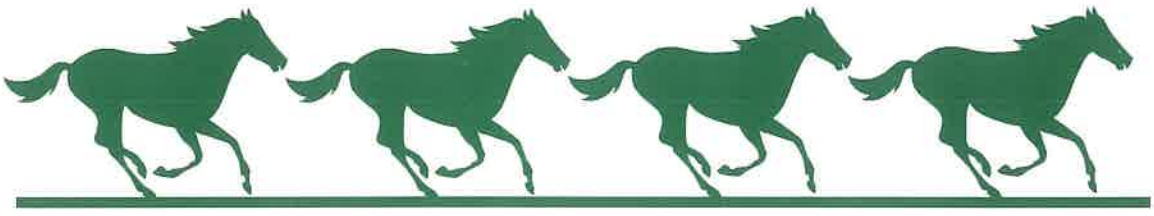
SOY 2000

8th Biennial Conference of the Cellular and Molecular Biology of the Soybean

AUGUST 13-16, 2000
LEXINGTON, KENTUCKY

COLLEGE OF AGRICULTURE
UNIVERSITY OF KENTUCKY
LEXINGTON, KENTUCKY 40546-0091

UK



WELCOME TO

SOY 2000

8th Biennial Conference
of the Cellular and Molecular
Biology of the Soybean

Hyatt Regency Hotel
Lexington, Kentucky

August 13-16, 2000

UK

HOSTED BY THE
UNIVERSITY OF KENTUCKY
COLLEGE OF AGRICULTURE

Glenn B. Collins, ***Conference Organizer***
Todd Pfeiffer, ***Program Co-Chair***
David Hildebrand, ***Program Co-Chair***

construction job came in under budget and the building was paid for by the time it was completed.

42. Union Pacific Railroad's museum is headquartered in Nebraska.

43. Buffalo Bill Cody held his first rodeo in North Platte, Nebraska July 4, 1882.

44. In 1950, Omaha became the home of the College World Series.

45. There are five army forts open to the public in Nebraska: Atkinson, Kearny, Hartsuff, Sidney, and Robinson.

46. Sidney, Nebraska was the starting point of the Black Hills Gold Rush.

47. Antelope and Buffalo are counties in Nebraska named after animals.

48. Dr. Harold Edgerton of Aurora, Nebraska is the inventor of the strobe light.

49. Kearney, Nebraska is located exactly between Boston and San Francisco.

50. Father Edward Flanagan founded Boys Town in Omaha, Nebraska in 1917.

Thanks to: Nancy Schreiner, Diane Robinson, Mike Kuhn, Scott Peterson, Stephanie Hamilton, rbrummers5

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SOY2000
8th Biennial Conference
of the Cellular and Molecular
Biology of the Soybean

We are delighted that you are attending the Soy2000 meeting this week. It is our pleasure to welcome you to the Bluegrass State and to have you staying in Lexington, where the University of Kentucky is located. The local city officials and numerous University of Kentucky faculty and staff have been generous with their time and energy in hosting this meeting, and they want you to enjoy it!

The program was organized by Drs. Todd Pfeiffer and David Hildebrand with the session chairs and co-chairs serving as a program committee. We appreciate the excellent support which they generated for the meeting.

We are indebted to the sponsors for their generous financial support for the meeting. We could never have put together such a top notch meeting at an affordable cost to the participants without such strong sponsor commitment.

We have provided a list of sponsors in the program so that you can thank them. A special thanks goes to United Agri Products and the Barnhart Fund for Excellence for providing the resources, financial and otherwise, that enabled us to have a keynote speaker, Sano Shimoda, and a reception to welcome him on Sunday evening.

Finally, we wish to thank the Local Arrangements committee and the many units they represent for all the vital efforts and activities they have contributed. This committee was chaired by Mr. Curtis Meurer, the real work horse behind the scenes for every aspect of this meeting. We sincerely appreciate all that you have done, Curtis! A list of the members of the local arrangements committee is also included in the program.

Enjoy the meeting and we hope you have a productive and enjoyable stay in Kentucky.

Sincerely,

Glenn B. Collins

Glenn B. Collins, Conference Organizer

Todd Pfeiffer

Todd Pfeiffer, Conference Organizer

Department of Agronomy University of Kentucky Lexington, Kentucky 40546-0091
Phone: 606—257-5333 gcollins@pop.uky.edu

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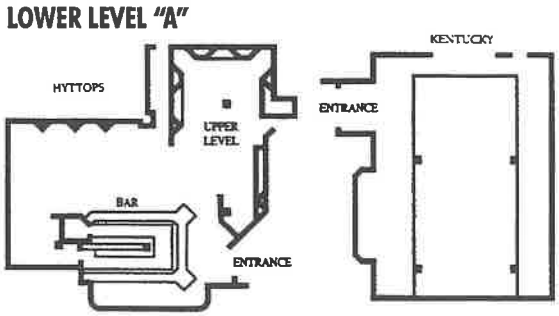

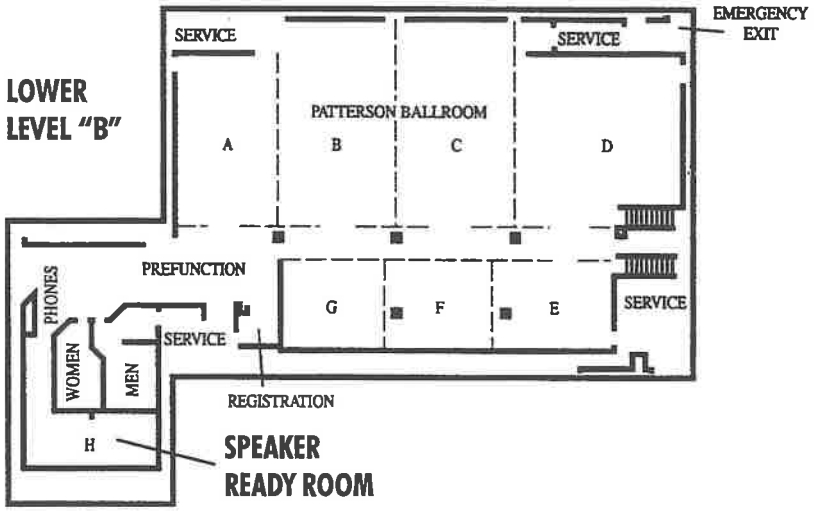

♿ ALL MEETING SPACE IS WHEELCHAIR ACCESSIBLE

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PROGRAM

SUNDAY, AUGUST 13, 2000

12:00-5:00 P.M.
Arrival and Check In

2:00-6:00
Registration — Patterson Foyer

6:00
Reception and Mixer with Keynote Speaker — Regency Ballroom

MONDAY, AUGUST 14, 2000

7:30 A.M.
Continental Breakfast — Patterson E/F/G

7:30
Registration Opens

Opening Remarks — Patterson Ballroom

8:30
Glenn B. Collins, University of Kentucky, Soy2000 Conference Organizer

8:35
James Boling, Vice Chancellor for Research, University of Kentucky

8:40
David Lord, President, Lexington Convention and Visitors Bureau

8:45
Keynote Address
Sano Shimoda, President, Bioscience-Securities, Inc.

Think You've Seen Changes in Agriculture? You Haven't Seen Anything Yet!

PLENARY SESSION I: RECENT SOYBEAN ADVANCES

Roger Boerma, Presiding — *Patterson Ballroom*

9:30
Introduction
Roger Boerma

9:35
Recent Soybean Advances: Genomics
Randy Shoemaker

10:10
Break

10:30
Recent Soybean Advances: Transformation
Wayne Parrott

11:05
Recent Soybean Advances: DNA Markers
Perry Cregan

11:40
Recent Soybean Advances: Molecular Breeding
Roger Boerma

12:15 P.M.
Luncheon — Regency Ballroom

CONCURRENT SESSION A: MOLECULAR BREEDING AND VARIETAL UTILIZATION

Brian Diers Presiding — Patterson Ballroom A/B

1:30
Approaches for Using Molecular Markers in Breeding Programs
Brian Diers

2:00
High Throughput Marker Assisted Selection
Donna Cahill

2:30
The Marketing Risks of Special Purposes Soybeans: A Farm Level Perspective
Gregory Ibendahl

2:50
Break

3:10
Progress in Improving the Cotyledonary-node Transformation Method
Paula Olhoft

3:30
Optimization of Growth of Embryogenic Soybean Tissue, Maintained on a Semi-solid Medium
John J. Finer

3:50
Evaluation of Soybean (*Glycine max* L. Merrill.) Somatic Embryo Growth and Gene Expression Using Image Analysis
Marco T. Buenrostro-Nava

4:10
Oxalate Oxidase: A Novel Reporter Gene for Transformation Studies
Daina Simmonds

4:30
A Novel System to Obtain Fertile Transgenic Soybean (*Glycine max* L Merr.) Plants at a High Frequency
Elibio Rech

4:50
Roundtable Discussion on Transformation
Tom Clemente, Moderator

5:15
Adjourn

5:30
Soycooking Demonstration – Health Seminars — Patterson E
Paula and Curtis Eakins

6:00 - 8:00
Poster Session and Reception, Authors Present — Patterson Ballroom D

MOLECULAR BREEDING AND VARIETAL UTILIZATION

SOYBEAN TISSUE CULTURE AND TRANSFORMATION

Bacterial Antigen Production in Transgenic Hairy Roots of Soybean **B10**
Johanna Preiszner

Production of Transgenic Soybean Using Glufosinate as a Selective Agent with an Improved *Agrobacterium*-mediated Transformation Protocol **B11**
Shaomian Yao

Genetic Studies of Somatic Embryogenesis in Soybean and Effects of Light Intensities **B12**
A. Orlando Mauro

Toward Establishment of a Soybean Transformation System Using Japanese Cultivars: Improvement in Regeneration Time and Efficiency **B13**
Norihiro Ohtsubo,

A Vector System for Creating and Transforming with Gene Artificial Clusters **B14**
Peter LaFayette

A Non-antibiotic Marker for the Selection and Propagation of Plant Transformation Vectors in *E. coli* **B15**
Peter LaFayette

Transformation of Multiple Genes into Soybean by Co-bombardment and by a 6-gene Cluster Plasmid **B16**
Monica Schmidt

A Promoter for Cell Specific Gene Expression in Soybean Pods **B17**
Martina Stromvik

Screening of Soybean Lines for Somatic Embryo Induction and Regeneration Capability from Immature Cotyledons **B18**
Elizabeth Tomlin

Using GFP as an Early Indicator of Soybean Transformation **B19**
Kathryn Larkin

Use of Isoxaflutole as Selectable Marker for Soybean Transformation **B20**
Christelle Muhr

Transformation and Characterization of Transgenic Soybean Expressing the Methionine Rich 15kDa Zein Storage Protein Gene from Maize **B21**
M.S. Srinivasa Reddy

Selection of Transgenic Somatic Embryos **B22**
Suryadevara Rao

GENOMICS

Single Nucleotide Polymorphism (SNPs) in Soybean Genes, ESTs, and Random Genomic Sequence **PIII 08**
Youlin Zhu

Paralogous Evolution of a Small Receptor-like Protein Kinase Gene Family in Soybean **PIII 09**
Etsuo Yamamoto

SoyBase 2000: Creation of a Composite Genetic Map for Soybean **PIII 10**
Marcia Imsande

Soybean Genomic Survey: Bac-end Sequence and Contig Building Near RFLP and SSR Markers **PIII 11**
Laura Marek

Detection of a LG-I Protein/Oil QTL in RIL and F2 Populations **PIII 12**
Heather Olsen

Statistical Analysis of Tissue-Specific Expression Data Using Soybean Microarrays **PIII 13**
Robin Shealy

QTL for Reproductive Traits in Soybean **PIII 14**
I.M. Tasma

TUESDAY, AUGUST 15, 2000

7:30 A.M.
Continental Breakfast — Patterson E/F/G

PLENARY SESSION II: SOY DIET AND NUTRITION
James Anderson, MD, Presiding — Patterson Ballroom

8:30
Health Benefits of Soyfoods
James Anderson

9:00
A Path Towards Increasing Isoflavone Levels in Soybean
Brian McGonigle

9:30
Communication with Consumers on the Health Benefits of Soy
Julie Tockman

10:00
Break

10:20
Soy Protein Protective Effects for the Kidney
Tammy Hanna

10:40
Metabolism of Isoflavones in Humans
Paolo Fanti

11:00
Nutritional Value of Soybean Meal from Genetically Enhanced, Low Oligosaccharide, Low Phytic Acid Soybean for Pigs and Chicks
Gary Cromwell

11:20
Compounds Contributing to the Odor of Aqueous Solutions of Soy Protein Isolates
William L. Boatright

11:40
Operation of the Oxylipin Pathway
David Hildebrand

12:15 P.M.
Luncheon — Regency Ballroom

CONCURRENT SESSION C: METABOLIC ENGINEERING AND VALUE ADDED TRAITS

Sean Coughlan and Jihong Liang, Presiding — Patterson Ballroom A/B

1:15
Modified Fatty Acid Composition in Transgenic Soy Seed
Sean Coughlan

1:45
Discovering and Delivering the Best Genes to the Feed and Processing Customers
Jihong Liang

2:15
Arginine Movement in Developing and Germinating Seeds: Insights From a Urease-negative Mutant
Elizabeth Hoyos

