a multidimensional pooling strategy for the oligos to reduce the number of samples that must be analyzed and then hybridizing the pooled overgos onto BACs arrayed onto a high density filter. These filters typically contain thousands of tiny spots, which make manual scoring of the hybridization data tedious and error prone. We have developed a Macintosh program - Filter Markup - that presents the user with an intuitive graphical interface for scoring and collecting the hybridization data, and for performing the deconvolution of the overgo pools to identify the specific BACs that contain the overgo sequence. The results are returned as a tab-delimited file ready to be imported into Excel or a local database. Filter Markup is freely available upon request.

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Poster 36

Gene Regulation during the Early Nodulation Process in Soybean Root Hair Cells

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Nodulation is a very specific symbiotic event between a host plant and a bacterium (e.g. *Glycine max – Bradyrhizobium japonicum*). The result of this interaction is the formation of a novel organ - the nodule - that can fix atmospheric nitrogen with high efficiency. During the first hours of the plant-bacterium interaction, both partners exchange chemical compounds (isoflavonoids secreted by the plant and Nod factor excreted by the bacterium). The Nod factors regulate the expression of genes involved in nodulation and induce specific morphological changes on the plant root hair cell (e.g., root hair curling). These responses are necessary for plant invasion by the bacterium. We have analyzed by microarray and quantitative RT-PCR reactions the expression level of genes during the first steps of the nodulation process (3, 6, 12 and 18 hours after inoculation). After root hair isolation, for each time point, we compared gene expression levels of inoculated plants vs mock plants by microarrays. The resulting analysis led to the identification of 5627 soybean genes significantly deregulated after bacterial inoculation. The microarray data was then confirmed by quantitative RT-PCR for selected genes. We are currently using RNAi to silence these genes to more fully explore their role in early nodulation.

Research was supported by a grant from the National Science Foundation, Plant Genome Program.

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Poster 37

A New Tool to Analyze the Expression of More Than 600 Soybean Transcription Factor Genes

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Transcription factors (TF) are the key regulators in the cell. Expression levels of TF genes are generally difficult to analyze by DNA microarray due to their low expression level. Quantitative RT-PCR (qRT-PCR) is >100-fold more sensitive in mRNA measurement than DNA microarray hybridization. We are developing a resource to utilize qRT-PCR to accurately analyze the expression levels of soybean TF genes. Primer sets were designed with PRIMEGENS software (http://digbio.missouri.edu/primegens/). This software is able to design primers and to compare them against library gene sequences to increase primer specificity. As a first step, a standard RT-PCR with each primer sets was performed to evaluate the expression pattern of the TF on different plant tissues, such as, flower, seed or young leaf. According to these results, qRT-PCRs were performed with one template to select each primer set according to its specificity and its amplification efficiency. Currently, 675 primers sets have been designed and selected to create the primer library. To validate the library, expression levels of these TF genes will be analyzed using different plant tissues and plants inoculated or not with B. japonicum, the bacterium responsible for soybean nodulation.

This research is funded by the Missouri Soybean Merchandising Council.

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Poster 38

Transcript Profiling of Soybean during Nitrogen Fixation Symbiosis

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The interaction between soybean (*Glycine max*) and the bacterium *Bradyrhizobium japonicum* (USDA110) leads to the establishment of a nitrogen fixing symbiosis. The bacteria induce the formation of a specific new organ in the plant, the root nodule, in which the bacteria reduce atmospheric nitrogen to ammonia which constitutes a source of nitrogen which can be used by the plant. A better understanding of this symbiosis is fundamental to its better exploitation in sustainable plant production systems. We used a microarray approach to identify genes regulated during the nodulation of the supernodulated GmNARK (*Glycine max* Nodule Autoregulation Receptor Kinase) mutant, obtained from the wild-type cultivar Sinpaldalkong2. The cDNAs obtained from RNA extracted 4, 8 and 16 day old roots uninoculated or inoculated with B. japonicum were hybridized to two 18 k cDNA microarray slides. Statistical analysis of the resulting data enabled the identification of more than 2000 genes significantly deregulated in response to B. japonicum inoculation. In order to confirm the microarray results, the transcriptional response of selected genes was confirmed using qRT-PCR. An RNAi approach was used on genes confirmed to be upregulated during nodulation demonstrating that some of these genes are essential for successful nodulation.

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Poster 39

Establishment of the Soybean Root Hair Proteome Reference Map

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Root hairs are single, tubular-shaped cells formed from the differentiation of epidermal cells, called trichoblasts, on primary and secondary roots. Root hairs improve the capacity of the root to absorb water and nutrients from the soil by increasing the surface area of the root. Root hairs are also the site of the infection of legumes by rhizobia leading to the establishment of a nitrogen fixing symbiosis (i.e., nodulation). As part of our effort to explore the early steps in symbiotic infection, we are establishing a root hair proteome reference map for soybean (*Glycine max*) by using 2D gel electrophoresis and MudPIT (Multidimensional Protein Identification Technology). Soybean was selected for this study due its agronomic importance and the larger root size, enabling the isolation of gram quantities of root hairs required for proteomic approaches. Proteins extracted from soybean root hairs were separated by 2D gel electrophoresis. Four replicates were performed and spots identified in at least 3 out of the 4 replicates were picked and their molecular weights and pIs determined. Tryptic peptides were analyzed on an Applied Biosystems 4700 MALDI TOF/TOF. Both MS and tandem MS spectra were acquired for each spot. Out of 1002 picked spots, 239 have been analyzed up to now. These 239 spots yielded 142 protein identifications with a confidence greater than 95%. MudPIT was also used to establish the reference map and enabled the identification of 307 proteins. Both methods were compared and showed an overlap of 54 %. In general, the proteins identified are mainly involved in primary metabolism, protein synthesis and processing, defense response and stress, and signal transduction.

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Poster 40

The Genetic Basis of the Low Phytic Acid Soybean Mutant CX1834: A Proteomics Approach

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It is estimated that over 2 billion people, mostly women and children in developing countries, suffer from iron deficiency. These deficiencies are due in part to iron uptake inhibitors found in staple foods, which are often of plant origin in developing countries. Phytic acid, a phosphorous storage molecule found in plant seeds, is the most important iron uptake inhibitor contributing to iron deficiency. Phytic acid strongly binds iron and other positively charged minerals in the small intestines and prevents their absorption. Phytic acid also poses several problems for agriculture. Monogastric livestock lack sufficient enzyme activity to metabolize the phytic acid present in cereal seeds, leading to inadequate phosphorous uptake and the need to supplement animal feed with expensive and non-renewable inorganic phosphorus. The undigested phytic acid that is excreted in the animal's manure (which is used as fertilizer) is the leading cause of phosphorus pollution. These undesirable properties make the development and characterization of low phytic acid crops a high priority in agricultural research. A previously developed low phytic acid soybean mutant, CX1834, is available for public breeding efforts. This line exhibits a 50% reduction in seed phytic acid levels and a concomitant increase in available phosphorus. Work from other labs has revealed that two independent recessive loci are responsible for the low phytic acid phenotype. To facilitate breeding efforts, we would like to identify the exact genetic lesions of these loci. This would result in "perfect" genetic markers for breeding efforts. We are taking a proteomics approach to identify the genetic basis of the low phytic acid phenotype of CX1834. Preliminary work indicates there are several differences in the 2D gel profile between wild-type soybeans and CX1834 soybeans. Candidate genes identified via proteomics will be sequenced from the CX1834 line to identify possible mutations in these genes.

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Poster 41

Genetic Diversity and Quality Attributes of Food-Grade Soybeans from North America and Asia

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Soyfood is becoming more and more widely accepted by U.S. consumers due to its nutraceutical value. The increasing soyfood demand in the domestic and international markets indicates that specialty soybean cultivars may potentially offer significant economic returns. Diversity of food-grade soybeans is critical for utilization of genetic resources in cultivar development, germplasm enhancement, and end-product commercialization. The objective of the study was to estimate the level of genetic variability and relationship within and between North American and Asian cultivars and lines by simple sequence repeats (SSR) analysis. The genetic diversity was accessed among 66 North American and 52 Asian genotypes including 63 large-seeded (> 20g/100 seeds) and 55 small-seeded (< 10g/100 seeds) soybeans. Ninety SSR primers were used for genotyping purposes, representing 40 flanking markers for quantitative trait loci (QTL) associated with seed size and calcium content and 50 core SSR primers selected using 80 Southern Chinese soybean accessions. Genetic distance between pairs of genotypes was calculated by means of Jaccard's coefficient. UPGMA dendrogram and principal coordinate were used to define the genetic relationship among genotypes. Molecular and seed quality attributes will be presented at the conference. Asian soybeans may serve as important genetic resources for broadening genetic base of U.S. specialty soybeans.

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Poster 42

QTL Hot Spots in the Soybean (*Glycine max* L.) Genome

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Certain regions in the soybean genome are hot spots for QTLs. In 1993 Mansur et al., detected three QTLs near markers A397, G173 and R79 in LG C2 (u9), L (u14) and M (u11), respectively. Recently, within 10 cM from A397, we found a QTL associated with seed length (Salas et al., 2006). Previously, this region was associated with R3 (Mansur et al., 1993; Orf et al., 1999, and Zhang et al., 2004), R8 (Mansur et al. 1993, 1996; Orf et al., 1999); reproductive period (Orf et al., 1999); plant height (Mansur et al., 1996; Orf et al., 1999; Zangh et al., 2004); lodging (Orf et al., 1999; Zangh et al., 2004); seed size (Hoeck et al., 2003); seed number (Orf et al., 1999); node number (Zangh et al., 2004); and yield (Mansur et al., 1993, 1996; Orf et al. 1999; Zangh et al., 2004). Similarly, within 10 cM of G173 we found a QTL controlling seed width and length where others detected QTLs for seed size (Hoeck et al., 2003); R3 (Mansur et al., 1993, 1996; Orf et al, 1999), plant height, lodging (Lee et al., 1996; Mansur et al., 1993, 1996; Orf et al, 1999); R8 (Mansur et al.,1996; Orf et al., 1999); seed protein and oil content (Mansur et al., 1996); leaf length and area, and yield (Orf et al., 1999). Finally, within 20 cM of R79 we found QTLs for seed length, width, and volume and others for R3 and R8, leaf area, and yield (Mansur et al., 1993, 1996; Orf et al., 1999); and plant height (Mansur et al., 1996; Orf et al., 1999; Zhang et al., 2004). These QTLs were detected in various environments and populations. Studies at the molecular level are needed to learn more about these QTLs hot spots genomic regions.