2:35
Break

2:55
Alteration of Trigonelline Concentration in *Glycine max* Under Drought and Irrigated Field Conditions
Andrew Wood

3:15
Down Regulation of the Soybean Embryo Specific Fatty Acid Desaturase FAD2-1
Tom Clemente

3:35
Enhanced Phosphorus Utilization by Development of Low Phytate Soybeans
Elizabeth Grabau

3:55
Regulation of UDP-Glucose Dehydrogenase in Developing Soybean Seeds
Lynn Litterer

4:15
Federal and State Initiatives in the US to Promote Biobased Industrial Lubricants from Commodity and Genetically Modified Seed Oils
Lou Honary

4:35
Adjourn

5:00-6:30
Poster Session and Reception, Authors Present Patterson Ballroom D

CONCURRENT SESSION D: MOLECULAR AND CELLULAR PATHOLOGY

Nevin D. Young, Presiding Patterson Ballroom C

1:15
Resistance Genes of the Coiled-coil NBS-LRR Gene Family in Soybean
Nevin Young

1:45
Lessons from the Protein, DNA and Locus Structure of rhg1
David Lightfoot

2:15
Signal Communication in the Rhizobium-Legume Symbiosis
Gary Stacey

2:35
Break

2:55
Developments of Phenylpropanoids in Soybean SDS Resistance
Vera Lozovaya

3:15
Identification of Multiple Phytophthora Resistance Genes and NBS-LRR-like Sequences at the Rps1-k Region
Madan Bhattacharyya

3:35
Gene Expression Analyses of a Resistance Gene Cluster on Linkage Group J
Michelle Graham

3:55
Expression of Bean Pod Mottle Virus (BPMV) Coat Protein Precursor Results in Resistance to BPMV in Transgenic Soybeans
M.S. Srinivasa Reddy

4:15
Genetic Diversity and Epidemiology of Bean Pod Mottle Virus
Said Ghabrial

4:35
Adjourn

5:00-6:30
Poster Session and Reception, Authors Present Patterson Ballroom D

DIET AND NUTRITION

Genetic Analysis of Phytoestrogen Content in Soybeans **PII 09**
Victor Njiti

High Isoflavone Soy Protein Alters Genes Involved in Glucose and Lipid Metabolism in Obese Rats **PII 10**
William Banz

METABOLIC ENGINEERING AND VALUE ADDED TRAITS

Two Clustered Genes Respond to Plant Hormones **C09**
Caglar Karakaya

Evidence of a Dehiscence Zone in Soybean Pods and Isolation of an Endopolygalacturonase Specific for that Zone **C10**
Lynge Christiansen

Localization and Processing of Bovine Beta-casein in Transgenic Soybean Seeds **C11**
Reena Phillip

Stokesia Desaturase/Epoxygenase-like Genes for Oil Improvement **C12**
Tomoko Hatanaka

Engineering Soybeans for the Production of Edible Vaccines for Poultry **C13**
Peter LaFayette

WEDNESDAY, AUGUST 16, 2000

7:30 A.M.
Southern Style Breakfast

PLENARY SESSION III: GENOMICS
Lila Vodkin and Randy Shoemaker, Presiding Patterson Ballroom

Note: Posters submitted in the Genomics session will be available for viewing with authors present on Monday evening from 6:00-8:00 P.M.

8:30
The NSF Functional Genomics Program for Soybean: An Update
Lila Vodkin

9:00
The Public Soybean EST Project: An Update
Randy Shoemaker

9:15
Development and Use of Soybean Microarrays for Analysis of Global Gene Expression
Steve Clough

9:35
Soybean Genomic Survey: What we are Learning about the Soybean Genome from BACs Identified by RFLP and SSR Markers
Laura F. Marek

9:55
Break

10:15
Development of Physical Maps Integrated with Genetic Markers and EST: Prelude to Genome Sequencing
Khalid Meksem

10:35
Development of the Universal Soybean Genetic Map
Jeongran Lee

10:55
Genomics Applications for Quality Traits
Rita Varagona

11:15
Open Discussion: "Where Do We Go From Here?"

11:30
Closing Remarks

12:00 NOON
Adjourn

MOLECULAR AND CELLULAR PATHOLOGY

RAPD Marker for Resistance to Cyst Nematode (race 3) in Soybean **D09**
A. Orlando Mauro

Tracing the Origin of QTLs for Resistance to *Sclerotinia sclerotiorum* in Soybean Ancestral Lines **D10**
Venancio Arahana

Identification of Early Induced Genes in Soybean-Soybean Cyst Nematode Interaction by Suppression Subtractive Hybridization **D11**
Gejiao Wang

Over-Expression of Anti-fungal Proteins in Transgenic Soybean **D12**
Wojcjech Ornatowski

Is Korea a New Source for Resistance Genes to *Phytophthora sojae*? **D13**
Kara Burnham

Confirmation of the Minor QTL from PI96354 Conferring Root-Knot Nematode Resistance **D14**
Zenglu Li

Genomic Analysis of Metabolic Pathways Conferring Partial Resistance to Fungi: *Fusarium solani* f. sp. *glycines* **D15**
M. Javed Iqbal

Propagation of the Soybean Cyst Nematode on Hairy Roots and Expression of Resistance in Transgenic Roots **D16**
Hyeon-Je Cho

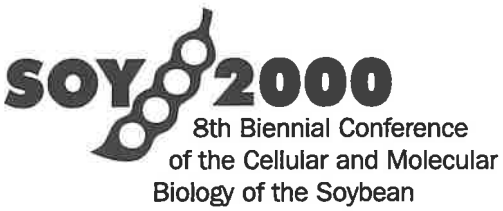
Isolation of Elicitor-inducible Cytochrome P450s from Tobacco Cell Suspension Cultures **D17**
Lyle Ralston

7:00
Buses Depart for Kentucky Horse Park

7:30
Bar-B-Q Dinner at Kentucky Horse Park
Bluegrass Music provided by Homer Ledford and the Cabin Creek Band

8:30
Private Viewing of "The Horse in Chinese Art" Exhibit

10:00
Buses begin returning to Hyatt Regency



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Deborah Witham
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KEYNOTE ADDRESS:

Sano Shimoda

President, Biosciences-Securities, Inc.

Think You've Seen Changes In Agriculture? You Haven't Seen Anything Yet!

8:45 am
Monday, August 14
Patterson Ballroom A/B/C

K 01 Keynote Presentation

Think You've Seen Changes in Agriculture?
You Haven't Seen Anything Yet!

Sano M. Shimoda, President
BioScience Securities, Inc.
2 Theatre Square, Suite 210, Orinda, CA 94563

Technological innovation in the biological sciences is expected to be one of the key catalysts of growth and a powerful engine driving corporate change in the 21st century. Agricultural biotechnology has the potential to transform and create a dynamic new era of growth for agriculture. Growing recognition of the power of agricultural biotechnology is expected to not only redefine the long-term value creation potential of agriculture, but also redefine the importance of agriculture to the broader economy.

Vision, changing corporate mindsets, and growing recognition of the power of agricultural biotechnology, is about to catalyze the creation of a new mosaic of corporate strategies that will not only ratchet up the long-term value creation potential of agriculture, but will also redefine the integrative linkages of agriculture to the broader economy. This realization is expected to lead to a proliferation of corporate strategies focused on creating new horizontal and vertical linkages between businesses, based on the growing recognition of the "Power of Convergence." Increasingly, converging markets will blur the boundaries, redefine existing linkages, and create new linkages between agriculture and the broader economy, which will redefine markets, industries, and companies. The convergence of multiple strategic drivers catalyzed by the development of agricultural biotechnology is expected to move the world's industrial base moves towards a "carbohydrate- or plant-based economy."

A growing investment commitment to research and development in agricultural biotechnology is spreading globally because of growing recognition of the long-term opportunities to enhance agricultural productivity and value creation. In addition, agricultural biotechnology is critical to promoting the long-term growth, health and structure of the U.S. farm economy, since the prospect of creating differentiated value-added holds long-term promise to provide market driven solutions to a number of key issues facing U.S. agriculture.

Unfortunately, the road to success is never a straight line. The commercial development of agricultural biotechnology is being hampered by the GMO controversy, which has pushed marketplace commercialization into a state of suspended animation that could exist for the next few years. Support for the development of this technology in the U.S. is expected to remains strong, however, the long road back toward regaining consumer confidence in many regions outside

the U.S. will take time and efforts by all stakeholders to improve their understanding of the risk and benefits of the technology.

Vision and focus, combined with perseverance, creates opportunities for those who recognize the road to success is sometimes fraught with unforeseen events that tests one's commitment and confidence in the future. While the near-term potential of agricultural biotechnology has become clouded by the GMO controversy, rewards will go to those with vision and a focus on the long-term potential to redefine the value creation of agriculture, recognizing that the world belongs to the optimists, who are also realists.

Biography of Sano M. Shimoda and Background on BioScience Securities, Inc.

Sano Shimoda, an investment banker and Wall Street analyst, is president and founder of BioScience Securities, a brokerage and investment banking firm located in the San Francisco Bay area. BioScience Securities was founded in 1994 and is unique in its focus on biotechnology and agriculture. The firm specializes in those sectors that will be impacted by this developing technology platform, as the industrial base of the worldwide economy moves towards a "carbohydrate- or plant-based economy." In addition to agriculture and related downstream sectors, the firm focuses on the growing impact the "agricultural biotechnology wave" will have on new developing areas, such as life sciences and a broad spectrum of non-conventional markets.

BioScience Securities is focused on the "knowledge power" since the firm's emphasis is to combine business and financial expertise with knowledge of agriculture and science, from both a technology and a marketplace perspective. The firm provides knowledge-driven corporate advisory and investment banking services to corporate clients, and value-added research for institutional investors managing large equity portfolios. From an investment banking perspective, BioScience Securities has provided strategic corporate advisory services to a number of companies involved in the recent wave of merger and acquisition activity, catalyzed by the development of agricultural biotechnology.

Sano Shimoda is well recognized for his forward-looking views of the changing dynamics agricultural biotechnology will have as it redefines the potential of agriculture, the implications for the existing agricultural infrastructure, and the growing integrative linkages agriculture will have with the overall economy. BioScience Securities' views are widely quoted in both general business and agricultural-oriented publications, on a global basis. Mr. Shimoda has made presentations in the U.S. and worldwide to a broad array of companies and industry groups focused on the impact, changes, and opportunities that will be created by agricultural biotechnology.

A Wall Street analyst by training, Mr. Shimoda has over 25 years of experience in the Wall Street community focusing on agriculture, agricultural biotechnology, and the agricultural chemical and chemical industries. Mr. Shimoda received a B.S. in Business Administration (magna cum laude) from Lehigh University (Bethlehem, Pennsylvania) and an MBA from the University of California (Berkeley, California). He serves as an Advisory Board member to the College of Natural Resources of the University of California in Berkeley.

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PLENARY SESSION I

Recent Soybean Advances

Roger Boerma, Presiding

9:30 am
Monday, August 14
Patterson Ballroom A/B/C

PI 01

Recent Soybean Advances: Genomics.

Randy C. Shoemaker

Department of Agronomy and USDA/ARS Field Crops Research Unit
Iowa State University, Ames, IA 50011

During the last few years we have watched soybean blossom into a model crop species. We have amassed a collection of tools and resources that are the equal of nearly any crop. We have a genetic map that contains well over a thousand markers. The producers have funded an EST project that has publicly deposited the largest number of entries from any plant species, approximately 100,000 in dbEST. Several laboratories have produced BAC libraries. We now have a many-fold redundancy in genome coverage, created in BACs with a variety of restriction enzymes and from numerous genotypes. The National Science Foundation has funded the development of a complete physical map. During the same year NSF funded development of functional genomic tools and resources (microarray and SAGE). A Soybean Microarray Workshop has already been held to pass on these technical advances to colleagues. Because of the historical cooperative and collaborative research among scientists and support from the Commodity Boards the soybean research community now enjoys an enviable position in plant genomics.

PI 04

Recent Soybean Advances: Molecular Breeding

H. Roger Boerma
Department of Crop & Soil Sciences
University of Georgia, Athens, GA 30602-7272

The genetic map of soybean [*Glycine max* (L.) Merril] contains approximately 700 simple sequence repeat (SSR) markers. As of May 2000, 243 quantitative trait loci (QTL) were reported. Less than 10% of these QTL have been confirmed. SSR markers are currently being employed by soybean breeders to improve breeding efficiency through: i) recovery of recurrent parent in backcrossing, ii) early generation selection of quantitative traits, iii) concurrent selection of multiple traits, and iv) mining exotic germplasm for desirable alleles. A major application of marker assisted selection (MAS) in commercial breeding programs has been for resistance to soybean cyst nematode (*Heterodera glycines*). Although MAS is being utilized in both commercial and public breeding programs, there is no documented release of a soybean cultivar or germplasm developed by MAS (Young, 1999). Cost, coarse mapping of QTL, and the lack of QTL confirmation have limited the application of MAS in soybean improvement. In addition, the physical separation of molecular breeders with knowledge of marker technology and classical breeders with knowledge of trait-specific selection limitations has restrained the development of novel applications of MAS in soybean improvement. In the future it is anticipated the cost of MAS will continue to decline and breeders will have access to trait-specific selection kits based on single nucleotide polymorphism (SNP) markers.

Reference

Young, N.D. 1999. A cautiously optimistic vision for marker-assisted breeding. Molecular Breeding 5:505-510.