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Poster 43

GmZFP1 is Expressed Predominantly in Reproductive Organs and Late Seed Development in Soybean

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The plant TF III A zinc finger proteins play important roles in plant growth and development. In this report, we isolated a single zinc finger gene, designated as GmZFP1, from soybean flowers by in silico mRNA subtraction strategy and RT-PCR. GmZFP1 is an intronless gene and encodes a protein of 210 amino acid residues with a predicted molecular mass of 23.5KDa. GmZFP1 contains a single zinc finger domain and a DLN-box/EAR-motif at the C-terminus. To localize GmZFP1 mRNA in various tissues, semi-quantitative RT-PCR assay was performed. GmZFP1 is expressed in all detected reproductive organs including flowers, developing seeds, pods, sepals, pistils with a low level, and more accumulated in petals, stamens, but not found in all detected vegetative organs with except of stem tips. Further, we found that the expression of GmZFP1 was higher in late seed development than in early seed development. To our knowledge, GmZFP1 is the first characterized gene encoding for single zinc finger protein in soybean, and may play a role as a transcriptional factor in reproductive organs development, especially functioning in petals, stamens and late developing seeds.

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Poster 44

Development and Verification of SNP Markers Associated with the Rcs3 Gene for Resistance to Frogeye Leaf Spot in Soybean

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Frogeye leaf spot (FLS), caused by Cercospora sojina Hara, is an increasing threat to many soybean [*Glycine max* (L.) Merr.] production regions in the USA. The Rcs3 gene has provided resistance to all known races of *C. sojina*. In order to provide resources for high throughput marker assisted selection of the Rcs3 resistance gene, we developed and evaluated several single-nucleotide (SNP) and insertion/deletion (InDel) polymorphisms for their linkage to the Rcs3 locus. Toward this end, we surveyed 13 Bacterial Artificial Chromosome (BAC) end sequences that were anchored with two SSR markers (Satt244 and Satt547) and the two SSR clones in a panel of six soybean cultivars for nucleotide variations. Three of the cultivars (Davis, Cook, and Young) were previously shown to possess the Rcs3 allele, with the other three cultivars (Blackhawk, Bragg, and Lee) possessing the rcs3. Nineteen nucleotide variants were identified and validated, but only 11 were mapped to the region of linkage group J (LG-J) containing the Rcs3 gene in a F2 population derived from Davis x Blackhawk. The Rcs3 gene was positioned in LG-J in the interval spanning Satt244 and two InDels (AZ573TA150 and AZ573CA393). None of the SNPs and InDels identified appears to contribute directly to the Rcs3 phenotype, but 11 cosegregated within a 3-cM interval surrounding the Rcs3 locus. These 11 markers were further validated for association with Rcs3 and for their potential in marker assisted selection in 64 lines and cultivars including ancestors and descendants of Davis. Our results suggest that the markers AZ573CA393 and AZ573TA150 could particularly be used successfully in marker assisted selection for soybean resistance to frogeye leaf spot. These two indels could be easily adapted for the direct hybridization procedure on a Luminex100 flow cytometry platform, which was shown to be more cost effective than the single base extension (SBE) we applied for genotyping these nucleotide variants.

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Poster 45

Functional Relationship between Fatty Acid and Tocopherol QTLs in Soybean

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Soybean tocopherols (*Glycine max* L. Merr.) have received a great deal of attention in recent years. Previous studies clearly outlined a strong correlation between individual tocopherols and fatty acids in soybean. Although fatty acid QTLs have been successfully mapped, little is known about genetic factors regulating tocopherol accumulation. For that purpose, two high yielding early maturing soybean cultivars were crossed to develop a RIL population for mapping these traits. A molecular linkage map was constructed from 120 SSR markers segregating in 93 F4:8 RILs. Phenotypic data was collected from 3 locations with two replications each in 2004. QTLs controlling *alpha* and *delta* tocopherol have been mapped on LG C2 and F. While no previous reports indicated fatty acid QTLs in these regions, research is currently being conducted to evaluate possible associations between these two component groups from a molecular genetic standpoint.

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Poster 46

QTL for Soybean Seed Yield and Agronomic Traits in Populations Derived from Exotic Lines

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Previous studies have reported that seed yield in soybean can be improved by using PIs as sources of favorable alleles. Therefore the objective of this study was to identify unique alleles in populations derived from exotic soybean lines. The A3 population consists of 133 F4:6 lines developed from four exotic lines and the adapted parent IA3023. The B3 population consists of 149 F4:6 lines developed from two exotic lines and the adapted parent U98-311442. Both populations were evaluated in 2005 at two locations in Illinois for plant height, lodging, maturity and seed yield. Both populations showed a significant genotypic effect and no environment by genotype interaction. High heritability for yield ranging from 0.78 to 0.83 was detected in both populations. In the A3 population 7 QTLs were detected for agronomic traits, including one QTL for yield in which the favorable allele, derived from the adapted parent, increased yield by 270 kg/ha. The B3 population showed transgressive segregation for yield with the top ten progeny lines exceeding the adapted parent by 536 - 670 kg/ha, with LSD of 295 kg/ha. A total of eleven QTLs were detected in the B3 population for agronomic traits including yield. Two QTLs for yield, one on LG A1 and the other on LG G, were detected consistently over environments. The LOD ranged from 2.7 to 5.2 and the QTLs explained 9.2 to 18.0 % of the phenotypic variation. The QTL on A1 is located 2 cM from a previously reported yield QTL. The QTL on G is located at Satt610, a location where, to our knowledge, a yield QTL has not been previously reported. The adapted parent contributes the favorable allele, causing a yield boost of 281 kg/ha. Results on E population developed from two backcrosses of Elgin to exotic germplasm will also be presented.

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Poster 47

Molecular Markers Associated with β-conglycinin Deficiency in Soybean

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The soybean seed storage protein β-conglycinin has a low amino acid score, shows lower functional gelling properties compared with glycinin and contains a major allergen. Therefore, decrease in the content of β-conglycinin is one of the objectives of soybean breeding programs. A β-conglycinin-deficient mutant QT2 was identified from a wild soybean in Kumamoto prefecture, Japan, and the phenotype was found to be controlled by a single dominant gene *Scg-1* (Suppressor of β-conglycinin). *Scg-1* was introduced into a soybean cultivar Fukuyutaka from QT2 and this near-isogenic line was designated as QY7-25. Segregation analyses of the progeny derived from a cross between QY7-25 and the wild type did not show any significant changes caused by *Scg-1* in the germination ratio and seed weight. Fukuyutaka and QY7-25 were used for development of DNA markers associated with β-conglycinin deficiency. Ten single nucleotide polymorphisms (SNPs) in the β subunit genes and four SNPs in the α subunit genes were detected. Two β subunit gene loci and an α subunit gene locus were found to cosegregate with β-conglycinin deficiency with the DNA marker based on the SNPs in a F2 population derived from a cross between Fukuyutaka and QY7-25. The DNA markers of β subunit genes also enabled to detect polymorphisms between QY7-25 and major soybean cultivars in Japan and could be used as a practical tool for the introduction of *Scg-1* gene into soybean. The chromosome region associated with β-conglycinin deficiency was located on linkage group I of a soybean genetic linkage map with the developed marker using a F2 population from the parents, Misuzudaizu and Moshidou Gong 503. We uncovered that a cluster region of β-conglycinin genes encode the β-conglycinin deficiency.

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Characterization of Raffinose Synthase Genes in Soybean

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Three of the oligosaccharides which are generally present in soybean meal are sucrose, raffinose, and stachyose. Of the three, only sucrose is nutritionally useful as a source of metabolizable energy by monogastric animals. Raffinose and stachyose are fermented by microbes present in the gut causing flatulence and discomfort ultimately leading to poor weight gain. The purpose of this project is ultimately to increase the value of soybean meal by elevating the metabolizable energy at the expense of raffinose and stachyose through the manipulation of raffinose synthase (RS). Biochemistry, molecular biology, and genetic approaches will be used in this study to develop new knowledge about oligosaccharide metabolism in developing soybean seeds. One aspect of this project is to determine which of several putative RS genes has authentic RS enzyme activity. An in-depth expression analysis of three putative RSs was completed for multiple tissues from the standard cultivar Williams 82. Each of the putative RS genes are highly expressed in all tissues examined, indicating that RS gene expression is not restricted to developing seeds. A low raffinose line, PI200508, has similar expression to Williams 82 for the raffinose synthases. However the RS enzymatic activity was previously shown to be significantly reduced in this line. Thus, it is our goal to identify mutations or polymorphisms in the RS sequences from this low raffinose line that explain the low levels of raffinose. A final goal is to use reverse genetics to identify low raffinose lines with associated RS mutations through TILLING the Williams 82 population. The identification of mutants and molecular markers could ultimately aid in breeding for higher sucrose/low raffinose seeds.

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Mapping *Fusarium solani* f. sp. glycines Resistant Loci in Soybean

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Sudden death syndrome (SDS) of soybean (*Glycine max* L. Merr.) is an important soybean disease caused by the soil-borne fungus *Fusarium solani* f. sp. glycines. Yield loss from SDS in severely affected areas can range from slight to nearly 100 percent. The most viable option for SDS disease management is to develop cultivars with field resistance to SDS. The main objective of the research was to identify simple sequence repeat (SSR) markers linked to SDS resistance in two RIL populations. The populations were derived from crosses between PI 243530 and Spencer, and PI 507531 and Spencer; PI 243530 and PI 507531 are partially resistant to SDS, and Spencer is susceptible to SDS. A total of 295 SSR primers from all 20 linkage groups were selected at 20-cM intervals from the soybean genetic linkage map for each parent. Subsequently, two populations were evaluated with polymorphic SSR markers identified during the parent screening. Plants were evaluated for disease severity (DS) in the greenhouse using cone layer inoculation method. Both populations were set up in a randomized block design with eight replications. Markers associated with SDS were found in both of the populations.

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Soybean QTLs for Yield and Yield Components Associated with *Glycine Soja* Alleles

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USA soybean germplasm has a narrow genetic base that could be augmented by alleles from the wild species *Glycine soja* which positively influence agronomic traits. The objective of this study was to identify such alleles for yield and yield component QTL. Two populations of 150 BC2F4 lines were generated from a mating between recurrent parent *Glycine max* '7499' and donor parent *Glycine soja* PI 245331 with one line in each population tracing back to the same BC2 plant. Population A was used for the QTL identification analysis and population B was used for the QTL verification test. The population A lines were genotyped at 120 SSR marker loci and one phenotype marker, covering a total map length of 1506 cM in 20 linkage groups with an average interval size of 12.5 cM. There were nine putative QTL significantly ($P<0.0001$, $LOD>3.0$) associated with yield and yield component traits across 3 environments. One QTL for seed yield was identified using the combined data; the *G. soja* allele at satt511 on LG-A1 was associated with increased seed yield (LOD=4.3) with an additive yield effect of 190 – 235 kg ha-1 depending on the QTL analysis method. The phenotypic variance accounted for by the QTL at satt511 was 12%. This QTL also provided a significant yield increase across environments in the validation population; lines that were homozygous for the *G. soja* allele at satt511 demonstrated a 6.3% ($P=0.037$) yield increase over lines that were homozygous for the *G. max* allele. One seed filling period QTL was identified at satt335 (LOD=4.0) on LG-F with an additive effect of +1 day. This QTL also provided a +1 day additive effect (LOD=3.3) on maturity. These results demonstrate the potential of using exotic germplasm to improve soybean yield.

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A PCR-based Marker for the Rsv1 Locus of Soybean Mosaic Virus Resistance

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A pair of DNA primers was developed from the candidate gene 3gG2 at the *Rsv1* locus for soybean mosaic virus (SMV) resistance in this study. This primer pair produced a 341 bp DNA fragment specific for soybean cultivars containing Rsv1. The same fragment was confirmed in genotypes with the *Rsv1* allele [Kwanggyo(*Rsv1-k*), Mashall (*Rsv1-m*), Ogden (*Rsv1-t*), PI 96983 (*Rsv1*), Raiden (*Rsv1-r*), Suweon 97 (*Rsv1-s*)] and two gene combinations [Zhao 18 (*Rsv1*, *3*), OX 670 (*Rsv1*, *3*), Tousan 140 (*Rsv1*, *3*), J05 (*Rsv1*, *3*), and PI 486355 (*Rsv1*, *4*)]. The fragment was neither amplified in *Rsv3* containing genotypes (L29 and Cordell) and *Rsv4* containing genotypes (V94-5152, PI 88788, and Columbia), nor in the susceptible genotypes (Essex and Lee 68). This primer pair was used to survey for *Rsv1* alleles in 99 SMV resistant genotypes. Thirty-two genotypes including Clifford, Corsica, Holladay, L78-379, L88-8431, Mercury, Pace, PI 61944, PI 61947, and Tsuronoko contain the *Rsv1* gene. All cultivars carrying *Rsv1-y* allele derived from York (including Calhoun, Musen and Ripley), and from Arksoy (including Brim, Cook, Davis, Dillon, Doles, Prolina, and Young) did not produce this fragment, indicating the absence of 3gG2 in these genotypes. Additional 14 SMV resistant genotypes (Beeson, Chuzu, CNS, PI 181550, PI 398289, PI 39833, 398479, PI 407975B, PI 43807, PI 90401, PI 96257, Suzuyutata, Virginia, and Yuwoltae) were not amplified by this primer pair as they likely contain different gene(s) than 3gG2 at the *Rsv1* locus. Analysis of an F2 population from J05 (*Rsv1*, *3*) x Essex (*rsv1*, *3*) showed co-segregation between the marker and the Rsv1 allele. Therefore, the specific DNA fragment can be used as a molecular marker to identify the resistance gene 3gG2 at the *Rsv1* locus in soybean germplasm and as a tool for marker-assisted selection in breeding for SMV resistance.

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AFLP Analysis of Popular Soybean Cultivars in the Past and at Present for Genetic Diversity

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Characterization of the genetic diversity among elite soybean cultivars that were developed and widely grown historically or presently at the molecular level is valuable for germplasm assessment and parent selection in a breeding program. Thirty-eight soybean cultivars widely grown in Arkansas in last 80 years were evaluated for their genetic divergence and relatedness with AFLP markers and compared with a wild type soybean and a Chinese cultivar. A total of 836 bands were amplified with 16 primer combinations. Among all the AFLP alleles examined, 50% were fixed in all 38 historical soybean cultivars. While 406 bands were common in all cultivars, 34 unique bands were detected and nearly half of these unique bands were found in three cultivars. In comparison with unadapted genotypes, 33 unique bands were produced in *G. soja* 'PI407046', while 8 specific bands were present (3) or absent (5) in a Chinese cv. 'Jiunong 12'. Cultivars developed before 1950s were more genetically distant from each other, as compared to those developed in 1960s. Genetic diversity increased in cultivars released during 1970s and 1980s, but decreased in 1990s and 2000s. Molecular profiling has provided complementary information to pedigree analysis and phenotypic dissecting of the genetic relationships among the 38 genotypes examined in this study that have been widely grown commercially and used as parents in many soybean breeding programs.

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Fine Mapping a Recessive Male Sterility Gene (Ms2) in an Effort to Improve the Male-Sterile-Facilitated Cyclic Breeding Scheme

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The most common breeding method used in soybean varietal development programs is to make many biparental matings (season 1), self the resultant progeny to near-homozygosity (seasons 2-6, and then evaluate the inbred progeny of each mating in performance trials (seasons 7-8) to identify the few progeny that are worthy of selection and release as new cultivars. This amounts to an 8-season recurrent selection cycle, but because a breeder will generate biparental matings every season, there are, in any given season, eight different groups of biparental progeny moving through the cycle, with no opportunity for genetic exchange amongst them. Is there an alternative to this method? Yes, a method we call male-sterile-facilitated cyclic breeding (MSFCB) could be used in soybean cultivar development to provide greater recombinational opportunities. The *ms2* allele for genetic male-sterility can be used to facilitate the annual biparental matings of elected elite germplasm lines (chosen each year) with male-sterile plants derived from a prior set of such matings. At present, we plant seeds of a mixture of elite breeding selections in the black checkerboard square rows of an intermating block, with 1600 F2 seeds (segregating 3MF:1MS) in the red checkerboard rows. About 400 of the latter are expected to be male-sterile, so the other 1200 must be rogued as soon as one flower opens and, upon inspection, is recognized as being male-sterile. To make MSFCB more convenient the F2 male-sterile genotypes could be identified by molecular markers flanking the Ms2 locus (located near the bottom of LG-O). A population of 513 F2 individuals segregating for male-sterility served as our mapping population. We evaluated 1200 RAPDs, 1280 SRAPs, 64 AFLP, 34 SSRs, 10 SNPs, and 24 markers derived from BAC end sequences near the bottom of LG-O, first for parental polymorphism, and then via bulk segregate analysis (BSA). We successfully identified bracketing markers. SRAP1241, S01296, SSR Sat_190, and CAPs marker 4770a_AciI were found to be linked to Ms2 at a distance of about 17 cM, 11 cM, 9 cM, and 4 cM, respectively. SNP markers S02269 and S04890 were found to be linked to *Ms2* at a flanking distance of about 4 cM and 10 cM, respectively. We are currently surveying elite materials that would be introduced in the MSFCB program to establish the degree of allelic polymorphism between the male-sterile parent and the elite parent materials at the six mapped molecular markers.

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Confirmation of Fatty Acid Modifier QTL in Soybean

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Producing soybeans which have specific fatty acid content is of prime importance for edible oil and industrial purposes. Several modifier genes have been found to influence palmitic, stearic, oleic, linoleic and linolenic acid content. The purpose of this study is to confirm previously reported quantitative trait loci (QTL) for fatty acid modifier genes in an independent 'Essex' x 'Williams82' population. Fatty acid analysis by gas chromatography of the methyl esters has been completed for 147 $F_{5:6}$ recombinant inbred lines (RIL) and the two parents. The 147 RILs averaged 105.3 g Kg^{-1} palmitic acid, 45.6 g Kg^{-1} stearic acid, 227.8 g Kg^{-1} oleic acid, 528.6 g Kg^{-1} linoleic acid and 727 g Kg^{-1} linolenic acid. Parents have been screened with 42 SSR markers on linkage groups (LGs) C2, D1b, D2, F, K and L, previously linked to fatty acid modifier QTL. Polymorphisms were found between the parents for 28 of 42 SSR markers screened. RILs have been screened with the polymorphic SSR markers and single factor ANOVA analyses used to identify QTL. Genomic regions where QTL were associated with fatty acid modifiers were: palmitic acid [Satt079 (LG C2), Satt372 (LG D2), Satt149 (LG F)], Satt362 (LG F)], stearic acid [Satt274 (D1b)], oleic acid [Satt372 (LG D2), Satt725 (LG K), Satt076 (LG L), Satt166 (LG L), Satt481 (LG L),Satt527 (LG L), Satt561 (LG L)], linoleic acid [Satt274 (LG D1b), Satt076 (LG L), Satt166 (LG L), Satt527 (LG L), Satt561 (LG L)], linolenic acid [Satt252 (LG F), Satt348 (LG F), Satt725 (LG K), Satt076 (LG L), Satt481 (LG L), Satt527 (LG L), Satt561 (LG L)]. RILs have been grown in Knoxville, TN (2006) for data collection and seed increase. In 2007 the population will be grown in three locations for multi-environment data analyses for the validation of QTL.

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Preliminary Characterization of BAC library PI399073

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Bacterial artificial chromosome (BAC) libraries have proven to be useful for a variety of genomic applications including physical mapping, positional cloning, and genome sequencing in a large number of systems. Soybean PI399073 was recently identified as a source of *Rps8* as well as partial resistance to *Phytophthora sojae*. In many libraries, gaps exist, including some gene-rich regions. This library was arrayed and colonies were picked at both 18 and 42 hours after plating and designated as fast- and slow-growing libraries, respectively. The combined library consists of 152,064 clones, of which 111,744 and 40,320 are from the fast and slow libraries, respectively. When the 152,064 clones were retested, 2.6% were blue indicating that there was no insert, 11.5% of the plate wells were dried or empty, and 6.9% of the white colonies had no insert. The average insert size is 155kb and 146kb for the fast and slow growing libraries, respectively. A total of 120,162 BACs have inserts, thus this library has approximately 16.7X genome coverage. To date, 132 plate pools were made. The plate pools were screened with Satt425, and thirteen of the 96 fast plate pools and 7 of 35 slow plate pools had bands corresponding to Satt425.