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CONCURRENT SESSION A

Molecular Breeding and Varietal Utilization

Brian Diers, Presiding

1:30 pm
Monday, August 14
Patterson Ballroom A/B

Poster Session 6:00—8:00 pm
Patterson Ballroom D

A 01

Approaches for Using Molecular Markers in Breeding Programs

Brian Diers

Department of Crop Sciences
University of Illinois, Urbana, IL

Over the last 15 years, genetic markers have expanded from being a basic science tool to now being useful in applied breeding programs. An example of the use of markers in breeding programs is marker assisted selection (MAS) for soybean cyst nematode (SCN) resistance. The major SCN resistance gene, rhg1, can be selected using microsatellite markers in most crosses. We found that MAS using the microsatellite marker Satt309 in a population segregating for PI 88788 resistance will improve the population's resistance to a female index of 6 for selected lines from a population average of 51. This selection can be done in our laboratory at a cost of about $ 0.33 per genotype. The identification and exploitation of genes from the germplasm collection is an additional use of markers in breeding programs. An example of this type of research is our mapping and selection of a gene from Glycine soja that confers high seed protein content. Through MAS, the gene was backcrossed into a high yielding soybean background and retested for its effect. We found that the gene's effect was the same magnitude in a backcross population as in the original interspecific cross.

A 02

High Throughput Marker Assisted Selection

Donna Cahill

Pioneer HiBred International
Johnston, IA 50131

Marker assisted selection (MAS) has promised to be a powerful selection alternative for traditional plant breeders to select for agronomically favorable alleles among segregating populations, particularly when a desired trait is affected by multiple genes and gene x environment interactions. High Through-put MAS gives the added advantage to screen a large number of progeny which hastens the advancement of an experimental line to become a possible commercialized product as well as conserves limited agricultural resources by eliminating unfavorable lines early in the breeding process. This presentation will give an overview of the High Through-put MAS system developed at Pioneer Hi-Bred.

A 03

The Marketing Risks of Special Purpose Soybeans: A Farm-Level Perspective

Gregory Ibendahl and Kimberly Zeuli

Department of Agricultural Economics
University of Kentucky, Lexington, KY 40506

Soybean growers across the country are adding value to their crop by producing specialty soybeans—soybeans grown for specific attributes. The trend towards producing special purpose soybeans for specific attributes (physical and chemical) is likely to continue. The new specialty soybeans present both production and marketing challenges for soybean farmers. Since no commodity market exists for these specialized soybean products, contract production is likely. Contractual arrangements between individual farmers and large agribusiness companies could easily result in sub-optimal contracts from the perspective of the farmer. Under some contracts, the growers may not fully realize the potential profits and risk mitigation benefits from specialty soybean production. This paper will show how farmers can evaluate the profitability and risk of specialty soybeans under contract production or by forming alliances to market their soybeans.

A 04

Molecular Breeding and Genetic Alteration of the Soybean Yield-to-Water Relationship - A Case Study.

James Specht, K.G Lark, and G. L. Graef

Department of Agronomy
University of Nebraska, Lincoln NE 68583-0915

Water is the paramount abiotic factor affecting crop productivity. Year-to-year variation in seasonal rainfall accounts for as much as 40% of the annual variation in Nebraska's rain fed soybean yields. Improving crop drought tolerance has been focus of attention for many of my breeder and physiologist colleagues during the last third of the last century. Though the term drought tolerance is used copiously in the literature, it does not intuitively invoke a universal definition in the minds of the scientists and producers who use it. Although a host of empirical and mechanistic approaches have been used to select genotypes with traits that presuppose a greater tolerance to water stress, it has been difficult to convincing demonstrate that drought tolerance as measured by yield performance under drought has actually been improved. Will the rapidly developing technologies of genomics finally provide the tools needed to successfully improve drought tolerance? This is certainly possible, given that the paradigm of calibrating phenotypes with genotypes is rapidly giving way to the paradigm of using marker-facilitated techniques to dissectively calibrate phenotypic variation with segments of the crop genome. This is clearly a tremendous technological advance in quantitative genetics. In this presentation, using my own research efforts as a case study, I will show why the distinction between absolute and relative drought tolerance is a critical one, and why, even with molecular markers, the genetic nature of drought tolerance will likely remain a "tough nut to crack".

A 05

QTL associated with salt tolerance in soybean

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Salinity can significantly inhibit both seed germination and plant growth, and in some areas of the world is a major environmental constraint to crop productivity. Most soybean [Glycine max (L.) Merr.] cultivars are sensitive to even moderate levels of salinity. Development of salt tolerant cultivars is an effective approach to avoid yield loss in a saline soil. DNA markers, as a tool, can be used efficiently for marker-assisted selection in a breeding program. This study was conducted to identify genomic regions that affect salt tolerance in soybean. An F2-derived population from the cross of 'S100' x 'Tokyo' along with parents and checks were evaluated for salt tolerance in the saline field in Hyde County, NC. Each F2-derived line was initially genotyped with RFLP markers and ultimately saturated with SSR markers on the regions of interest. A major quantitative trait locus (QTL) on Linkage Group N with the favorable allele contributed from S100 was found to be significantly associated with salt tolerance. This marker, Satt237, accounted for 31% of total variation. Moreover, two minor QTL were found on the Linkage Groups D1a and L. Tokyo contributed the allele for salt tolerance at the QTL on LG-D1a, while an allele from S100 increased salt tolerance at the QTL on LG-L. When combined, the three QTL explained 40% of total phenotypic variation. Both S100 and Tokyo are ancestral lines of modern soybean cultivars in the southern U.S.A. These results could be very useful for maker-assisted selection in the S100 derived progenies. Confirmation of the QTLs in this population is being conducted

A 08

Restructuring Plant Type to Enhance Yielding Ability in Soybean (*Glycine max*).

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A 10

Application of DNA Markers to Select High Protein High Yield Soybean Lines

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A soybean [Glycine max (L.) Merr.] population of Benning x Danbaekkong was developed to map quantitative trait loci (QTL) conditioning traits associated with tofu and soymilk quality. Benning is a high yield, multiple pest resistant cultivar adapted to the southeastern USA (Maturity Group VII) with approximately 42% seed protein content. Danbaekkong is a Maturity Group IV cultivar from South Korea developed for soy food use. It has a seed protein content of approximately 51%. In 1998 and 1999, 180 F2-derived lines were grown in a replicated field experiment in order to map the protein QTL in this population. A major protein QTL conditioning 49% of the phenotypic variation was identified on Linkage Group I near Satt239. Lines that were homozygous for the Danbaekkong allele at Satt239 averaged 35g/kg (3.5%) higher protein content than lines homozygous for the Benning allele. In a second experiment conducted in 1999, lines from this population were selected to evaluate the association between seed yield and protein content. Twenty-eight F2-derived lines (14 with high protein and 14 with low protein) along with four entries each of Benning and Danbaekkong were evaluated in replicated field plots at two locations. Results indicated that one F2-derived line (G98SF-114) may possess both high protein content and high seed yield. This would suggest a genetic recombination in the Satt239 genomic region. G98SF-114 averaged 48.6% protein and was equal in yield and maturity to Benning.

A 11

Soybean Cultivar Identification with Trinucleotide Simple Sequence Repeat Markers

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Soybean [*Glycine max* (L.) Merr.] cultivars are described for purposes of Plant Variety Protection (PVP) by standard pigmentation and morphological traits. However, many commercial soybeans arise from a limited number of elite lines and are often indistinguishable based on these traits. A system based on DNA markers could provide unique DNA profiles or fingerprints of cultivars. Simple Sequence Repeat (SSR) or microsatellite allele size profiling is used in human forensics to provide unique DNA fingerprints of an individual. Allele sizing technologies are well established and can be readily used to size SSR alleles from any organism. The purpose of the work presented here was to select and evaluate a small set of trinucleotide SSR markers with maximum reliability and repeatability that would provide a high level of discriminatory power to distinguish soybean genotypes. A total of 48 fluorescently labeled SSR primer sets was used to amplify genomic DNA of the 35 ancestors of N. American soybeans as well as a diverse group of elite N. American soybean cultivars. Only loci with allele size ranges that showed no overlap in size over a series of analyses and in which adjacent alleles differed by at least three base pairs were maintained for further statistical analysis via a clustering procedure. Cluster analysis was performed on the remaining loci and resulted in the identification of a subset of 13 loci, from 12 different linkage groups, that easily produced unique SSR allele size profiles for each of the 66 elite N. American soybean cultivars. This set of 13 loci was used to characterize four independent sets of elite cultivars that were selected based upon identical maturity, morphological, and pigmentation traits. Based upon these analyses, all cultivars could be distinguished using the set of 13 selected SSR loci. This set of loci is proposed as a standard set for use in DNA profiling of soybean cultivars for purposes of obtaining PVP.

A 12

Towards the Evaluation of Pyramided Insect Resistance Genes

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The deployment of insect resistance genes in soybean may enhance seed yields and reduce pesticide usage. The ideal insect resistant cultivar would exhibit adequate resistance levels to an array of pests and would possess durable resistance, such that the development of resistant pest populations would be reduced. Native insect resistance has been shown to be effective against certain pests. Insect resistance derived from *Bacillus thurengensis* (Bt) via synthetic cry1Ac has been incorporated into soybean through transgenic technology. cry1Ac expressing Bt endotoxins has been shown to be very effective against certain soybean pests; however, widespread use of Bt without the implementation of effective resistance management strategies could lead to the development of resistant pest populations. By combining the two sources of resistance, the range of pest controlled may be increased, the level of resistance may be elevated, and the durability of the resistance system may be enhanced. The combination of cry1Ac and a major insect resistant QTL derived from PI229358 has been shown to elevate resistance to corn earworm and soybean looper, as determined by antibiosis assays. The objective of the current research is to evaluate the effectiveness of a combination of one or two major insect resistant QTLs and cry1Ac for control against insect defoliation in field grown plants. Eight different BC2F3-derived lines possessing the presence or absence of the resistance genes are being evaluated under corn earworm and soybean looper infestations

A 13

Soybean Genetics Newsletter

David Lohnes and Ronald Fioritto

OARDC
The Ohio State University, Wooster, OH 44691

The Soybean Genetics Newsletter is an online journal found at http://www.soygenetics.org. Information in the Soybean Genetics Newsletter is of an informal nature, to stimulate thought and to exchange ideas among soybean scientists. Newsletter articles may be preliminary in nature and speculative in content. They should not be regarded as equivalent to papers in scientific journals. Even so, such reports can be exceedingly helpful and valuable if viewed in the proper perspective

A 16

Quantitative Trail Loci for Insect Resistance in Recombinant Inbred Soybean Lines.

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A soybean RI population ('Noir 1' X 'Minsoy'), developed for genetic mapping purposes, showed transgressive segregation for resistance/susceptibility to several different insect pests, including corn earworm and soybean looper. In this RI population, several quantitative trait loci (QTLs) were determined for resistance/susceptibility through their linkage to molecular markers. To confirm these loci, another RI population derived from 'Minsoy' X 'Archer' (an elite cultivar) was tested for resistance and then analyzed for QTLs. Of the 20 linkage groups (LGs) of the composite genetic soybean map, five LGs in the Minsoy- Noir1 and four in the Minsoy-Archer RI have QTLs associated with resistance. The most important QTL identified in both populations is found on LG U2, linked to Satt575 and Sat_112. It accounts for the largest fraction of phenotypic variation (> 12% of the Minsoy-Noir RI and > 28% of Minsoy-Archer RI) and affects larval weight, pupal weight, and development rate. Because the resistance allele from Minsoy remains active in a different genetic background (Minsoy X Archer RI), it is likely that the resistance will not be lost if introgressed into other elite germplasm. Specific plant traits are being examined for their association with this resistance and susceptibility.

A 17

A Transgene Locus in Soybean Exhibits a High Level of Recombination

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Our laboratory previously described the transformation of soybean by particle bombardment with a construct containing bovine B-casein under the control of the soybean lectin 5' and 3' regulatory elements. Analysis of primary regenerated plants indicated that four copies of the transgene had integrated into the genome. Southern hybridization analysis of over 200 T1 progeny plants revealed that approximately 15% of the plants had lost two or three of the original four casein-hybridizing bands. Further DNA hybridization experiments on T2 progeny showed a continued loss of hybridizing bands in lines originally possessing the original four-copy pattern. Isolation of the transgene locus using restriction enzymes that do not cut the transformation plasmid showed that all four transgene copies are at a single locus no larger than approximately 40 kb in size. Therefore, the recombination events resulting in the loss of transgene DNA are taking place within a limited physical distance on the host chromosome. Potential mechanisms are discussed.

A 19

Density Effects of Waterhemp (*Amaranthus rudis*) Competition on Cultivated Soybean.

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Populations of waterhemp (*Amaranthus rudis*) have increased in no- or reduced-tillage soybean fields, extensively reducing biomass in late planted soybeans. We examined the effects of competition between soybean and various population densities of waterhemp upon various growth parameters and trigonelline (TRG) biosynthesis. Four soybean cvs. Essex, Forrest, Stressland and PI471938 were planted under controlled conditions and 14-d-old soybean seedlings were then transplanted to 3.8-liter pots sown with waterhemp at densities of 0, 1, 4 or 16 seeds per pot. Measurements were taken for plant height and total biomass in both plant species and for leaflet number, node number, growth stages and biomass in soybean plants. TRG was extracted from soybean leaf samples and purified by ion exchange chromatography. Significant genotypic variation was found for plant height ($P<0.01$). Total dry biomass in all soybean genotypes significantly decreased up to 4-fold (range 1.0- to 4.0- fold) as waterhemp population density increased per unit area. The number of nodes in soybean decreased slightly (up to 1.4-fold; range 0.9- to 1.4-fold). TRG accumulation and its association with growth characteristics will be examined. This work was supported, in part, by grants from the Illinois Soybean Program Operating Board to AJW.

A 20

Comparative Molecular Marker Analysis of Yield QTL in Soybean Cultivars.

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Yield is a complex trait with perhaps 30-100 genes contributing. Some yield genes may be of large effect and portable across genetic backgrounds. Maturity group and growth habit clearly confound yield QTL analysis therefore we have mapped QTL in adapted cultivars with high coefficient of common parentage with no maturity or growth habit segregation. Coinheritance of increased yield and field resistance of soybean (*Glycine max* (L.) Merr.) to sudden death syndrome (SDS) (caused by the fungus *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & Hans.) and soybean cyst nematode (SCN) (caused by *Heterodera glycines* Ichinohe) occurs in crosses among PIs and adapted cultivars. Linkage group G where major loci for SCN race 3, race 14 and SDS resistance appear to cluster is largely responsible for this coinheritance. We have studied yield in Essex x Forrest (ExF) Hartwig x Flyer (HxF) and Pyramid x Douglas (PxD). We analyzed yield with disease pressure in 5-8 environments. Using the ExF and HxF RIL and NIL populations we have tested yield in five environments free from major disease pressure to compare with yield data from environments infested by pathogens. There was no evidence of yield depression caused by linkage group G in adapted cutivars. Two major yield QTL were identified that were consistent across multiple populations and multiple years. The yield beneficial alleles of some linked markers are over-represented in elite breeding material at SIUC. The QTL were further isolated in NILs. The yield effects are not caused by resistance to any known pathogen. Isolation of candidate genes by shotgun sequencing from contiguous overlapping clones of genomic BACs and microarray expression analysis is in progress. This research was supported by the Illinois Soybean Program Board.

SOY 2000
8th Biennial Conference
of the Cellular and Molecular
Biology of the Soybean

CONCURRENT SESSION B

Tissue Culture/Transformation

Ted Klein and Paul Umbeck, Presiding

1:30 pm
Monday, August 14
Patterson Ballroom C

Poster Session 6:00—8:00 pm
Patterson Ballroom D

B 01

Transformation of Soybean by Bombardment of Embryogenic Cultures

Ted Klein

Dupont Agricultural Products,
Newark, DE 19714

B 02

Versatility in the Transformation of Elite Soybean Germplasm

Brian Martinell, Lori Julson, Laura Crow, Michael Petersen, and Paul Umbeck.

Monsanto Company, Agracetus Campus, 8520 University Green, Middleton, WI 53562.

The result of any good soybean transformation system should be a process that is capable of delivering high quality events efficiently and without the burden of freedom-to-operate issues. Building on that premise, researchers have probed many alternatives for enhancing commercially valuable traits. The result has been development of a versatile collection of markers and methods useful for creating transgenic soybean lines through the use of Agrobacterium and particle mediated transformation. In order to bring sanity to a process that many times appears mystical, in-depth analyses of many variables is required. In this presentation, we will review some of the parameters that have driven creation of a robust system for transforming elite commercial varieties of soybean.

B 03

Efficient Plant Regeneration From Various Explants of Soybean (Glycine max L. Merr.)

Sharard Tiwari and M..K. Tripathi

J.N. Agricultural University, Jabalpur, India

In vitro culture is an important experimental tool for crop improvement, only when efficient and reproducible plant regeneration systems are available. In this study, induction of callus cultures and regeneration in soybean from immature embryos, immature cotyledons, mature cotyledons, hypocotyls, leaf discs and anthers have been undertaken. Six separate experiments were conducted involving different explants from eleven genotypes on different modifications of culture media to examine genotypic influences, to select responding culture medium and to search genotype x culture medium interactions for totipotent callus induction and plant regeneration. Different explants from all the genotypes initiated callus at varying frequencies which later regenerated plantlets except from anther culture where plant regeneration was observed from only two genotypes. Besides genotypic differences, various explant cultures were influenced by culture media that seems to be a major determinant of callus formation and regeneration. In addition to genotype and culture medium influences strong genotype x medium interactions were also observed. Results from these experiments reveal that an efficient and productive in vitro culture system is available for soybean that has potential for immediate utilization in crop improvement programme by conventional or biotechnological means.

B 04

Transgenic Soybean via Hypocotyl-Based Organogenic Method

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An organogenic regeneration protocol for soybean (Dan and Reichert, In Vitro Cell. Dev. Biol. 34P:14-21, 1998) was coupled to biolistics-based transformation using PDS-1000/He. Double bombardments introduced plasmid pLMBAR (contained the *bar* coding sequence controlled by the CaMV 35S promoter), into hypocotyl tissues of genotypes representing three maturity groups. Post-bombardment, tissues were placed on selective shoot initiation and elongation media which contained Ignite (phosphinothricin-based herbicide) for at least five months. Stable integration of *bar* was confirmed via polymerase chain reaction (PCR) analyses, with positive shoots rooted, transplanted to soil, acclimatized and maintained in the greenhouse. All were noted to be fully fertile, to date. Additional molecular analyses are currently being conducted on original transformants and resulting progeny.

B 05

Progress in Improving the Cotyledonary-node Transformation Method.

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The soybean cotyledon (cot)-node transformation method would be improved if the efficiency of producing transgenic plants were increased. We hypothesized that resistance of the cot-node region of young soybean seedling explants to Agrobacterium infection limits Agrobacterium-mediated DNA. We increased Agrobacterium infection rates by amending the solid co-cultivation media with various compounds. In eight independent experiments, the percent of explants showing GUS transient expression at the cot-node 5-days post-inoculation increased from 37% on non-cysteine control explants to nearly 100% on explants cultured with cysteine. More striking was the significant increase in GUS+ foci on those explants that underwent the cysteine treatment. This increase in Agrobacterium infection was also evident after 28-days on shoot induction media. Not only was there a significant increase in GUS+ sectors in the callus growth but the percent of explants containing GUS+ shoots increased from 4.5% in non-cysteine controls to 20.6% in one cysteine treatment. We will present data on transformation efficiencies of these ongoing experiments and discuss potential mechanisms for cysteine enhancement of Agrobacterium infection.

B 06

Optimization of Growth of Embryogenic Soybean Tissue Maintained on a Semi-solid Medium.

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Embryogenic tissue is one of the two main target tissues used in most laboratories for soybean transformation. Proliferative embryogenic tissues, maintained using either a solid or liquid medium are typically either bombarded or subjected to Agrobacterium-mediated transformation. The growth of embryogenic soybean tissues is believed to be more rapid in a liquid medium as the tissue is constantly bathed in the liquid medium rather than sitting on a semi-solid medium, which allows only partial contact of the tissue with the nutrients. Unfortunately, liquid cultures of soybean can be difficult to initiate and maintain, and are more prone to contamination problems. We have recently developed transformation systems for soybean using embryogenic soybean tissue (D20 tissue, maintained on a semi-solid medium containing 20 mg/l 2,4-D) with both the particle gun and Agrobacterium. In order to increase transformation rates of D20 tissue, efforts were made to optimize growth of this tissue by evaluating environmental conditions and media addenda. No increase in growth was obtained following placement of tissue under various light conditions or when using different types and concentrations of carbohydrate in the media. Surprisingly, maintenance of D20 cultures at 23°C or 25°C, rather than our standard laboratory conditions of 27°C, resulted in both enhanced growth rates and higher tissue quality (as judged by tissue morphology and color). Tissues maintained at 23°C and 25°C also appear to be more responsive to transformation. Addition of 5-15 mM asparagine to cultures maintained at 25°C also resulted in increased growth rates with an additional increase in transformation competency. We believe that further optimization of growth conditions for D20 tissue will give rise to higher and more consistent transformation rates, making this tissue most suitable for transformation by many other laboratories.

B 07

Evaluation of Soybean (*Glycine max* L. Merrill.) Somatic Embryo Growth and Gene Expression Using Image Analysis.

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The current development of sophisticated imaging systems and complex computer algorithms have increased the use of image analysis for quantitative evaluation of biological processes, such as plant growth and development. Using the proper reporter gene such as the green fluorescent protein (gfp) and the appropriate molecular techniques, image analysis can be used to quantify levels and patterns of gene expression over time. In this work, the growth and development of soybean somatic embryos was initially evaluated using the image analysis approach. Proliferative embryogenic tissue was placed on a semi-solid development medium under different environmental conditions (27°C and 23°C) and images were collected every 24 hrs for analysis. Images were analyzed using Visilog software and subsequently manipulated using Adobe Photoshop. The color of the embryos was designated according with the standard CIE 1931 chromaticity diagram (x, y). Strong differences were observed in the rate of growth and rate of differentiation of the embryos tested under the two different environments. Despite the higher rate of growth of embryos at 27°C, these embryos showed the lowest germination rate compared with embryos growing at 23°C. These results also suggest that the pale green (0.37, 0.62) embryogenic tissue has a higher differentiation rate compared with pale yellow (0.38, 0.36) tissue, which suggests that color may be a good characteristic for selection of starting material that will have a high differentiation rate and produce high quality embryos. Efforts are currently underway to introduce the green fluorescent protein gene into soybean under regulatory control of various promoters in order to use image analysis for quantification of gene expression in various soybean tissues.

B 08

Oxalate Oxidase: a Novel Reporter Gene for Transformation Studies

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The ideal reporter gene has no background activity, no detrimental effects on development, is very simple to detect in both histochemical and quantitative assays and requires no complex instrumentation. None of the reporter genes currently available meet all these requirements.

Oxalate oxidase (OXO) encoded by the wheat germin gene (*gf-2.8*)[1] is a heat stable protease-resistant glycoprotein. Detection of H_2O_2 generated from OXO oxidation of oxalate provides simple, rapid detection of gene expression. OXO activity was detected histochemically by the production of an insoluble purple precipitate as a result of H_2O_2 oxidation of 4-chloro-1-naphthol. Activity can be observed directly in assay buffer, in minutes, without chlorophyl clearing procedures. It has been used for both transient and stable expression in monocots and dicots. For determination of levels of transgene expression, a simple quantitative enzyme activity assay was used and required only a spectrophotometer. Inexpensive substrates are used for both assays. Histochemical detection of transgene activity was used to optimize transformation procedures and to screen subsequent generations for segregation of enzyme activity. The simplicity of the assays allowed large-scale screening for primary transgenics and for segregating populations in field studies.

[1] Lane BG et al. (1991) J. Biol. Chem. 226:10461-10469

B 09

A Novel System to Obtain Fertile Transgenic Soybean (*Glycine max* (L.) Merril) Plants at a High frequency.

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Brazil is the second largest soybean producer, with an annual average production over 13 million tons. There is a worldwide interest in the utilization of the recombinant DNA technology to introduce new traits in soybean, which in turn, should allow cost reduction, productivity increase, among others benefits. Over the past ten years, several attempts have been made in order to obtain transgenic soybean plants. The protocols so far published, have failed to reproducibility, simplicity to conduct, high efficiency and variety independency. The combination of: a) genes which codify herbicide-active polipeptides, capable of translocate systemically and concentrate in the apical meristematic region of the plant and b) a short multiple shooting induction protocol, have allowed the development of a simple and routine system to obtain transgenic soybean plants at a high frequency and variety independent. The apical meristematic region of mature soybean embryonic axes were excised, and bombarded with the plasmid DNA. Then, the bombarded embryonic axes were transferred to the culture medium containing MS basal salts, sucrose, cytokinin and the selective agent. After three to five weeks in culture, putative transgenic shoots were excised and transferred to the greenhouse. The plants were allowed to set seeds and progeny analysis were performed. Four elite commercial soybean varieties (Doko RC; BR16; Celeste and Conquista) were transformed. The frequency of transformation (number of transgenic plants/number of bombarded embryonic axes) varied from 5-20%, depending on the cultivar. Virtually no chimeras were observed. We have already been utilizing this novel system to generate elite events carrying usefull traits and introduced these plants in the soybean breeding program. Field release experiments have been carried out over the last two years.

B 10

Bacterial Antigen Production in Transgenic Hairy Roots of Soybean

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Many intestinal bacteria which cause dysentery produce specific proteins to invade intestinal cells. These proteins induce an immune response at the intestinal mucosa and the application of these antigens may induce a transient immunity that prevents re-infection. While manufacturing vaccines through conventional fermentation requires high technology systems, oral immunization through edible-plant based vaccines are safe and easy to administer even in developing countries. Since soy-based food can be used in several form (soymilk, granulate, etc) transgenic soybean seeds producing the antigen could provide a suitable vehicle for oral administration. We introduced a gene encoding the invasion protein antigen of *Shigella flexneri*, into an Agrobacterium vector. Generating transgenic hairy roots to study gene expression is a fast alternative to the labor-intensive, whole-plant regeneration methods. To test if the gene is properly transcribed and the protein is produced in soybean tissue, we used *Agrobacterium rhizogenes* transformation to generate antigen expressing hairy root lines. Following soybean cotyledon transformation, high frequency of hairy root production could be observed. We could detect the proper transcription of the gene in all of the examined hairy root lines. The antigen protein was detectable in three of the lines by immunoblotting. We can conclude that the production of the antigen is possible in soybean tissues. As a next step we are performing soybean cotyledonary node transformation to regenerate whole transgenic plants.