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Development of a Backcross Population to Assess Allelism between *Rps3* and *Rps8*

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Rps3 and *Rps8*, resistance genes to *Phytophthora sojae*, both map to major linkage group (MLG) F. The current map positions place *Rps8* between Satt516 and Satt114 with *Rps3* placed below Satt114 near Satt510. Six backcross populations were made with PI399073 (*Rps8Rps8*) from crossing BC5F1 plants as the male parent with Kottman (*Rps1k, Rps3*) as the female parent. The BC5F1 plants were crossed without confirmation of genotype (*Rps8rps8* or *rps8rps8*). The BC6F1 plants were selfed and 10 plants from each of the six populations were selected for further analysis. The purpose of this study was to determine which of these populations would be suitable for fine mapping of both *Rps3* and *Rps8* and to determine if *Rps3* and *Rps8* are allelic or distinct genes in a resistance gene cluster on MLG F. Each family was inoculated with *P. sojae* isolates which have virulence to soybeans with *Rps8* alone, *Rps1k, Rps3a*, and the combination of *Rps1k* and *Rps3a*. There are few SSR markers which are polymorphic between the Kottman and PI399073 parents (6.7% polymorphism), but several markers in this region are segregating in one of the populations. Based on preliminary analysis, one of these populations is suitable for advancement and fine mapping of both *Rps3* and *Rps8*.

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Agriculture Biotechnology Pipeline: Evaluating Agricultural Traits Derived from Genetic Engineering in an Academic Environment Agriculture Biotechnology Pipeline

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Plant genetic engineering has received much attention as a means to improve germplasm of crop plants. Evaluating plant traits derived from biotechnology requires extensive field-testing, beyond the characterization of the target phenotype, to ensure the agronomic qualities of the experimental material have not been compromised. Field tests conducted on regulated transgenic material must be carried out in accordance with Federal guidelines governing the movement and release of regulated transgenic seed. The Federal guidelines are crafted to limit the possibility of an unexpected contamination into the environment or food chain. The University of Nebraska has recently strengthened its regulated transgenic trait field-testing capabilities by building infrastructure to ensure identity preservation, containment and chain of custody tracking of the regulated seed. These resources include a Field Coordinator who is responsible for training of personnel and oversight of all field-testing of the regulated material, isolated storage facility, separate planting and harvesting equipment and dedicated acreage. This infrastructure permits the researcher to evaluate transgenic traits from the lab bench to the field under strict identity preservation. More recently we have established a down-stream processing center for preparation of experimental feed and oil extrusion. This infrastructure, collectively referred to as the agriculture biotechnology pipeline, uniquely positions the University of Nebraska in the area of agriculture biotechnology and will serve as a strong complement to the University's teaching, research and extension missions.

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Identification of Unique Alleles Associated with Soybean Seed Protein Content in Wild Soybean

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Many studies have been recognized the potential of using *Glycine soja* as a source of genes for increased soybean seed protein content. And, the relationship between wild and cultivated soybean and the alleles associated with an increase in protein content in wild soybean were conducted. Using three simple sequence repeat (SSR) markers, 192 wild soybean (*G. soja*) and 160 cultivars populations were surveyed. These SSR markers were quantitative trait loci (QTL) detected as significant markers associated with soybean seed protein content by our previous association mapping study. From 3 SSR loci, 66 and 29 alleles were detected in wild and cultivated soybean, respectively. The gene diversity averaged 0.82 (range from 0.72 to 0.91) in wild soybean, and cultivated soybean showed an average of 0.53 (range from 0.40 to 0.72) in the gene diversity. In Satt384, 20 alleles were distributed without showing difference of allele frequencies, except one allele of 131bp in wild soybean. In contrast, two alleles of five alleles present in wild soybean showed very high frequency in cultivated soybean. Especially, the frequency of one allele in Satt182 was so high accounting for 76% of alleles in cultivated soybean. This suggested mutation leaded to domestication of soybean. Some alleles were observed at a higher frequency in wild soybean than cultivated soybean. The frequency of the 131bp allele in Satt384 was high as above 50% of alleles in wild soybean, but cultivated soybean obtained this allele as only 2.9%. Although more studies are needed to be conducted to identify the role of the alleles for soybean seed protein, it could be thought these alleles present only in wild soybean with higher frequency would be unique alleles for soybean seed protein

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Analysis of the Soybean Genome for Seed Protein QTL using SSR Markers

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Association studies based on linkage disequilibrium (LD) can be used to test for association between molecular markers and quantitative trait loci (QTLs) in various crop species. We conducted an SSR marker scan of the soybean genome for seed protein QTLs. An association map consisting of 159 markers was constructed on the basis of differences in allele frequency distributions between two subpopulations differing in protein content. The association studies identified markers tightly linked markers to QTLs for soybean seed protein content that had previously been detected by linkage mapping. Some markers that had highly significant P-values were not close to known soybean seed protein QTLs. Thirty-five SSR markers on 16 linkage groups (LGs) had P values <0.001, suggesting possible linkage to seed protein QTLs. Eleven putative QTLs were identified on the basis of highly significant markers. Two of the markers (Satt431 on LG J, and Satt551 on LG M) may be linked to unreported seed protein QTLs. Two additional population sets with different protein contents were used for confirmation of the QTLs detected in our initial analysis. Satt405 and Satt571 showed significant P-values < 0.05. As in the association study with the original population set, Satt551 and Satt431 identified QTLs associated with seed protein content. In conclusion, the results suggest that our association analysis approach, which was based on LD among SSR markers, could be a viable alternative to linkage mapping for the identification of new QTLs in soybean.

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Genetic Diversity and Sexual Reproduction in the Soybean Sudden Death Syndrome Pathogens

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The symptoms of soybean sudden death syndrome (SDS) include leaf chlorosis, leaf necrosis, root rot, defoliation and death. Four members of the *Fusarium solani* species complex are known to cause these symptoms on soybean. Three of these pathogens are found only in South America (*F. tucumaniae*, *F. brasiliense*, and an as yet unnamed species). The fourth SDS pathogen (*F. virguliforme*) is found in the U.S. and S. America. DNA sequence analysis of 13 loci indicated that *F. virguliforme* isolates are nearly identical genetically to each other while those of *F. tucumaniae* are relatively diverse. These results suggested that the *F. virguliforme* isolates represent a clonal lineage whereas those of *F. tucumania*e may reproduce sexually. Consistent with these ideas, mating experiments between 17 U.S. *F. virguliforme* isolates never produced perithecia while *F. tucumaniae* crosses frequently were abundantly fertile, making it possible to assign mating type and assess female fertility in 25 representatives of the species. Genotyping of progeny from four *F. tucumaniae* crosses confirmed that sexual recombination had occurred in these mating experiments. The red, warty perithecia and two-celled, oblong-elliptical ascospores produced by *F. tucumaniae* indicate that it produces a Nectria-like sexual stage typical of the *F. solani* species complex. In inter-species crosses, *F. virguliforme* isolates induced infertile perithecia to form in only one of the two *F. tucumania*e mating types, suggesting that all U.S. *F. virguliforme* isolates are of a single mating type. We conclude that the *F. virguliforme* population in the U.S. is asexual and likely to evolve slowly, and that the *F. tucumaniae* population in S. America is sexual and thus capable of relatively rapid evolution.

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Identification of Soybean for Resistance to Six Strains of Soybean Mosaic Virus

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Soybean mosaic virus (SMV) causes one of the most destructive viral diseases in soybean worldwide. Ninety-eight SMV isolates identified in the U.S. were classified into seven strain groups (G1 to G7). Three independent loci (*Rsv1*, *Rsv3*, and *Rsv4*) have been identified for SMV resistance. In an initial study, 209 soybean genotypes were inoculated with G1 or G7, and the results showed that 129 genotypes were resistant to one or two strains. The objective of this research was to screen the 129 resistant soybean genotypes with G2 through G6 to differentiate specific alleles for SMV resistance in these genotypes. The results indicated the existence of new alleles for SMV resistance gene. Soybean genotypes Corsica, Yuwoltae, PI 398289, PI 407975B, Clifford, and PI 398833 carry new alleles at the *Rsv1* locus based on the comparison of their differential reaction pattern against published eight *Rsv1*-alleles. Bryan and CNS carry new alleles at *Rsv3* locus. PI 61947, PI 398479, Suzuyutaka, Beeson, and Virginia likely carry *Rsv1-h*, *Rsv1-r*, *Rsv1-s* or new alleles at *Rsv4*. Research is ongoing to confirm the new SMV resistance alleles via genetic study.

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Mapping QTLs Resistant to SCN Race 1 and Race 4 in Some New Resistance Sources

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SCN Race 1 and Race 4 were identified as the two predominant races in Huang-Huai Valleys in China and ZDD2315 and ZDD2226 were identified as elite resistance sources to Race 1, 3, 4, and 5. Genetic analysis under major gene + polygene mixed inheritance model indicated that the resistance to Race 1 was controlled with two or three major genes without polygenes detected and the resistance to Race 4 was conditioned by two or three major genes plus polygenes in the four BC1F2 populations derived from Essex×ZDD2315, Peking×ZDD2315, PI88788×ZDD2226 and Peking× ZDD2226. Total 114 BC1F1 plants of the backcross (Essex×ZDD2315)×ZDD2315 was used to construct a genetic linkage map with 250 SSR markers spanning 25 linkage groups (LG), each with 2 to 20 markers, at a total distance of 2963.5 cM and average marker distance about 11.8 cM. By using Win QTL Cartographer Version 2.5, three QTLs conferring resistance to SCN Race 1 and five QTLs conferring resistance to Race 4 were mapped with CIM (Composite Interval Mapping), while only one QTL resistant to Race 1 and two QTLs resistant to Race 4 with MIM (Multiple Interval Mapping). Comparing the results from CIM and MIM, it was confirmed that rhgR1-1 locus on linkage group G was resistant to Race 1 and co-segregated with Sat_210 explaining more than 20% of the total phenotypic variance, rhgR4-2 locus on linkage group G resistant to Race 4 and co-segregated with Sat_168 explaining more than 20% of the total phenotypic variance, and rhgR4-3 locus on linkage group H resistant to Race 4 located 3.5 cM away from Sat_401 explaining more than 10% of the total phenotypic variance. Other QTLs detected with CIM need to be further confirmed.

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Molecular Characterization of the Asian Soybean Rust Disease

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Asian soybean rust (ASR), caused by the fungus *Phakopsora pachyrhizi*, is a major disease of soybean that can have disastrous consequences for soybean production under favorable conditions. Currently, no durable resistance has been identified and ASR can only be combated with diligent scouting and by timely fungicide applications. Our goal is to acquire critical information about the ASR infection process of soybean in susceptible and resistant interactions to assist in the development of molecular tools for controlling the disease. The rationale is that ASR infection causes changes in gene expression and that the identification of such soybean genes will provide insight into the molecular mechanisms of the disease. Our approach was to detect gene expression over a 7-day time course in both ASR- and mock-infected leaves from a susceptible and resistant cultivar by using Affymetrix GeneChip arrays. Statistical analysis showed that 1516 genes in the susceptible cultivar and 894 genes in the resistant cultivar tested significantly different in either the infection treatment main effect or the infection by time interaction effect at a false discovery rate of 5%. Most genes were differentially expressed within the first 36 hours after infection, followed by the time period up to 3 days after inoculation (dai), in which very few genes were differentially expressed. Between 3 and 7 dai, host gene expression diverged again between mock- and ASR-infected leaves. *Arabidopsis* homologs of differentially expressed genes were identified for 1302 and 773 genes in the susceptible and resistant cultivar, respectively. This allowed us to perform functional classification and revealed that disease/defense, signal transduction, transcription, protein storage, primary and secondary metabolism are among the classes to which many differentially genes belong. Even though many genes are differentially regulated in both the susceptible and resistant cultivar, a large proportion of the genes is unique to either cultivar.

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Poster 64

Quantitative Trait Loci Analysis and Fine Mapping of Resistance to Soybean Cyst Nematode in PI438489B and PI567516C

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Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is the most destructive pathogen of soybean [*Glycine max* (L.) Merr.] in the United States. Genetic resistance is the most effective measure to control this pathogen. However, soybean breeding, which has been largely dependent upon one major source of SCN resistance genes, may result in genetic vulnerability to SCN. Thus, evaluations of exotic soybean germlasm have been conducted to identify new plant introductions (PI) with great level of resistance to multiple SCN races. Among hundreds of PIs evaluated, PI438489B and PI567516C were reported to be resistant to all five SCN races widely distributed in soybean production areas in the US. The objectives of the present study were to identify novel quantitative trait loci (QTL) associated with SCN resistance in these two soybean PIs, to conduct fine mapping of these QTL for cloning candidate genes, and to develop gene-based molecular markers for marker-assisted selection. Two hundred fifty F2:3 families derived from the crosses of the two resistant PIs and a susceptible cultivar, Magellan, were assayed for resistance to five different SCN race isolates (1, 2, 3, 5, and 14) in a greenhouse. Female index (FI%) was utilized to measure SCN reproduction on individual plants of each F2:3 family and the differential lines. Around 400 simple sequence repeat (SSR) markers were surveyed for genetic polymorphisms between the parents, Magellan and PI438489B. Of these, 182 markers (~45%) were polymorphic and were used to genotype the populations. Greenhouse bioassays were completed. Phenotypic data collection and molecular data generation are in progress.

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Poster 65

Potential of Detached Soybean Leaves for Evaluation of Rust Resistance

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Soybean rust is one of the most important foliar diseases of soybean caused by the fungus *Phakopsora pachyrhizi*, and it is a threat to Illinois soybean crops and to all other U.S. soybean growing areas. A detached leaf assay experiment was developed to optimize the detached leaf assay to screen soybean lines for resistance to rust. Field based screening is not allowed in Illinois. The detached leaf assay can serve as an alternative to field evaluation for rust resistance. Treatments consisted of all different combinations of water agar media containing different sources of agar and different concentrations of plant hormones (gibberellic acid, 6-benzylaminopurine and benizimidazole). Unifoliate leaves from 14 d old seedlings were removed and immersed in sterile water, then placed on agar media with 5 mm long petiole surfaced on agar. The detached leaves were inoculated with spore suspension of single uredium isolates collected from various locations in US. Following inoculation, detached leaves were incubated in growth chamber for 14-21 days to detect partial or RB disease reaction. Disease reaction was determined in a range of soybean cultivars. The most appropriate agar media (1.5% water agar and 10mg/l benzylaminopurine) was identified which retarded senescence, retained green color longer than 30days by stimulating roots. Another detached leaf assay experiment was used for testing various reactions of isolates on different soybean genotypes to rust inoculation are being in progress. Result of detached leaf evaluation could be used in selecting the resistant parents based on the disease reaction to develop mapping population for QTL study.

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Poster 66

Resistance Evaluations of Germplasm Accessions to Georgia Races of *Phakopsora pachyrhizi* (Asian Soybean Rust)

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Asian soybean rust (ASR), caused by the biotrophic fungus *Phakopsora pachyrhizi*, is one of the most destructive foliar diseases of soybean. Since no North American cultivars are resistant to ASR, identification of germplasm accessions with resistance is a crucial first step in the development of resistant cultivars. In late 2005, 778 *Glycine max* accessions which had exhibited putative resistance to ASR in greenhouse assays conducted at the Fort Detrick (MD) biocontainment facility were evaluated in the field at Attapulgus, GA for resistance to local races of *P. pachyrhizi*. Artificial lighting was used to synchronize flowering among the accessions, which ranged from Maturity Groups 000 to X. A 5-point scale was used to rate the accessions for resistance on the basis of canopy disease incidence, which ranged from no lesions found (score of 1) to abundant lesions in the upper canopy (score of 5). In early 2006, 328 of the accessions which appeared to possess resistance in the Attapulgus field test were assayed in a greenhouse at Griffin, GA for resistance to pooled isolates of *P. pachyrhizi* from several Georgia locations. Although at least 40 accessions had appeared to have a useful level of resistance in the field study, only a subset of these also showed a high level of resistance in the greenhouse assays. Further field and greenhouse evaluations are needed, but data from these experiments should be useful to breeders who wish to begin crossing to accessions likely to have ASR resistance genes that will provide some level of protection against ASR in North America.

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Poster 67

Affymetrix Chip Hybridization Identifies Many Transcripts Up-Regulated By Soybean Cyst Nematode Infection

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Soybean cyst nematode is currently the most devastating pathogen of soybean. SCN penetrates the root and migrates toward the central vascular bundle where it establishes a complex multinucleated feeding structure that withdraws nutrients from the plant to support development and growth of the nematode. To identify genes that may play significant roles in formation and maintenance of the feeding structure, RNA from SCN inoculated and non-inoculated root pieces were hybridized to the Affymetrix soybean genome chips. RNA for probe was collected from 8 days post inoculation (dpi) root pieces (1 to 3 mm) that displayed multiple swollen female SCN. Branch roots and root tips were trimmed from the root pieces to minimize the amount of RNA contributed by these organs. We also collected RNA from similarly trimmed root pieces from non-inoculated roots. Of the 35,611 transcripts represented on the chip, approximately 29,000 of these are indicated as being present in the SCN colonized root pieces. 234 transcripts are up-regulated by 8-fold or greater (>3.0 log base 2) in SCN colonized root pieces compared to non-inoculated root pieces. Of the transcripts to which a function can be assigned, a large proportion of these were associated with cell wall structure, which might be expected since the formation of the feeding structure requires significant changes to the cell walls of host cells. Other functional categories that included a large number of up-regulated transcripts were defense and metabolism, and a smaller group of transcripts were identified that are associated with signal transduction and transcription.

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Poster 68

Simple Sequence Repeats for the Soybean Pathogen, *Phytophthora sojae*

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*Phytophthora soja*e is the most important pathogen of soybeans in regions with soils that remain saturated for long periods of time. *P. sojae* is a diploid oomycete which is homothallic and is limited to primarily to one host - soybean. Oospores are readily produced in susceptible soybean roots and can survive in crop residues and in soil for many years. There are a limited number of population genetic studies with *P. sojae* and none from a narrow geographic region. Recently, 72 different pathotypes were identified in a collection of *P. sojae* isolates from Ohio with as many as 54 pathotypes identified in one field. Microsatellite markers (SSRs) which are co-dominant markers and easily transferred to multiple laboratories have been utilized to investigate the genetic structure of populations as well as mapping studies. The overall objective of this study is to determine if these large numbers of pathotypes identified in Ohio are due to mutation, random genetic drift, gene and genotype flow, or through widespread outcrossing. The *P. sojae* genome sequence is now available and an assessment of the simple sequence repeats (SSRs) has been completed. SSRs were identified in the genome, and repeats > 15 in size were selected. Primers, 18 to 20 bp in length, were designed flanking SSRs and evaluated on *P. sojae* races 1, 2, 3, 4, 7, and 25. Of the 95 SSR primer pairs tested to date, 19 are polymorphic on these isolates. However, only Race 1 and 7 could readily be distinguished from the remaining five isolates for 1 and 18 SSRs, respectively. Further analysis of isolates from one field will also be presented to examine the diversity that exists within a field and compare this to the diversity among a set of standard isolates.

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Poster 69

A Proteinaceous Phytotoxin Causes Sudden Death Syndrome in Soybean

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Sudden Death Syndrome (SDS), caused by *Fusarium solani* f. sp. *glycines* (*Fsg*) is one of the most important fungal diseases of soybean in the United States. The fungus attacks only the roots and it has not been identified from the above ground parts of the diseased plants. Therefore, it has been hypothesized that the foliar symptoms of the disease are caused by the translocation of a phytotoxin(s) released by the fungus into the roots. Cell-free *Fsg* culture filtrates cause foliar SDS symptoms, known as leaf scorch, in cut soybean seedlings. Treatment of cell-free *Fsg* culture filtrates with an endolytic protease, Proteinase K, abolished the activity of culture filtrates to cause leaf scorch, suggesting that the phytotoxin(s) is proteinaceous. To identify the specific protein that causes leaf scorch, cell-free culture filtrates were fractionated thorough column chromatography and then run in native-polyacrylamide gels. Individual protein bands were eluted from the gels and tested for SDS activity in cut soybean seedlings. A low molecular weight protein band (less than 10 kDa) was found to produce SDS symptoms in the cut seedlings. Characterization of this putative proteinaceous toxin is in progress.

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Poster 70

Identification of Proteins That Interact With Phytophthora Resistance Soybean Protein *Rps1-k-2*

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In the United States, the annual soybean yield loss suffered from the root and stem rot disease caused by *Phytophthora sojae* is valued over quarter of a billion dollars. Very little is known about the molecular basis of Phytophthora resistance in soybean. In order to identify signal transduction factors involved in the expression of Phytophthora resistance, a yeast two-hybrid system has been applied and isolated 12 candidate signalling factors that interact with the Phytophthora resistance protein *Rps1-k-2 in vivo* in yeast and *in vitro*. Candidate *Rps1-k-2*-interactors include 26S proteasome AAA-ATPase subunit RPT5a and a type II metacaspase. RNA interference experiments have been conducted in soybean cotyledons transformed by *Agrobacteriaum rhozogenes*. Silencing of some of the putative *Rps1-k-2* interactors including the type II metacaspase abolished the resistance against *P. sojae*.