B 11

Production of Transgenic Soybean Using Glufosinate as a Selective Agent with an Improved *Agrobacterium*-mediated Transformation Protocol

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Although *Agrobacterium*-mediated gene transfer is now well established for routinely transferring genes into many crops, transformation of soybean remains inefficient with *Agrobacterium*-mediated gene transfer system. Only handful of laboratories in the world is able to produce transgenic soybean plants consistently. Meurer et al. (1998) reported that only 1-2% of regenerated shoots was transformed with *Agrobacterium*-mediated cotyledonary node transformation. Improvement of soybean transformation technique is necessary before transgenic soybean plants can be produced routinely in the laboratories.

Transgenic soybean plants were obtained with *Agrobacterium*-mediated genetic transformation in this research. The bar gene and glufosinate were incorporated in the selection system. The binary vector PBIMC-B containing *bar* and *nptII* genes driven by 35S promotor were introduced into *Agrobacterium* stains EHA105, GV3101, KYRT1 and LBA4404. Cotyledonary node of soybean with 1/3 of cotyledon attached was found more sensitive to glufosinate selection than the node with whole cotyledon attached; therefore was used as target explants in the transformation protocol. After *Agrobacterium* co-cultivation, explants were selected on the medium containing glufosinate for multiple shoot induction. Low concentration of plant growth regulator TDZ in the medium could efficiently induce multiple shoots, which were morphologically normal, and easy to be recovered into fertile plants. Glufosinate at 3.0 mg/l and up totally inhibited the formation of multiple-shoots from untransformed explants. The putative transformed shoots were further selected on glufosinate containing medium until plantlet was more than 2 cm in length. We have determined that glufosinate concentration of 1-1.2 mg/l was sufficient to select the transgenic shoots at this stage. Comparing the different *Agrobacterium* strains, KYRT1 produced more glufosinate resistant plantlets. Recovered plants were transplanted into soil, and screened with 0.3-ml/l solution of Liberty herbicide. Southern hybridization analysis of Liberty resistant plants confirmed the stable transformation.

B 12

Genetic Studies of Somatic Embryogenesis in Soybean and Effects of Light Intensities

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To investigate the genetics of somatic embryo formation and to determine the effects of light intensities on soybean somatic embryogenesis capability, several crosses between contrasting embryogenic (IAS-5 and Embrapa 1) and non-embryogenic (Parana) cultivars were performed to obtain F1 and F2 generations. Backcrosses (RC1P1 and RC1P2) were also performed. Immature cotyledons, 4-6 mm in length, derived from parental lines, F1, F2, RC1P1 and RC1P2 were grown in Petri dishes containing the inductive N10 medium, for 90 days, in a growth chamber. Somatic embryos derived from the induction were counted and the number used to obtain genetic parameters. The results showed that the somatic embryogenesis trait is of quantitative nature, controlled by approximately 10 genes. The distribution of somatic embryo formation in the F2 generations was normal, reinforcing the quantitative nature of the trait. Parental immature cotyledons cultivated in N10 medium and submitted to two light intensities (8-12 and 27-33 uEm-2s-1) showed that variations of light intensities in the growth chamber had no effects on the somatic embryo formation process.

B 13

Toward an Establishment of Soybean Transformation System Using Japanese Cultivars; Improvement in Regeneration Time and Efficiency

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Soybean [Glycine max L. Merrill.] is one of the most important crops in many countries. Many useful genes have been isolated, and capability of the seeds as a storage system of foreign gene product has been reported. Though there is a great demand for soybeans with resistance to insect attack, disease and other environmental stress that could not be solved by traditional breeding procedure, there's still no efficient transformation system adapted for routine laboratory work due to the low efficiency of gene introduction and selection for transformed tissues. Our goal is to establish the system using Japanese important cultivars.

As a first step, we tried to find cultivars that efficiently produce somatic embryos on immature seeds as materials for the Agrobacterium-mediated gene transfer. Various sizes of immature seeds from 15 cultivars (11 Japanese cultivars, Fayette, Harosoy, Peking and Picket) were subjected to the induction of somatic embryo under high 2,4-D concentration (40mg/l). Wasemidori and Akishirome showed higher efficiency of somatic embryogenesis than Fayatte, which is generally thought to be the most suitable cultivar for the induction of somatic embryo. Suzuyutaka, despite its average efficiency in somatic embryogenesis, appeared to be suitable for the experiment because of its complete elimination of interruptive wet callus formation during the auxin treatment and the high regeneration efficiency. Suzuyutaka is one of the most popular cultivars in Japan and has strong resistance to soybean mosaic virus and soybean cyst nematode. Difference in the efficiency between cultivars was 8 fold at maximum, and the efficiency was significantly dependent on the size of immature seeds.

We are now trying to introduce GUS or GFP genes in these cultivars using various procedures of Agrobacterium infection.

B 14

A Vector System for Creating and Transforming with Gene Artificial Clusters

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The breeding and development of soybean cultivars with multiple transgenes for stacked traits could be facilitated if all the transgenes were linked together as one segregating unit. Furthermore, transformation with multiple genes in a coordinated fashion can facilitate the engineering of metabolic pathways. Until now, placing multiple genes in one vector via standard restriction endonuclease digestion has been very difficult. To remedy this, a new vector system for the multiple integration of at least nine independent DNA constructs has been created. This system is based on a multiple cloning site containing a tandem array of homing endonuclease cleavage sites. Such homing endonuclease have DNA recognition sites which are between 10 and 19 bp long, making them extremely rare cutters. Two versions are available; pUGA for microprojectile-mediated transformation, and pPZP203 for *Agrobacterium*-mediated transformation. In addition, there is a series of unidirectional shuttle vectors. Each vector has a pair of homing endonuclease restriction sites which flank 34 unique 6 or 8-base cutter sites. At least one gene cassette can be cloned into each individual shuttle and transferred to either pUGA or pPZP203 to construct gene clusters for transformation into plants. We have transformed tobacco plants with a pPZP203-based binary vector containing six gene constructs, each containing a promoter, coding region, and terminator. PCR indicated the presence of the transgenes in the tobacco genome. Plants are currently flowering and seed from these lines will be analyzed for the presence of the transgenes.

B 18

Screening of Soybean Lines for Somatic Embryo Induction and Regeneration Capability from Immature Cotyledons.

E.S. Tomlin[1], S.R. Branch[1], C.N. Stewart[1], Jr., D. Chamberlain[1], H. Gabe[2], S.V. Evola[2], M.S. Wright[2].

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Introduction

Somatic embryos of soybean have proven amenable to transformation by microprojectile bombardment. Capacity for somatic embryogenesis and conversion into plants, however, is highly dependant on the breeding line used. Our goal was to screen a number of elite lines of soybean from different maturity groups to assess their capacity for somatic embryogenesis, and conversion into plants. Embryos were induced and proliferated on solid MS media containing 2,4-D, and differentiated, matured and germinated on solid MS media. Previously we reported that the breeding lines used differed both qualitatively and quantitatively in their capacity to produce immature embryos. In this paper, we report on maturation and conversion of these lines. Number of differentiated embryos/ cluster, embryo morphology, time taken to maturity and percent germination based on appearance of roots, shoots or both (conversion) were recorded. Measures of differentiation, maturation and conversion were correlated with earlier measures of immature embryo quantity and quality. The 18 breeding lines differed with respect to quantity and morphology of differentiated embryos produced, time to maturation and percent germination and conversion. Quality of immature embryos, based on shape and color, was positively correlated with the number of differentiated embryos produced by immature clusters, but not with the morphology of differentiated embryos or germination frequency. Also, rapid maturation time was positively linked to germination and conversion frequency. There was no relationship between immature embryo quality or quantity and percent germination or conversion. In fact, two of the most embryogenic lines, Jack and MOO4131, had the lowest levels of germination. These data suggest that for purposes of transformation, there is a tradeoff between high production of embryos for bombardment and subsequent recovery of plants for many breeding of these breeding lines.

B 19

Using GFP as an Early Indicator of Soybean Transformation: GFP as a Reporter Gene Displays Variability in Expression During the Early Stages of Soybean Transformation.

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Green fluorescent protein (GFP) from jellyfish (Aequorea victoria) is becoming a popular reporter gene in many plant transformation laboratories. The GFP reporter system allows direct viewing of gene expression in living tissues and does not require destructive staining procedures. GFP therefore permits early monitoring of transformation events and the dynamics of gene expression can be easily followed over time. In some transformation systems, GFP expression has been used as the only "selective agent", resulting in the recovery of clones without the use of antibiotics or herbicides. In this study, an mgfp5-ER gene was introduced into embryogenic soybean tissue maintained on semi-solid media using a technique called SAAT or sonication-assisted Agrobacterium-mediated transformation. This technique utilizes sonication to create micro-wounds on the surface and deep within the plant tissue, creating an entry point for the bacterium. SAAT-treated, embryogenic tissue was monitored for transient, chimeric, and stable GFP expression. Unexpectedly, GFP expression could not be followed from a single transformed cell, but seemed to arise only after a critical mass of tissue was obtained. GFP expression was initially observed 4 days following SAAT (transient expression), disappeared in all cases after 2 weeks, and then reappeared in selected clones 3-5 months later. The use of GFP as a reporter gene in embryogenic tissue of soybean may not be ideal for selecting early transformation events, but may prove useful for monitoring gene expression dynamics.

B 20

Use of Isoxaflutole as Selectable Marker for Soybean Transformation.

Christelle Muhr, Frédéric Garçon, Stéphanie Betka, Sophie Le-Roux, Bernard Lestrade, Luc Portelatine, Alain Sailland and Bernard Pélissier.

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Isoxaflutole, (*5-cyclopropyl isoxazol-4-yl-2-mesyl-4-trifluoromethylphenyl ketone*) the active ingredient of Balance™, has been evaluated as selectable marker for engineering soybean. The compound inhibits 4 hydroxyphenyl pyruvate dioxygenase (HPPD), an enzyme involved in tyrosine degradation and plastoquinone biosynthesis (1). A mutated HPPD gene isolated from *Pseudomonas fluorescens* has been transferred into Arabidopsis and tobacco conferring tolerance to isoxaflutole (2). Investigations to evaluate isoxaflutole as selectable marker for soybean transformation have then been conducted using "FNL"soybean somatic embryogenic tissues. Tissues were bombarded with the HPPD gene and after two rounds of selection, green spots among white tissues were easily detected using a binocular microscope. As soon as green tissues were big enough they were amplified in liquid medium before entering the stages of regeneration towards a plant. Molecular analysis confirmed stable integration of the HPPD. This new selectable marker allows us to regenerate routinely 40-80 primary events/person/year. The potential of isoxaflutole as a new selectable marker, the quality of different embryogenic tissues and pre-treatment of tissues prior bombardment are presented and discussed in respect to hygromycine.

(1) K.E. Pallett, J.P. Little, M. Sheekey and P. Veerasekaran. The mode of action of isoxaflutole. Pesticide Biochemistry and Physiology 62, 113-124 (1998).
(2) WO 99/24585. Mutated HPPD, DNA sequence and method for obtaining herbicide tolerant plants containing such gene.

B 21

Transformation and Characterization of Transgenic Soybean Expressing the Methionine Rich 15kDa Zein Storage Protein Gene from Maize.

M.S. Srinivasa Reddy, Michael Hayes, Curtis Meurer, Randy Dinkins, and Glenn B. Collins

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Three fertile transgenic lines of soybean [*Glycine max*.(L) Merr.] cv. 'Jack' were recovered using *Agrobacterium*-mediated transformation with a phaseolin:15 KD maize zein storage protein gene construct. Southern blot hybridizations confirmed the integration of the zein gene in the three transgenic plants recovered. However all three plants displayed the same T-DNA integration pattern suggesting that they originated from the same transgenic event. Analysis of forty-eight progeny from one T_0 plant revealed that three copies of the transgene were integrated and that these were inherited as a single Mendelian locus. RNA and immunoblot analyses indicated that the accumulation of zein was developmentally and tissue specifically regulated. RNA blot analyses of leaf, root, stem and seed showed that expression of the maize zein gene was mostly confined to the seeds. RNA analysis also revealed higher expression of zein in cotyledons compared to embryos, pods and the seed coat. Immunoblot analysis of cotyledons from different developmental stages showed slightly higher accumulation of zein in young embryos compared to the mature embryos. Amino acid analysis of the homozygous T_2 seed revealed an increase in the sulfur containing amino acids.

B 22

Selection of Transgenic Somatic Embryos.

Suryadevara Rao and David Hildebrand

Department of Agronomy
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The lack of effective selective agents is one of the major bottlenecks in the identification of transformed somatic embryos/ plant tissues. This renders the selection process inefficient and tedious and effectively curtails the pace of genetic transformation. Several escapes could be easily detected owing to the inefficient selection process.