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Poster 71

Putative *Arabidopsis* Mutants Showing Loss of Non-host Resistance against the Soybean Pathogen, *Phytophthora sojae*

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Plants are immune to most phytopathogens because of the species-specific nonhost resistance. The mechanism of nonhost resistance is largely unknown. Recently, the *Arabidopsis pen1-1* mutant carrying a mutation in a syntaxin protein showed enhanced penetration by the barley powdery mildew pathogen, *Blumeria graminis* f. sp. *hordei.* It was noted that the same mutation also allowed the soybean pathogen *Phytophthora sojae* to penetrate and cause hypersensitive death in single *Arabidopsis* cells. *Arabidopsis* is otherwise immune to *P. soaje*. Following mutagenesis of the *Arabidopsis pen-1* mutant with the chemical mutagen, ethyl methane sulfonate, approximately 3,000 individual M_2 families were generated. Around 60 plants of each M_2 family were then screened for increased cell death following *P. sojae* infection. Twenty-nine families were found to contain certain number of plants having cell death in two or more continuous cells. These 29 families have been advanced to the M_3 generation in order to identify the homozygous mutants. Currently, progeny testing of these putative mutants is in progress.

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Poster 72

Alterations in Phenylpropanoids in Soybean Leaves Caused by *Pseudomonas syringae*

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We studied changes in phenolic metabolism of soybean leaves caused by *Pseudomonas syringae* pv. *glycinea* and compared a compatible (C) pathogen that caused disease to an incompatible interaction (I) that led to hypersensitive response and cell death. Soybean leaves were infiltrated with *P. syringae* pv. *glycinea* (with or without *avrB*), or with 10 mM MgCl2 as a control. Soluble and cell wall bound phenolics were analyzed using HPLC-UV/DAD and HPLC/MS-MS 8, 24 and 48h after inoculation. There were marked differences in the speed and magnitude of soybean leaf responses to the pathogens compared, even though phenolic metabolism was activated in both cases. The I interaction was accompanied by an increase in lignin level with the highest increase (compared to the control) found 48h after inoculation. There were no notable differences in lignin concentrations between control and C samples. Great differences were found in concentrations of isoflavonoids and pterocarpanoids between I and C responses. Results of the phenylpropanoid profiling nicely correlated with gene expression data generated in microarrays.

This study was supported in part by funds from the Illinois Soybean Program Operating Board, the United Soybean Board, the North Central Soybean Research Program, the Soybean Disease Biotechnology Center, the Illinois Agricultural Experiment Station, and the USDA Agricultural Research Service.

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Poster 73

Glyceollin Accumulation and FSG Resistance of Soybean Roots

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Sudden death syndrome (SDS) is one of the most destructive soybean diseases in Illinois. There are cultivars available that show resistance as measured by the visible foliar symptoms, but root infection still occurs that results in yield loss. If root resistance was found and was utilized in breeding programs then the damage caused by SDS would be markedly reduced. It is also important to determine the cause of root resistance and to develop fast and reliable assays for resistance. The major objective of this study is to determine if we can identify root resistance to SDS and determine compounds that may be important for defence responses to *Fusarium solani* f. sp. *glycines* (FSG) that causes SDS. The specific objectives are to use selected commercial cvs. and Plant Introductions with known susceptibilities to SDS and a) induce hairy root cultures, b) develop hairy root assays for root colonization, c) correlate FSG growth on hairy roots with contents of glyceollin, the soybean phytoalexin that has been associated with pathogen resistance and d) to perform global metabolic profiling to determine soluble compounds that are associated with root resistance. The hairy root lines have been initiated from 18 genotypes following infection with *Agrobacterium rhizogenes* and a hairy root growth assay was developed to evaluate root SDS resistance of the different genotypes with known field and greenhouse rankings based on foliar symptoms. We carried out experiments with FSG treatment of selected hairy root genotypes followed by fungal growth tests and estimation of glyceollin production. Tests with selected lines showed different FSG growth that generally was inversely correlated with the root glyceollin concentration. The cv. A5560 was considered the most SDS resistant based on foliar symptoms of the 24 cvs. being studied, however, the glyceollin level detected in A5560 hairy roots did not differ notably from that of susceptible Spencer. We found that the A5560 hairy roots contained an unknown phenolic compound not seen in extracts of the other lines. LC-MS and GC-MS data generated for selected lines will be presented.

This study was supported in part by funds from the Illinois Soybean Program Operating Board, the Illinois Soybean Disease Biotechnology Center, the Illinois Agricultural Experiment Station, and the USDA Agricultural Research Service.

Session:Biotic Stress

Poster 74

Effect of Temperature on *omega*-6 Desaturase Gene Expression and Linoleic/Oleic Acid in Soybean seed Seeds

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Microsomal *omega*-6 desaturases (FAD2s) catalyze the first extra-plastidial desaturation, converting oleic acid (18:1) to linoleic acid (18:2). Environmental temperature modulates the 18:2/18:1 ratio of membrane and storage lipids through effects on desaturase enzyme activity and possibly gene expression. We measured changes in steady state transcript levels of the seed-specific FAD2-1A and FAD2-1B genes in developing soybean seeds from plants grown at 22/18°C (cold), 26/22°C (normal), and 30/26°C (warm) temperatures, and analyzed associations between temperature, FAD2-1 gene expression, and linoleic/oleic acid levels. FAD2-1A and FAD2-1B gene expression was comparable at the normal temperature, but growth at a temperature to either side of the norm resulted in increased expression of FAD2-1B over FAD2-1A, slight at the warm temperature, but more pronounced at the cold temperature. The response to temperature across varieties was also more pronounced for FAD2-1B compared to FAD2-1A. The increase in FAD2-1B transcript accumulation with decreasing temperature was associated with increasing 18:2 content in two of three soybean varieties examined.

Session:Abiotic Stress

Poster 75

Does High Temperature Reduce Seed Mass and Vigor in Soybean by Repressing Gene Expression for Sucrose Binding Protein?

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Sucrose is the major carbon source that is transported from soybean photosynthetic tissues into developing seed for synthesis of storage reserves, such as protein and oil. Accumulating storage reserves in developing seed determines seed mass and vigor. Sucrose binding protein (SBP) is a vehicle for sucrose transport across plasma membranes. Soybean plants grown in our laboratory under high temperature (37/30°C, day/night) produced mature seed of smaller mass and reduced vigor, relative to mature seeds from soybean plants grown under control temperature conditions (27/18ºC). Proteomic analysis of the mature soybean seed revealed that seed from the high-temperature growth condition to exhibit decreased SBP levels. We hypothesize that high temperature represses SBP gene expression and restricts sucrose transport into developing seeds, resulting in mature seed with less mass and reduced seed vigor. To test our hypothesis, we are conducting real time RT-PCR to compare SBP gene expressions in seeds developed on soybean plants grown under control or high temperature conditions. Comparisons include examinations of seed in developing stages (R5-early R8) and mature seed (R8). Varied sucrose concentrations of water-based solutions will test whether seeds impaired by high temperature can have their vigor restored when imbibed in an exogenous sucrose source.

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Poster 76

Role of Inositol Phosphate Kinases in Phytic Acid Biosynthesis

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Phytate or *myo*-inositol hexakisphosphate is the major storage form of phosphorus present in plant seeds. Bound phytate phosphorus and chelated mineral cations are largely unavailable in the diets of non-ruminants due to a lack of digestive enzymes to remove phosphate groups. Phytate phosphorus is excreted in manure, accumulates in soil, and eventually enters watersheds. As a result, phytate is considered both an antinutrient and an environmental pollutant. Altering seed composition to reduce phytate, improve nutrient availability, and reduce phosphorus pollution will require an understanding of the phytate biosynthetic pathway. Demonstration of one such pathway in the model system *Arabidopsis* (1) has provided a foundation for comparative studies in economically important crops. In *Arabidopsis* the pathway involves the conversion of IP_3 to IP_6 via a *myo*-inositol (1,4,5) P_3 6/3-kinase and *myo*-inositol (1,3,4,5,6) P_5 2-kinase. However, the demonstration that a different enzyme, *myo*-inositol (1,3,4) P_3 5/6-kinase, as the site of a low phytate mutation in maize (2), illustrates that the biosynthesis of phytate must be more complex. We have isolated several *myo*-inositol phosphate kinase genes in soybean as possible candidates for steps in the biosynthetic pathway. We have characterized the genes for four *myo*-inositol (1,3,4) P_3 5/6-kinases, one *myo*-inositol (1,4,5) P_3 6/3-kinase, and one *myo*-inositol (1,3,4,5,6) P_5 2-kinase. We have examined expression in developing seeds and other tissues by Northern blot analysis and quantitative RT-PCR. We have expressed all six genes as GST fusion proteins in *E. coli*, and verified enzyme activity on the proposed substrates. We are conducting biochemical characterization to determine enzyme kinetics and substrate specificities. We are utilizing soybean embryogenic cultures to test for alterations in inositol phosphate profiles as a result of down-regulating the kinase genes by RNA interference.

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2. Shi, J., et al. (2003). Plant Physiol. 131: 1-9.

Session:Metabolic Engineering

Poster 77

Seed-specific Expression of the *Perilla frutescens* γ-tocopherol Methyltransferase Gene in Soybean Results in Increased α-tocopherol Content

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Tocopherols, with antioxidant properties, are synthesized by photosynthetic organisms and play important roles in human and animal nutrition. In soybean, γ-tocopherol, the biosynthetic precursor to α-tocopherol, is the predominant form found in the seed, whereas α-tocopherol is the most bioactive component. This suggests that the final step of the α-tocopherol biosynthetic pathway catalyzed by γ-tocopherol methyltransferase (γ-TMT) is limiting in soybean seed. Soybean oil is the major edible vegetable oil consumed, so manipulating the tocopherol biosynthetic pathway in soybean seed to convert tocopherols into the more active α-tocopherol form could have significant health benefits. In order to increase the soybean seed α-tocopherol content, the γ-TMT gene isolated from *Perilla frutescens* was overexpressed in soybean using a seed-specific promoter, vicilin. One transgenic plant was recovered and the progeny analyzed for two generations. The data obtained on the tocopherol content demonstrated that the seed specific expression of the *P. frutescens γ-TMT* gene resulted in a dramatic change in the tocopherol composition of the transgenic soybean seed compared to wild-type seed. On average a 10.4-fold increase in the α-tocopherol content and a 14.9-fold increase in the β-tocopherol content in the T2 seed. Given the relative contributions of the different tocopherols to vitamin E activity, the activity in T2 seed was calculated to be 4.8-fold higher than in wild-type seed. In addition, the data obtained on lipid peroxidation indicate that α-tocopherol may have a role in preventing oxidative damage to the lipid components during seed storage and seed germination. The increase in the α-tocopherol content in the soybean seed could have the potential to significantly increase the dietary intake of vitamin E.

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Poster 78

Genetic Engineering of Soybean Isoflavones

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To alter isoflavonoids in soybean plants we carried out a bombardment of embryogenic cultures (cv. Jack) with a mixture of cassettes, harboring important genes of the phenylpropanoid pathway, such as PAL5 (phenylalanine ammonia-lyase), CHS6 (chalcone synthase), IFS (isoflavone synthase) and CHR12 (chalcone reductase) driven either by lectin (seed-specific) or CsVMV (constitutive) promoters, and a cassette with the selectable marker HPT gene under the 35S promoter. We have obtained 16 lines transformed with different combinations of genes under the lectin promoter and 11 lines with genes under the CsVMV promoter in these experiments. Several lines have shown the segregation of genes in the T1 generation, including the selectable marker gene, and the progeny of these plants kept new combinations of genes in the next generations. We demonstrated that several foreign genes delivered into embryogenic cultures by particle bombardment can be simultaneously expressed in different parts of the plants. One homozygous line transformed with PAL5, CHS6 and IFS genes under the lectin promoter and selected with the HPT gene, showed the expression of target genes in immature seeds and a marked reduction of isoflavones in mature seeds compared to untransformed plants. We found that the cotyledons of untransformed seeds contain about 70-80% of the total seed isoflavones. Interestingly, embryos account for about 3% of the whole seed weight, and contain 20-30% of the total seed isoflavones. The main isoflavones of cotyledons are genistein and daidzein and only trace amounts of glycitein were detected in cotyledons, however, embryos contain a high glycitein proportion. The expression of phenolic genes resulted in decreased total isoflavone content in cotyledons only, while there were no changes in total isoflavones content in embryos of seeds collected from transgenic plants compared to the control. This may indicate that the lectin promoter drives genes in cotyledons and not in embryos. We also found alterations in leaf isoflavones of 3 lines expressing phenolic genes (CHS6, IFS, CHR12) under the CsVMV promoter: one line showed the reduction of isoflavones and two others showed increased levels of isoflavones in leaves compared to the control.

This study was carried out with funds from the United Soybean Board, the North Central Soybean Research Program, Illinois Soybean Program Operating Board, and Illinois Soybean Disease Biotechnology Center.

Session:Metabolic Engineering

Poster 79

Does Leaf Iron Reductase Activity Contribute to Seed Iron Accumulation in Soybean?

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Iron is one of the most important micronutrients in human and plant nutrition, with plant foods providing a considerable amount of dietary iron in certain population groups. However, due to the poor bioavailability of iron in most plant foods, strategies are needed to generate crops with higher levels of iron using biotechnological or conventional breeding approaches. To assist this effort, we would like to have a better understanding of how iron is handled in leaves, prior to its delivery to developing seeds. Iron is transported to soybean leaves as ferric citrate, but we don't know if a reduction step is required before the iron is phloem loaded. Thus, we are studying a transgenic line in which the ectopic expression of AtFRO2 potentially confers iron reductase activity throughout the plant. Cultivar Thorne was transformed with a construct containing the *Arabidopsis* FRO2 gene. Quantitative RT-PCR revealed that the CaMV 35S promoter drove AtFRO2 expression in different organs of the transgenic plants, including seeds. Protoplasts isolated from leaf cells of 35S-FRO2 and wild-type plants showed that AtFRO2 expression increased leaf iron reduction capacity up to 3-fold. Iron analysis of different shoot tissues revealed increased iron concentrations in the transgenic plants, including up to a 100% increase in leaves, a 60% increase in pod walls, and a 10% increase in seeds. The 35S-FRO2 plants also had a higher expression of the ferritin gene, suggesting that at least some of the excess iron was stored in the ferritin form. We will use these and other results to discuss possible mechanisms of iron mobilization within leaves, and the potential of using enhanced leaf iron reduction to increase iron concentration in soybean seeds.

Session:Nutritional Genomics

Poster 80

Isogeneic Soybeans with Genetically Altered Phytoestrogen Protein and Oil Content

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Soybean seed derived protein products contain compounds that have been shown to have physiological effects on humans and animals that ingest these products. Soy phytoestrogens in combination with soy protein and other matrix components may have beneficial effects in humans. Jointly they reduce the risk of certain cancers and cardiovascular disease, as well as ameliorating symptoms of menopause, including osteoporosis. In contrast, hormone replacement therapy (HRT) with non-plant derived estrogens can have negative effects on cardiovascular health. In non-human animals it is evident that phytoestrogens also affect reproduction, immune function, and growth. We identified eight loci (QTL) controlling isoflavone content, six controlling protein content and four underlying oil content. Here we report the development of isogeneic germplasm with elevated and reduced isoflavones content in an otherwise isogeneic protein and oil matrix. In addition we have sought to isolate genes underlying isoflavone, protein and oil content. An ortholog of KASI is found underlying a protein and oil QTL. The research has lead to the identification of nearly isogeneic soybeans, in which phytoestrogen amounts and profiles can be managed for specific needs within the research community, and later the human and animal health industries. This research may expand the usefulness of soy products in the health functional food industries.

Session:Industrial Uses for Soybean

Poster 81

QTL for Japanese Beetle (*Popillia japonica*, Newman) Resistance in Soybean [*Glycine max* (L.) Merr.], Isoflavones, Protein, and Oil Identified through Positional Cloning and Map-based Programs

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Quantitative trait loci (QTL) discovery through positional cloning using microsatellite markers helps in the construction, definition and saturation of the soybean genome map. The usage of several hundred new markers has considerably helped in reducing gaps in both the physical-genetic maps. Among the favorable elite cultivars, Essex by Forrest (ExF) is a favorite choice in terms of contrasting QTL for Isoflavones, yield, and resistance to disease and manganese toxicity etc. Near Infra-Red Analyzer (NIR) analysis yielded ample data for the four traits, mean protein, mean oil, mean water and mean seed weight content of 100 ExF recombinant inbred lines (RILs). Among the one hundred RILS different degrees of resistance to Japanese beetles (JB) was observed. The data from the NIR analysis helped select RILs into three categories as high, mid and low for protein, oil, and water respectively. The one hundred RILs were scored for pest severity (PS), pest incidence (PI), pest number (PN), and pest index (PX). The NIR data and field data was included in the marker data for ExF and finally compared against the ExF composite map data. This analysis provided valuable QTL information in the form of composite data from Mapmaker EXP and Mapmaker QTL programs and, single point analysis using Statistical Analysis Systems (SAS) that showed very strong marker trait associations for JB resistance, Protein, oil and, water . The presence of 7 new QTL was confirmed through SAS single point analysis that showed very high probability values for the 7 biochemical traits tested in 2001 and 2003. Three QTL were identified for PX with high LOD scores (4 to 5). PX also correlated to the protein trait for the year 2001. Strong correlation was also observed between PI percentage and protein trait for the year 2001. PS was characterized by four QTL with very high LOD scores (10 to 11). Potential QTLs for JB resistance and biochemical traits were based on high LOD scores (>2.5) and probability scores (≤0.005).

Visit us at http://soybeangenome.siu.edu for more information on the Physical-Genetic map.

Session:Genomics/Functional Genomics

Poster 82

Three Minimum Tile Paths from Bacterial Artificial Chromosome Libraries of the Soybean (*Glycine max* cv. 'Forrest'): Tools for Structural and Functional Genomics

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The creation of minimally redundant tile paths (MTP) from contiguous sets of overlapping clones (contigs) in physical maps is a critical step for structural and functional genomics. Build 4 of the physical map of soybean (*Glycine max* L. Merr. cv. 'Forrest') showed the 1-Gbp genome was composed of 0.7 Gbp diploid, 0.1 Gbp tetraploid and 0.2 Gbp octoploid regions. MTP sub-libraries from the soybean cv. Forrest physical map builds 2 to 4 were developed to represent the unique genome (about 0.8 Gbp). MTP2 was 14,208 clones (of mean insert size 140 kbp) picked from the 5,597 contigs of build 2. MTP2 was constructed from three BAC libraries (BamHI (B), HindIII (H) and EcoRI (E) inserts). MTP2 encompassed the contigs of build 3 that derived from build 2 by a series of contig merges. MTP2 encompassed 2 Gbp compared to the soybean haploid genome of 1 Gbp and does not distinguish regions by ploidy. The second and third MTPs, called MTP4BH and MTP4E, were each based on build 4. Each was selected from 2,854 contigs. MTP4BH was 4,608 B and H insert clones of mean size 173 kbp in the large (27.6 kbp) T-DNA vector pCLD04541 suitable for plant transformation and functional genomics. MTP4E was 4,608 BAC clones with large inserts (mean 175 kbp) in the small (7.5 kbp) pECBAC1 vector suitable for DNA sequencing. MTP4BH and MTP4E clones each encompassed about 0.8 Gbp, the 0.7 Gbp diploid regions and 0.05 Gbp each from the tetraploid and octoploid regions. MTP2 and MTP4BH were used for BAC-end sequencing, EST integration, micro-satellite integration into the physical map and high information content fingerprinting. MTP4E will be used for genome sequence by pooled genomic clone index. Each MTP and BES will be useful to deconvolute and finish the whole genome shotgun sequence of soybean.

Session:Genomics/Functional Genomics

Poster 83

An Updated 'Essex' by 'Forrest' Linkage Map and First Composite Interval Map of QTL Underlying Six Soybean Traits

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DNA marker maps based on single populations are the basis for gene, loci and genomic analyses. Individual maps can be integrated to produce composite maps with higher marker densities if shared marker orders are consistent. However, estimates of marker order in composite maps must include sets of markers that were not polymorphic in multiple populations. Often some of the pooled markers were not codominant, or were not correctly scored. The soybean composite map was composed of data from six separate populations based on northern US germplasm but does not yet include 'Essex' by 'Forrest' recombinant inbred line (RIL) population (ExF) or any southern US soybean cultivars. Here we update the ExF map with co-dominant markers, and compare marker orders among this map, the Forrest physical map and the composite soybean map and compare QTL identified by composite interval maps to the earlier interval maps. Three hundred and thirty six codominant markers were used to construct the ExF map. The majority of marker orders were consistent between the maps. However, thirty-eight putative marker inversions were detected on 14 of 20 linkage groups (LG). The number of inverted markers ranged from 1 to 6 per affected LG Thus, comparison of the ExF population map with the composite map suggests marker order inversions are common in soybean maps. A total of 51 QTL among 35 measures of six traits were detected. Thirty QTL were new. Twenty-one of 29 QTL previously detected by interval maps were detected using composite intervals. The genomic locations of the QTL were more closely delimited. A genome sequencing project to compare Southern and Northern US soybean cultivars would catalog and delimit inverted regions and the associated QTL. Gene introgression in cultivar development programs would be accelerated.