One of the commonly used selection markers, Kanamycin monosulfate is known to be ineffective for use in soybean transformation even at high (300 mg/L) concentrations. In the present study two selective agents, Basta® (phosphinothricin), a known herbicide and Hygromycin were employed individually and in combination to assess their efficacy in the selection of transformed somatic embryos. At 80 mg/L Hygromycin or 40 mg/L Basta complete tissue death was observed. Our data suggests 30 mg/L Basta or 50 mg/L Hygromycin could be used as effective concentrations for selection of transformed somatic embryos. Alternatively, a combination of 25 mg/L Hygromycin and 15 mg/L Basta would also serve the same purpose. Soybean somatic embryos have also been transformed using green fluorescent protein (GFP) as a reporter gene. GFP offers the convenience of visual identification of the transformed embryos (non-destructive assay) even at an early stages of transformation. GFP expression was seen one hour after bombardment and could be detected up to one week in most initial experiments. Efforts to obtain stable transformants are under way.

SOY 2000

8th Biennial Conference
of the Cellular and Molecular
Biology of the Soybean

PLENARY SESSION II

Soy Diet and Nutrition

James Anderson, Presiding

8:30 am
Tuesday, August 15
Patterson Ballroom A/B/C

Poster Session 5:00–6:30 pm
Patterson Ballroom D

PII 01

Health Benefits of Soyfoods

James W. Anderson, MD

VA Medical Center and University of Kentucky, Lexington, KY

In the past five years the interest in soyfoods among consumers has grown exponentially. Nutrition, basic and clinical research has expanded dramatically. Soyfoods and their isoflavones appear to have clear protective effects related to CHD and probable protective and therapeutic effects related to osteoporosis. The effects on the kidneys are clear and the protective effects are under study. While the greatest interest may relate to chemopreventive effects related to cancer, much more research is required. The effects of soyfoods on cognitive function are unclear and also require further research. The use for menopausal symptoms appear promising and postmenopausal women who cannot or choose not to take HRT may be ideal candidates for daily soyfood use. Soy foods contain protein and isoflavone components that have specific effects on reducing risk for CHD. The isoflavones or soy estrogens contribute an estimated three-fourths of the protective effect while the soy protein may be responsible for the remaining one-quarter of the protection. Soy protein and its isoflavones exert at least five anti-atherogenic effects. These effects are itemized and will be reviewed in greater detail. 1 Soy foods have favorable effects on all the blood lipid levels. 2. Soy protein and its isoflavones are important antioxidants that prevent oxidation of LDL. 3. Soy isoflavones have anti-inflammatory effects. 4. Soy foods decrease tendency to form blood clots or thromboses. 5. Soy isoflavones have health promoting effects on blood vessels

PII 02

A Path Towards Increasing Isoflavone Levels in Soybean

Brian McGonigle,

Dupont Agricultural Products, Wilmington DE

Isoflavones are of significant interest due to their potential for promoting human health. We have cloned the gene encoding the enzyme responsible for the initial step of isoflavone biosynthesis isoflavone synthase (IFS) using an EST expression strategy. Using RT-PCR we have cloned IFS from eight other legume species as well as the non-legume sugarbeet. Expression of soybean IFS in Arabidopsis results in the accumulation of genistein in Arabidopsis leaves. We have also expressed soybean IFS in tobacco plants and BMS cells and accumulation of genistein in these non-legume plants is related to the level of the endogenous phenylpropanoid pathway. Isoflavone accumulation in soybean seeds is variable depending on both the cultivars of soybean and the environmental conditions under which they are grown. We have used stable gene expression in somatic embryos as a model system for transgenic soybean seed development. We expressed IFS in the somatic embryo system and detected isoflavone levels approximately two times higher than in wild type embryos. We have gone on to introduce an IFS coding region regulated by a seed storage gene promoter in soybean plants.

PII 03

Communicating with Consumers the Heath Benefits of Soy

Julie Tockman
Protein Technologies International, Inc. St. Louis, MO 63188

PII 04

Soy Protein Protective Effects for the Kidney

Tammy J. Hanna, Paolo Fanti, James W. Anderson
VA Medical Center and University of Kentucky, Lexington, KY

Diet plays an important role in the prevention and treatment of renal disease. The pivotal role of dietary protein in renal health has been recognized for more than 150 years. Most recently research has found that not only protein restriction, but also modifications in the type of protein consumed, has a favorable effect on renal health. Soy protein may be superior to animal protein for kidney health.

Soy and Diabetic Nephropathy

Nearly one-third of the eighteen million Americans with diabetes mellitus develop renal disease. Diabetic nephropathy is a major contributor to death in those with diabetes, primarily from end-stage renal disease and cardiovascular disease. The soy protein hypothesis states that substitution of soy protein in individuals with diabetes results in less hyperfiltration and glomerular hypertension with resultant protection from diabetic nephropathy. Two short-term and two long-term clinical trials have specifically examined the effects of soy protein intake, compared with animal protein, on proteinuria and the progression of renal disease in Type 1 and Type 2 diabetes patients. From these preliminary reports it is evident that soy protein has beneficial effects on diabetic nephropathy characterized by proteinuria. The mechanisms by which soy protein exerts renal protective effects have yet to be elucidated. However, from preliminary in vitro and in vivo evidence we postulate that the unique amino acid composition, isoflavone and lipid-lowering and antioxidant properties of soy are all important contributors.

Soy and End-Stage Renal Disease

Chronic renal failure is associated with impressively high morbidity and 24% first-year mortality in the United States. Disorders of the immuno-inflammatory system in this population may play an important part in the poor outcome of these patients. It has been postulated that genistein and daidzein, the isoflavones particularly concentrated in soyfoods, may offer benefits in end-stage renal disease. Specifically, the isoflavones may antagonize the immuno-inflammatory system.

PII 06

Nutritional Value of Soybean Meal from Genetically Enhanced, Low Oligosaccharide, Low Phytic Acid Soybeans for Pigs and Chicks.

G.L. Cromwell, E.G. Xavier, L.W.O. Souza, S.L. Traylor, L.A. White, M.D. Lindemann, T.E. Sauber, H.L. Stilborn, and D.W. Rice; University of Kentucky, Lexington, and DuPont Speciality Grains, Des Moines, IA.

Soybeans with reduced oligosaccharides and phytate phosphorus have been developed. Lowering the phytate content of oilseeds should be beneficial to pigs and poultry because they lack intestinal phytase and are unable to degrade phytate. Feeding studies with growing pigs and broiler chicks were conducted to assess low phytate, low oligosaccharide soybean meal (LP-SBM) and a near-isogenenic, normal soybean meal (N-SBM). The total, phytate, and non-phytate phosphorus, and the total oligosaccharides in the LP-SBM and N-SBM were (%): .77, .22, .55, 1.08 vs .70, .48, .22, 6.28. Slope-ratio analysis of bone traits indicated that considerably more of the phosphorus in LP-SBM was bioavailable to pigs (49 vs 19%) and chicks (58 vs 28%) than the phosphorus in N-SBM. Additional studies compared diets with normal and low-phytate corn (this corn contained the *lpa-1* mutant gene and possessed less phytate phosphorus than its near-isogenic counterpart; .10 vs .20%) supplemented with N-SBM and LP-SBM. Optimal growth and bone mineralization were achieved with less dietary supplemental inorganic P when LP-corn and LP-SBM diets, compared with N-corn and N-SBM diets, were fed. Also, pigs and chicks fed LP-corn and LP-SBM diets excreted 50% less P than those fed conventional corn and SBM, an important aspect from an environmental standpoint.

PII 08

Operation of the Oxylipin Pathway

David Hildebrand

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Oxylipins are a diverse group of oxygenated fatty derivatives that includes products of lipoxygenase. Lipoxygenase is a ubiquitous enzyme of plants and animals that catalyzes the peroxidation of polyunsaturated fatty acids. Soybean seeds appear to be the most abundant natural source of this enzyme. The high lipoxygenase activity of soybean seeds has been a major impediment to utilization of soybean protein in foods as products of lipoxygenase and hydroperoxide lyase such as hexanal give many food products containing soy protein an undesirable flavor and aroma. Soybean seeds normally contain three lipoxygenase isozymes designated LOX 1, 2 and 3. Breeders have succeeded in making single, double and triple nulls for soybean LOX. LOX2 is the most active isozyme in hexanal formation and LOX3 can actually reduce hexanal levels in some soybean protein products at least in part by catalyzing formation of linoleic acid hydroperoxide isomers that do not lead to hexanal formation. Allene oxide synthase also competes with hydroperoxide lyase for hexanal formation and over expression of allene oxide synthase leads to a reduction of this compound. The LOX/hydroperoxide lyase catalyzed formation of hexanal usually requires free linoleic acid to start the process. Minimizing free fatty acid formation in handling and processing soybean seeds can reduce hexanal formation and improve the quality of soybean protein products. Large reductions in total linoleic acid levels can also suppress the problem of hexanal formation. Thus high oleic acid soybeans not only have improved oil quality, they have improved quality of many protein products as well. Products of LOX/hydroperoxide lyase from linolenic acid (18:3) such as E-2-hexenal and Z-3-hexenol, in stark contrast to the linoleic acid (18:2) product, hexenal, are desirable flavor and aroma components of many foods and beverages, although not in the case of many soy protein products, imparting a fresh, fruity, "green" aroma. Use of soybean genotypes with the appropriate LOX isozyme constitution together with high hydroperoxide lyase activity of the correct stereochemical specificity and a source of high linolenic acid can lead to commercial production of E-2-hexenal and Z-3-hexenol in a bioreactor system.

PII 09

Genetic Analysis of Phytoestrogen Content in Soybeans.

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Departments of Plant Soil and General Agriculture1; Animal Science, Food and Nutrition2, Physiology3
Southern Illinois University, Carbondale, IL 62901

The abundance of phytoestrogens in soybean seed can vary up to five fold. Phytoestrogen content and profile can vary by year, soybean geneotype and location. The objective of this study was to determine the proportion of this variation that could be explained by genetic inheritance and to identify genes underlying the inheritance of phytoestrogen content and profile. We have analyzed samples from within a recombinant inbred line population derived from two popular soybean cultivars, 'Essex' and 'Forrest'. The cultivars contrast for disease resistance, resistance to abiotic stress and phytoestrogen content and so are suitable for inheritance studies for phytoestrogen profile and amount. We found a consistent differences between Essex and Forrest across years and environments whereby Forrest accumulated more total phytoestrogen, daidzein and genistein, but Essex accumulated more glycitin. The recombinant inbred lines showed transgressive segregation for total phytoestrogen, daidzein, genistein and glycitin content. Broad sense heritability estimates indicated that 22-79% of this variability was genetic. By DNA marker mapping and map integration the location of genes involved in the enzymatic synthesis of phytoestrogens from phenylalanine were compared with inheritance data. We have shown that the 6 loci explain up to 10-50% of the variability of total daidzein, genistein and glycitin content. The genomic regions that control overall content are additive and can be used to manipulate phytoestrogen content and profile. Individual soybean phytoestrogens can effect a number of physiological events in mammals. Using DNA markers in breeding selection we can reduce detrimental phytoestrogens and enhance beneficial phytoestrogens. This research was supported by the Illinois Council on Food and Agricultural Research and the Illinois Soybean Program Board.

PII 10

High Isoflavone Soy Protein Alters Genes Involved in Glucose and Lipid Metabolism in Obese Rats.

W Banz, M Iqbal, R Steger, M Roeder, M Peluso, C Stiglitz, B Kinney, T Winters, M Shanahan, D Lightfoot

Southern Illinois University, Carbondale, IL 62901

Previous data from these researchers demonstrated a marked effect of high- and low-isoflavone soy protein diets on platelet, lipid, and liver parameters in male lean and obese rats. This study was designed to further investigate genes involved in the effect of soy diets on glucose tolerance and liver parameters in female obese rats. Female obese Zucker rats were assigned to one of three diet groups: High-Isoflavone Soy Protein (HS); Low-Isoflavone Soy Protein (LS); or Non-Soy (Casein) Protein (C) diets. During the 10-week study, body weight, feed intake and feed efficiency ratio (FER) were assessed. Additionally, a Glucose Tolerance Test was performed and plasma, abdominal fat pads, liver and reproductive organs were collected, weighed and frozen for subsequent analysis. The HS diet attenuated ($P<.025$) fatty liver as compared to LS and C diets. The HS diet also improved ($P<.005$) glucose tolerance relative to the LS and C diets. Differential display analysis of 2,500 rat liver mRNAs revealed a modulation (5% responded) of key genes that were protein- and/or isoflavone-enriched fraction-specific. Genes involved in metabolism, the regulation of gene expression and oncogenes were identified. In conclusion, a diet rich in high-isoflavone soy protein provided protective benefits regarding development of fatty liver and glucose intolerance in female obese rats and key genes are modulated in this process. *This project was supported by the Illinois Council on Food and Agricultural Research and Protein Technologies International.*

SOY 2000
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CONCURRENT SESSION C

Metabolic Engineering and Value Added Traits

Sean Coughlan and Jihong Liang, Presiding

1:15 pm
Tuesday, August 15
Patterson Ballroom A/B

Poster Session 5:00 – 6:30 pm
Patterson Ballroom D

C 01

Modified Fatty Acid composition in Transgenic Soy Seed.

Sean. J. Coughlan, Anthony Kinney, Edgar Cahoon & William D. Hitz.

Dupont Agricultural Products, Wilmington DE 19880

The combination of an efficient soy transformation system and cloning virtually all of the enzymes involved in fatty acid biosynthesis has enabled us to create an array of transgenic soy plants with modified fatty acid composition. We are thus in a position to commercialize plants with low (<5%) total saturated fat, and high (>80%) oleic acid. Regulatory hurdles to commercialization (antibiotic selection, complexity of transgenic insertions) will be discussed. We have also modified the co-product composition of transgenic soy oil, in particular the tocopherol (vitamin E) and isoflavone content to add value to the seed.