Session:Molecular Breeding

Poster 84

The Development of BAC-end, Sequence-based, Microsatellite Markers, and their Placement in the Physical and Genetic Maps of Soybean

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The composite map of soybean shared among Soybase, LIS and SoyGD (March, 2006) contained 3,073 DNA markers in the "Locus" class. Among the markers were 1,019 class I microsatellite markers with 2-3 bp simple sequence repeats (SSRs) of >10 iterations (BARC-SSR markers). However, there were few class II SSRs (2-5 bp repeats with < 10 iterations; mostly SIUC-Satt markers). The number of class I and II SSR markers was increased, and bacterial artificial chromosome (BAC) clones were integrated onto the soybean physical map using the markers. We used was 10 Mb of BAC-end sequence derived from 13,474 reads from 7,050 clones constituting minimum tile path 2 of the soybean physical map (SoyGD; http://soybeangenome.siu.edu). Identified were 1,053 1-6 bp motif, repeat sequences, 333 from class I and 720 from class II. Potential markers were shown on the MTP_SSR track at Gbrowse. Primers were designed as 20-24mers that had Tm of 55+1 C that would generate 100-500 bp amplicons. About 853 useful primer pairs were established. Motifs were not randomly distributed with biases toward AT rich motifs. Strong biases against the GC motif and all tetranucleotide repeats were found. The markers discovered were useful, about 60% of class II markers and 75% of class I markers were polymorphic among on the parents of four recombinant inbred line (RIL) populations. Many of the BES-SSRs were located on the soybean genetic map in regions with few BARC-SSR markers. Therefore, BES-SSRs represent useful tools for genetic map development in soybean. New members of a consortium to map the markers are invited.

Session:Genomics/Functional Genomics

Poster 85

The Soybean Genome Database (SoyGD): A Tool for Integrated Legume Biology.

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Genomes like *Glycine max* (soybean) that have been highly conserved following increases in ploidy present challenges for genome analysis. At http://soybeangenome.siu.edu the Soybean Genome Database (SoyGD) genome browser has, since 2002, integrated and served the publicly available soybean physical map, BAC fingerprint database and genetic map associated genomic data (1). Duplicated regions have been identified and catalogued with a-d suffix to marker anchor names and contig names that communicate ploidy (ctg>8000 are tetraploid, ctg>9000 are octoploid). DNA sequence data has been used to separate DNA marker anchors from homologs of DNA marker anchors in BAC pools. In recent additions, about 3,840 minimum tiling path (MTP4) BIBAC clones provided BAC end sequences (BES) to decorate the physical map in addition to the 13,747 BES from MTP2. MTP2 and MTP4 clones were added to the database as separate tracks. Predicted gene models were developed for about 15% of the BES. From these models candidate genes underlying disease resistance, seed yield and seed protein, oil or isoflavone content were detected and fine-mapped. In genome evolution analyses more than a thousand additional microsatellite marker anchors were developed for contigs, 353 on the map and about 700 still in Queue (awaiting placement). Linkage analysis placed one hundred of the 1,053 new microsatellite markers on the genetic map with contigs and associated features. About half of the markers mapped to regions of the genome that formed gaps in earlier maps suggesting marker clustering biases. SoyGD represents the new build 5 for the physical map with 800 contigs from the 76,749 fingerprinted clones publicly available. New QTL data has been incorporated from the newly released ‘Essex’ by ‘Forrest’ and ‘Flyer’ by ‘Hartwig’ RIL populations. Gene expression data has been added. NSF project #9878635 and USB 2218-6218.
(1) Shultz et al., 2006. Nucleic Acids Res. D758-D765.

Session:Genomics:Functional Genomics

Poster 86

High Throughput Metabolic Profiles by FT-ICR-MS using Near-Isolines that Contrast for Resistance to SCN and SDS.

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Cataloging the organic components of plants and relating them to genotype is a mammoth task for the post-genomic era. Metabolite profiling by Fourier transformed ion cyclotron mass spectrometry (FT-ICR-MS) may be a useful high-through-put tool. We sought to test the validity of FT-ICR-MS derived metabolite profiles (1) with *Glycine max* (soybean). We compared isogeneic lines with altered disease resistance (soybean). Full scan FT-ICR-MS analysis of all metabolites between 200-961 d, showed there were 55 metabolites in root extracts and 122 metabolites in leaf extracts that were observed to have changed among 1,054 metabolites detected. We demonstrate that FT-ICR-MS is a rapid and economical method for the comprehensive analysis of the changes caused by alleles in cell composition and by inference metabolism. Chemicals associated with disease resistance in soybean were identified and 60% were not previously identified in any plant. We have therefore begun analysis by quadropole MS to verify the broad utility of the technique to plant systems and to develop a new database of plant related chemical compounds. (1) Mungur R., Glass AD, Goodenow DB, Lightfoot, D.A. 2005.Metabolic Fingerprinting by FT-ICR-MS in transgenic plants: *Nicotiana tabacum* altered by the *Escherichia coli* glutamate dehydrogenase gene. J. Biomed. and Biotech. 2005: 32-40

Session:Metabolic Engineering

Poster 87

Genetic Components of Water Stress Tolerance in Soybean

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At SIUC there are two groups (Lightfoot, Wood) working on the genetics and physiology of water deficit tolerance using biochemical markers and root growth characteristics. Aims to map QTL for yield in double cropped beans in relation to phenotypic traits related to root growth and vigor. Yield on July planted beans, Yield and trigonelline content of dry and irrigated beans, growth in 200 ml pots in greenhouse and root hair density have been measured in ExF96. QTL controlling full season yield, plant height resistance to manganese toxicity or trigonelline content were found. QTL for root architecture root hair density, aluminum toxicity and resistance to salt toxicity are being mapped. To date we have found 8 QTL in ExF for yield in 8 linkage groups (at SATT385 (A1), SATT133 (A2), SATT251 (B1), SATT294 (C1), SATT408 (D1a), SATT369 (E), SATT334 (F), SATT440 (I)). For root architecture QTL are found for Basal Root LGs A2, B2, and C2. Satt509, Sat_083 Satt316. One QTL for Root Dry Weight (and Shoot DW) was identified on LG F Satt554-CAA19 One QTL underlying RDW/SDW (a composite trait) was identified by the marker Satt214 on LG G. During water deficit we have discovered that soybeans accumulate the low molecular weight compatible solutes trigonelline, rather than glycine-betaine as in grasses. Trigonelline (TRG), nicotinic acid betaine, is the N-methyl conjugate of nicotinic acid and is synthesized by S-Adenosyl-L-methinoine:nicotinic acid-N-methyltransferase (EC 2.1.1.7) (NNMT). NNMT is a soluble enzyme that catalyzes the transfer of the methyl group from S-adenosyl-methionine to nicotinic acid. Trigonelline has been shown to stabilize enzyme activity in vitro, to act as a cell cycle regulator and is postulated to function as a compatible solute in response to salinity- and water deficit-stress. We have purified NNMT from soybean and aim to clone the corresponding gene.

Session:Abiotic Stress

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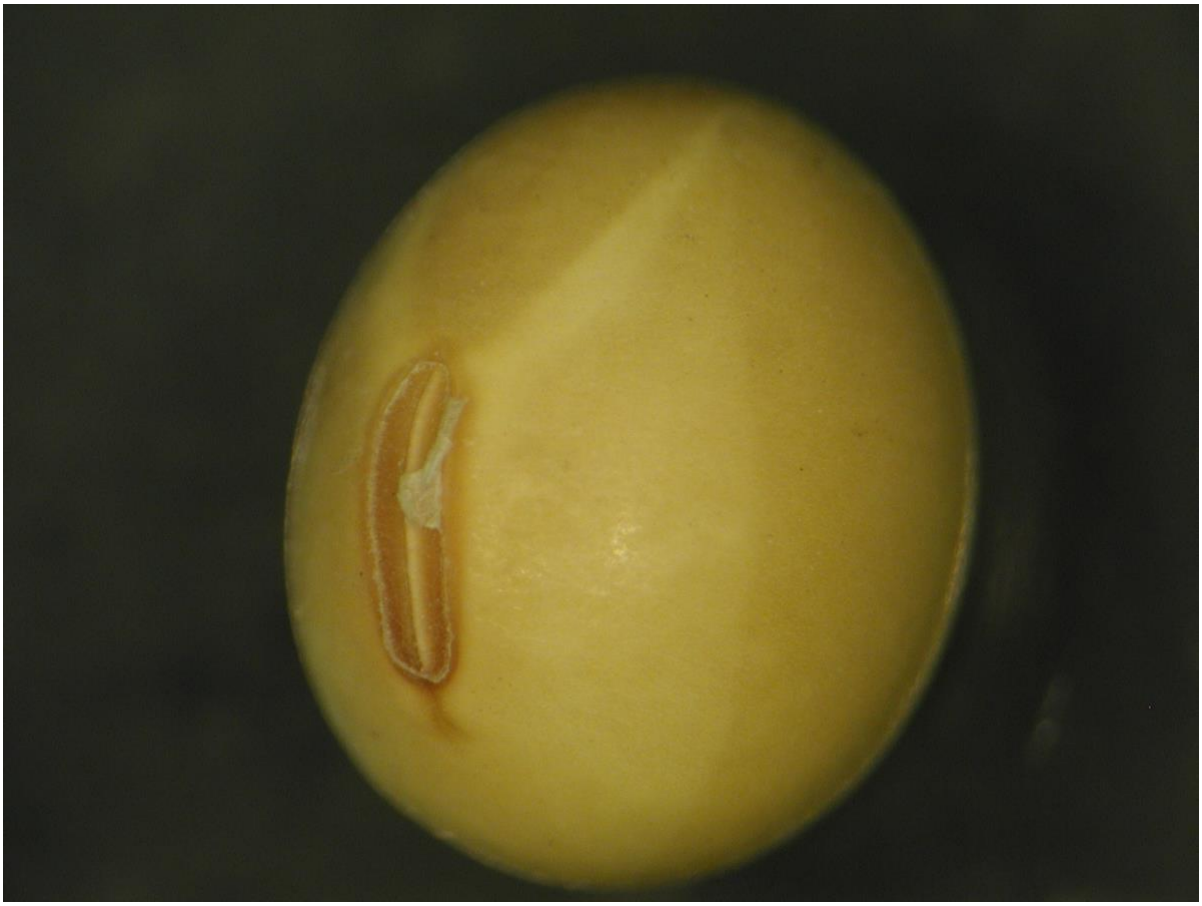

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