Finally, we have taken advantage of the in house sequencing capability of DuPont to clone out a variety of diverged Fad 2 cDNAs (hydroxylases, epoxidases, acetylenases) as part of our industrial oil programme. For example, overexpression of the *Vernonia galamensis* epoxidase in transgenic soy seed accumulates a maximum of about 5% vernolic acid. Possible metabolic reasons for this shortfall in comparison to the wild type Vernonia seed (70% vernolate) will be discussed.

C 02

Discovering and Delivering the Best Genes to the Feed and Processing Customers

Jihong Liang

Renessen, Mystic CT

Renessen is a freestanding joint venture between Monsanto and Cargill, which marries biotechnology innovations with processing know-how. We are the first global alliance to provide the value of biotechnology across the entire food chain. In collaboration with our agribusiness partners, Renessen will develop quality traits and customized products to improve and increase the functionality of the world's grains, feed, processing and food. We will develop, commercialize and market grains, oilseeds and other crops that have been improved through biotechnology for the feed, grain processing and food industries. Biotechnology is transforming the way that crops are grown, traded and used to produce food and feed. Renessen's goal is to increase value and choice for farmers, livestock and poultry feeders, grain processors and consumers. To achieve this, we will leverage the strengths of our parent companies by drawing upon Monsanto's global capabilities in biotechnology, crop seed production and farming practices and by drawing upon Cargill's global capabilities in the areas of agricultural inputs, handling processing risk management, logistics and animal nutrition qualities of major crops. The combination will rapidly move biotechnology's benefits from the laboratory to the end user.

C 03

Arginine Movement in Developing and Germinating Seeds: Insights From a Urease-negative Mutant

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Arginine (Arg) is the major Nitrogen source for germinating soybean seeds and it is one the predominant aminoacids in angiosperm seed protein. Therefore control of Arg synthesis is important to seed protein yield and control of Arg breakdown is important to mobilization of seed N during germination. In order to be utilized arginine is degraded via arginase to ornithine and urea. Ornithine is a precursor of proline, and urea is degraded into ammonia by urease reaction. Arginase transcripts accumulate in germinating soybean cotyledons and its activity is coincident with in-vivo accumulation of Arg-derived urea in the urease-negative soybean mutant eu3-e1/eu3e1. Conversely, no urea accumulation was detected in developing urease-negative embryos, indicating that arginase is inoperative in vivo, in spite of the presence of a large pool of free Arg. It is known that plant arginases are mitochondrial. Thus, during germination, Arg uptake into the mitochondrion appears to be important for channeling its N and C skeletons into other compounds through arginase activity. On the other hand, in developing embryos, blocking Arg uptake into the mitochondria or Arg degradation is important to avoid a futile cycle resulting from simultaneous Arg synthesis and degradation by arginase and urease. Our working hypothesis is that mitochondrial membrane is a barrier to arginine uptake during embryo development. Uptake experiments with isolated mitochondria from pre and post-germination embryos yield similar uptake rates. However, captured Arg was converted to urea in post but not in pre germination embryos. Although these results contradict our working hypothesis, study of transporters is important because there may be in vivo controls that we do not yet understand, because arginine has other fates (polyamines, NO, etc.) and transport into other organelles (chloroplasts for polyamines and peroxisome likely for NO) will have a large effect on its fate. Experiments are currently in progress to clone the soybean mitochondrial arginine transporter to study differential expression of transcripts in pre and post germination embryos as well as in different organelles. We will also characterize the transporter by using heterologous systems to express the protein.

C 05

Down Regulation of the Soybean Embryo Specific Fatty Acid Desaturase FAD2-1.

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Abstract: Down regulation of endogenous gene expression in soybean is useful to ascertain gene function, or to manipulate metabolism to generate novel plant phenotypes. Two common strategies to down-regulate gene expression in plants are RNA antisense and co-suppression. A third approach, retention of transcripts in the nucleus, has not been tested in plant systems but has been demonstrated in animal systems as an effective way to specifically down-regulate gene expression. Blocking export of mRNA transcripts out of the nucleus can be achieved by replacing the 3' termination signal with a self-cleaving ribozyme in animal cells. We assembled a series of plant expression cassettes designed to specifically down-regulate the embryo-specific, omega fatty acid desaturase, FAD2-1, or co-down-regulate the fatB thioesterase and FAD2-1. The objective of the study was to compare the efficiency of RNA antisense, co-suppression and nuclear-targeted strategies in soybeans. The FAD2-1 open reading frame (ORF) was subcloned between the ß-phaseolin promoter and the CaMV 35s termination signal (T35s) in plus and antisense orientation. A third cassette carried the FAD2-1 ORF in antisense orientation between the ß-phaseolin promoter and a self-cleaving ribozyme (RZ). Two additional expression cassettes under the control of the ß-conglycinin promoter harbored fatB and FAD2-1 ORFs in plus sense orientation separated by the RZ, with the fatB ORF proximal to the promoter element. These dual ORF cassettes carried either the T35s terminator or RZ at the 3'end. The five expression cassettes were joined to a binary vector that carried a bar cassette for plant selection. The resultant vectors are referred to as pPTN166 (ß-phaseolin-anti-FAD2-1-RZ), pPTN167 (ß-phaseolin-FAD2-1-T35s), pPTN170 (ß-phaseolin-anti-FAD2-1-T35s), pPTN300 (ß-conglycinin-fatB-RZ-FAD2-1-T35s) and pPTN303 (ß-conglycinin-fatB-RZ-FAD2-1-RZ). The binary vectors were mobilized into Agrobacterium tumefaciens strain EHA101 and subsequently used for transformation. Six to 15 independent soybean events were derived from each binary vector and integration was confirmed by Southern blot analysis. The fatty acid composition of soybean seed from the respective transformants was ascertained by gas chromatography. We identified soybean lines with oleic acid levels in the seed ranging from 45%to 75% in the pPTN166 transformants, 55% to 85% in the pPNT170 transformants, up to 90% in the pPTN300 transformants, and up to 89% in the pPTN303 transformants. None of the pPTN167 events characterized to date displayed elevated oleic acid levels.

C 06

Enhanced Phosphorus Utilization by Development of Low Phytate Soybeans

Elizabeth A. Grabau, Dept. of Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, VA 24061

Elevated soil phosphorus levels can result from the application of animal manure as fertilizer in areas of intensive livestock production. Phosphorus in manure originates from phytate (myo-inositol hexakisphosphate), the storage form of phosphorus found in plant seeds. Phytate is inefficiently utilized by monogastric animals and can lead to enviromental phosphorus pollution of critical watersheds through run-off. The dietary inavailability of phytate phosphorus necessitates supplementation of animal feed with inorganic phosphate or phytase to meet animal growth requirements. Reduction of seed phytate levels should improve phosphorus availability, overcome the additional antinutritional effects of phytate, and provide better nutrient management strategies. We are using two approaches to to obtain reduced soybean phytate levels, including the alteration of phytate biosynthetic and degradative pathways. We have isolated cDNAs for myo-inositol 1-phosphate synthase (MIPS), a key step in the de novo synthesis of myo-inositol, and have examined MIPS expression patterns in soybean. We have also cloned the gene for a soybean phytase from germinating cotyledons as a candidate transgene for future attempts to lower phytate levels in developing seeds.

C 07

Regulation of UDP-Glucose Dehydrogenase in Developing Soybean Seeds.

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Soybean seeds contain more cell wall fiber than other dry beans. Reducing the amount of fiber could increase the economic value of soybean seeds. UDP-glucose dehydrogenase (UDP-Glc DH) activity was measured to determine if this enzyme regulates non-cellulose cell wall polysaccharide synthesis in developing seeds. Soluble proteins were extracted from seeds harvested at five-day intervals during the linear growth period of seed development. UDP-Glc DH activity was measured spectrophotometrically by reduction of NAD+. A UDP-Glc DH cDNA was cloned and used to examine gene number and mRNA expression over the same period of development. UDP-Glc DH enzyme activity and mRNA levels changed significantly during seed development. Enzyme activity increased rapidly between 20 and 30 days after flowering (DAF), and remained high from 30 to 35 DAF. UDP-Glc DH activity was 45-fold higher in mid-maturation phase seeds than in rapidly expanding leaves. Enzyme activity did not directly! correlate with mRNA level. These observations suggest that UDP-Glc DH is important in cell wall polysaccharide synthesis in soybean seeds.

C 08

Federal and State Initiatives in the US to Promote Biobased Industrial Lubricants from Commodity and Genetically Modified Seed Oils

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A sequence of executive orders issued by President Clinton form a basis for expanding commercial availability of crop-based industrial lubricants in the USA. EO 12873, issued in 1993, requires EPA to "issue guidance" relating to federal acquisition, recycling and waste prevention; it established definition for "environmentally preferable" products.

The second EO 13101 (1998) is an extension of EO 12873; it calls for preferential federal purchasing that requires procurement officials to justify using products that are not considered "biobased: and listed as such in the Federal Register. The third EO 13134, (1999) established joint councils of the US departments of Energy and Agriculture and calls for a national policy for implementing biobased purchasing.

These actions provide early-market support that is intended to expand availability and symbolize future expectations of industry. Providing introductory markets for these products establishes incentive for businesses to develop technologies and processing facilities necessary to supply to commercial markets as well. Because "Lubricants" are one of the primary targets of EO 13101, the sequence of orders provides a "staging area" in which the specific features and competencies of soybean based industrial lubricants can be publicly demonstrated and proven.

It also provides indirectly subsidized capital investment necessary to reduce production costs and "alerts" industry of the long-term importance placed on environmental issues by the current administration.

Government initiatives combined with advances in the technology of biobased lubricants are expected to expand the market for such products. This presentation will focus on the key issues and opportunities in the USA.

To date, growth in biobased lubricants in the US has been relatively slow compared to that displayed in Europe. This shows signs of changing however, as the US government expands its incentives for producers to develop technologies and capacities for supplying biobased alternatives. Advances in the genetic industry too have resulted in better performance and more economical products.

C 12

Stokesia Desaturase/Epoxygenase-Like Genes for Oil Improvement.

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Plants are known to accumulate a wide diversity of unusual fatty acids, some of which have industrial use. Epoxy fatty acids, such as vernolic acid, are an example of an uncommon fatty acid that accumulates in triacylglycerol of a few plant species that is valuable for various industrial uses including as plasticizers. *Stokesia laevis* seed oil is made up of about 60 – 70% vernolic acid (12,13-epoxy-9-octadecenoic acid). cDNAs encoding epoxygenases from *Crepis palaestina* (Lee. et al., 1998) and *Vernonia galamensis* (Hitz, 1998) have been cloned and found to be members of a growing family of Δ12 fatty acid desaturase-like analogs that also includes hydroxylases, acetylenases and conjugases. Degenerate primers were designed on the basis of conserved sequences of these Δ12 desaturase-like genes, and an apparent full-length epoxygenase gene from *S. laevis* was isolated using RT-PCR and RACE strategies. The cDNA is 1.4 kb, the ORF 1134 bp and it encodes 378 amino acids. The similarities of this gene with epoxygenase of *Vernonia* and *Crepis*, Δ12 desaturase of soybean, FAD 2-1 and FAD 2-2 are 84.3%, 69.4%. 50.4% and 56.2%, respectively. The function of this gene was tested in the yeast expression system first, however no epoxygenase or Δ12 desaturase activity was detected as is the case with other known plant epoxygenase genes. Secondly, the gene was cloned into a TDNA-vector, pCAMBIA 1201. In this construct, the gene is driven by a seed specific phaseolin promoter. The recombinant plasmid was transformed into soybean somatic embryos by a particle delivery system and Arabidopsis thaliana plants by *Agrobacterium tumefaciens*. The lipid compositions of the embryos are being analyzed to test the effect of the *Stokesia* gene on epoxy fatty acid production and accumulation in the oils of these seeds.

C 13

Engineering Soybeans for the Production of Edible Vaccines for Poultry.

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The poultry industry is a significant component of world agriculture. The success of the poultry industry depends on the ability to maintain healthy birds. The current industry approach to poultry disease management is to immunize the birds using live vaccines, which leads to mild disease symptoms during which productivity is lost. The use of edible vaccines for the poultry industry should result in significant cost savings by greatly simplifying the current vaccination strategies and avoiding the practice of immunization via live vaccines. The soybean seed is the site of endogenous protein accumulation as well as the main component of poultry feed. The use of soybean for the production of edible vaccines could allow for much higher levels of expression than that obtained with typical model systems. We have transformed soybean via particle bombardment with DNA from the infectious bronchitis virus (IBV) S1 glycoprotein gene, the avian influenza (AI) HA5 gene and the Newcastle disease virus (NDV) P and F genes. Two different tissue-specific promoters, phaseolin or soybean lectin, were employed to direct viral antigen gene expression to the seed. Forty-seven transgenic lines have been identified. PCR and Southern blotting confirmed the presence of the viral antigens in the soybean genome. Plants which show protein and mRNA expression will be used to test the immune response of the plant-synthesized proteins.

SOY 2000
8th Biennial Conference
of the Cellular and Molecular
Biology of the Soybean

CONCURRENT SESSION D

Molecular and Cellular Pathology

Nevin Young, Presiding

1:15 pm
Tuesday, August 15
Patterson Ballroom C

Poster Session 5:00 – 6:30 pm
Patterson Ballroom D

D 01

Resistance Genes of the Coiled-Coil NBS-LRR Gene Family in Soybean

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Most plant resistance genes are members of an ancient gene family that encodes nucleotide binding site, leucine rich repeat (NBS-LRR) proteins. This family can be split into two groups, those with N-terminal homology to Toll and IL-1R of animals (TIR), and second exhibiting a coiled-coil (CC) motif. CCs are bundles of two to five helices with a distinctive packing of amino acid side chains at the helix-helix interface, of which leucine zippers are one example. Soybean has hundreds of copies of both types of NBS-LRRs. We have amplified CC-NBS-LRR sequences of soybean by degenerate primer PCR and examined their sequence diversity, phylogeny, genetic map location, and physical organization. There are at least four classes of soybean CC-NBS-LRR sequences, three that are closely related. Most soybean CC-NBS-LRR sequences examined to date reside in a series of small clusters on a 15 cM segment of linkage group F. This genomic region is known to contain many classically defined resistance genes. Physical analysis of these sequences places them on at least eight bacterial artificial chromosome (BAC) contigs. At least one of these CC-NBS-LRRs is expressed in seedling roots. A detailed comparison of phylogenetic and physical relationships indicates that most CC-NBS-LRR clusters are composed of closely related sequences. However, some clusters are mixtures with sequences from distinct phylogenetic branches. A parallel series of experiments with the model legume, Medicago truncatula, also uncovered many CC-NBS-LRRs. When used as hybridization probes against a soybean BAC library, these M. truncatula sequences uncovered dozens of additional copies not previously discovered. Genomic analysis of NBS-LRRs will provide candidates for resistance gene cloning, as well as insight into the evolution of plant gene families.

D 02

Lessons From The Protein, DNA And Locus Structure Of *rhg1*.

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Candidate gene sequences for *rhg1* and *Rhg4* have been isolated from soybean cv. 'Forrest' after a positional cloning project involving three laboratories over four years. From 512 primer combinations a total of 36,800 loci were screened and the frequency of polymorphism was 6.5% representing 2,396 polymorphic bands. Saturation mapping of rhg1 and Rhg4 with ten AFLP markers showed that AFLPs can effectively be targeted to any interval of the soybean genome by bulked segregant analysis. Failure of AFLP amplification from BAC DNA to be specific made it necessary to convert all of those markers to SCARS. Methods for AFLP band sequencing and sequence extension using BACs demonstrated that essentially all AFLP bands can be recovered as SCARs (25% is common in press). AFLP bands proved to be unusually rich in SNPs and insertions. Polymorphic AFLP bands contained 1.4 SNPs and 0.4 insertions per 100bp and allele frequencies ranged from 0.4-0.6 across soybean germplasm. Duplication of codominant AFLP alleles around *Rhg4* indicated that the gene derives from a insertion and duplication event involving *rhg1.* Saturation of the rhg1 interval with recombinants derived from 5,300 F2 NILs allowed the positioning of 50 recombinants within a 2 cM interval and seven recombinants in a 150 kbp interval containing *rhg1*. One class of recombinants was favored, that with Forrest alleles to the proximal side of rhg1, inferring meiotic drive operates in the region. Shotgun gene sequencing (105 kbp) cDNA hybridization and primer amplification identified eight genes in the interval containing rhg1. Hairy root transformation with all eight is ongoing. Three genes proved to be leucine rich repeat sequences separated by a membrane spanning domain with homology to Xa21 and Cf2 and are therfore *rhg1* gene candidates. The Forrest allele contains 76 leucines in 13 extra-cellular GXXP motifs and a kinase on the intracellular domain. PI88788 appears to separate the kinase to a second gene function (possibly rhg5). A gene closely homologous to the PI88788 rhg1 is found in the Forrest *Rhg4* interval. This gene duplication and the large number of LRRs implies the rhg genes function by dimerization, which can explain the temperature sensitivity of host- pathogen recognition. This research was supported by the United Soybean Board and the Illinois Soybean Program Board.

D 03

Signal Communication in the Rhizobium-Legume Symbiosis

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Our laboratory (in conjunction with a variety of collaborators, too many to mention above) has been using a variety of experimental approaches to analyze the regulatory signals exchanged between legumes (e.g., soybean, *Medicago truncatula*) and their rhizobial symbionts. One such signal is the lipo-chitin nodulation signal, produced by the bacterium, which induces nodule formation. Apyrases, nucleotide phosphohydrolases, have been proposed as a possible receptor for the Nod signal. Apyrases are encoded by a multigene family in legumes. A few of these genes are rapidly induced upon rhizobial inoculation. Interestingly, these inducible genes are clustered on the *M. truncatula* genome in a region that shows synteny with the soybean genome. Antibody directed against a membrane bound apyrase blocks nodulation. These data suggest that apyrases play an important role in nodulation. The work with lipo-chitin nodulation factors has led us to examine the general ability of soybean to respond to chitin, a well-known elicitor in other plants. We have identified an 85 kDa, plasma membrane protein that binds chitin at high affinity. The specificity of this binding correlates well with the ability of chitin to elicit plant defense responses (e.g., medium alkalinization, oxidative burst). In addition to these responses of the plant to bacterial signals, the plant also produces a variety of signals that affect bacterial gene expression. Recently, we have identified a novel, plant-produced signal that acts to repress nodulation. It is likely that this signal is important for down-regulating bacterial nodulation functions after infection. Recently, our efforts to analyze signal communication between host and symbiont have been aided by new genomic methods. Our most recent results using these technologies will be presented.

D 04

Developments of Phenylpropanoids in Soybean SDS Resistance

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Phenylpropanoid metabolism plays an important role in providing leguminous plants resistance to various pathogens. Our goal is to identify the alterations in phenolic metabolism that are critical for soybean resistance to sudden death syndrome (SDS), caused by the soil-borne fungal pathogen Fusarium solani P. sp. glycines (FSG). SDS has become a widespread and consistent problem worldwide. Some sources of resistance have been identified that do not show foliar symptoms but still have damaging root infection. Using soybean plant and hairy roots we study a) what changes occurs in phenolics in SDS infected root tissues, b) how these changes impart resistance to cultured roots and c) what genes are effective for SDS resistance in roots.

The development of SDS infection was monitored by sampling the roots for biochemical assays of soluble and wall bound phenolics. Simple pulse and pulse-chase radioactive labeling (from exogenous labeled phenylalanine - 14C-PA) of phenolic compounds was applied, which helped to detect the dynamics of phenolic metabolism alteration in soybean root tissues during pathogenesis. Considerable changes were found both in compounds of isoflavone and lignin branches of phenylpropanoid pathway. Considerable decrease in isoflavone concentrations was found in FSG infected roots (13d after treatment of plant roots and 4d after treatment of hairy roots) of both partially resistant (PI 567.374) and susceptible (Spencer) genotypes. The accumulation of isoflavones in hairy root culture medium was observed, the total level of isoflavones and glyceollin being much higher in infected samples especially of the partially resistant genotype. Activation of lignin synthesis as a response to FSG infection was found in resistant but not in susceptible plant roots. Degradation of lignin took place in the roots of both genotypes as infection developed which resulted in a decrease in the lignin level. These results indicate that FSG is capable of degrading lignin and that lignin may be important in the resistance response. Genetic manipulation of phenolic metabolism that are effective in providing resistance to SDS will be discussed.

D 05

Identification of Multiple Phytophthora Resistance Genes and NBS-LRR-like Sequences at the *Rps1*-k region.

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Phytophthora root and stem rot is the second most important disease of soybean. It is caused by *Phytophthora sojae*. A series of *Rps* genes provide soybean with resistance to *P. sojae* races. We are interested in understanding the recognition process involved in the expression of race-specific resistance conferred by *Rps* genes. We have mapped the *Rps1* locus that carries six functional *Rps1* alleles. We have screened a large segregating population in mapping one of these alleles *Rps1*-k (Kasuga et al. 1997). This gene has been shown to be highly stable and has been successfully used in most soybean lines grown in the north-central United States for nearly two decades. We have used recombinants in studying the complexity of this gene. *P. sojae* isolate 3-3 is virulent to hypocotyls but avirulent to roots of *Rps1*-k lines, whereas race 1 is avirulent to both roots and hypocotyls of lines carrying *Rps1*-k. This observation and analysis of recombinant lines indicate that most likely two functional *Rps* genes are physically linked at this locus. In addition to these two genes a third minor *Rps* gene, showing only root tolerance against this pathogen, was mapped ~0.2 cM from *Rps1*-k. These data indicate that *Rps1*-k is comprised of multiple functional homologues and these genes together provide soybean with stable resistances against this root pathogen. We are also close to cloning *Rps1*-k and its functional homologue that mapped in between two AFLP markers TC1 and CG1 (Kasuga et al. 1997). Screening of several BAC libraries indicated that the *Rps1* region is highly under represented in BAC libraries. We have constructed two BAC contigs of ~400 kb that span 0.7 cM genomic region at the *Rps1* locus. Sequencing of a BAC clone next to *Rps1*-k revealed a disease resistance gene like sequence (LRR). This sequence is highly repetitive and polymorphic in the soybean genome. Using this LRR fragment we have constructed a contig of three overlapping BAC clones at the *Rps1* region. These three BAC clones carry two copies of the LRR-like sequences. Sequence analysis of all three candidate copies indicates that *Rps1*-k could be a member of the NBS-LRR type of disease resistance gene. Current experiments include complementation analysis.

D 07

Expression of Bean Pod Mottle Virus (BPMV) Coat Protein Precursor Results in Resistance to BPMV in Transgenic Soybeans

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Somatic embryos of Jack, a cultivar of *Glycine max* (L.) Merr. were transformed with the gene for bean pod mottle virus (BPMV) coat protein precursor (pCP) by particle bombardment. The binary vector pHIG/BPMV-pCP used in these experiments contained the BPMV-pCP, intron-GUS and hygromycin resistance genes. Twenty independent transgenic soybean lines resistant to hygromycin were screened for the BPMV-pCP gene by Southern blot hybridization. Seven of the transgenic soybean lines obtained initially were studied in detail. Five of these lines (137, 139, 157, 183 and 186) had three copies of the T-DNA and were derived from the same transgenic event while the other two lines (200 and 407) had one copy of the T-DNA. Transgenic line 200 contained the intron-GUS and hygromycin resistance genes but lacked the BPMV-pCP gene and thus served as a negative control. Immunoblot analysis showed that the expression of BPMV-pCP was high in the five transgenic lines with three copies of T-DNA but undetectable in line 407. The steady state transcript levels of the BPMV-pCP gene were also high in lines with three copies of the transgene but very low in line 407. In detached leaf assay experiments, all the transgenic lines except line 200 were resistant to infection with the mild strain of BPMV. However transgenic line 407 and to a much lesser extent the transgenic lines with three copies of T-DNA were also resistant to infection with a virulent strain of BPMV.

D 08

Genetic Diversity and Epidemiology of Bean Pod Mottle Virus

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Bean pod mottle virus (BPMV) is widespread in many of the soybean growing areas in the southeastern United States. Increased incidence of BPMV has recently been observed in several major soybean-growing regions in some central and southeastern states. Soybean yield losses of l0-55% have been reported as a consequence of BPMV infection. Disease management through genetic resistance is not possible at present because no soybean cultivars with resistance to BPMV are commercially available. A limited number of transgenic soybean lines that express the capsid polyprotein (CPP), comprising the two capsid proteins (CP), have been generated and are being evaluating for field performance.

Knowledge of genetic diversity is essential for developing broad resistance to BPMV. The complete nucleotide sequence of BPMV genome has been determined and cloned cDNA probes are available for BPMV detection and strain relationship studies. Northern hybridization analysis of RNA from several BPMV isolates collected from soybean fields in three states indicated the occurrence of at least two distinct subgroups of BPMV strains.(I and II). Genetic diversity was more evident with BPMV RNA 1 than with RNA 2, and naturally occurring reassortants between subgroups I and II were detected.

The primary sources of BPMV inoculum in soybean fields early in the season have not been critically studied. We examined the roles of overwintering beetles and seeds from infected soybean plants as possible primary sources of BPMV inoculum. None of the virus-containing naturally overwintered beetles transmitted the virus to healthy soybeans. Likewise, beetles exposed to artificial overwintering conditions showed little or no transmission of BPMV. Overwintered beetles regained the ability to transmit virus following acquisition feeding on infected plants. Regurgitants from overwintered beetles were infectious by mechanical inoculation and viral RNAs extracted from such regurgitants showed no apparent changes in their integrity. Limited proteolysis of the large capsid protein, however, was detected and is believed to be related to the loss of beetle transmissibility. Evidence to date from seedling grow-out and ELISA tests of seeds collected from fields with high BPMV incidence failed to demonstrate seed transmission of BPMV. Perennial leguminous weeds growing near soybean fields are suspected as sources for BPMV inoculum early in the season.

D 09

RAPD Marker for Resistance to Cyst Nematode (Race 3) in Soybean

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The nematode *Heterodera glycines*, the soybean cyst nematode (SCN), is considered to be the most important problem for soybean cultivation, with rotation between resistant cultivars and no-host species being the most economic and reliable control method. Breeding programs for the development of resistant cultivars have to evaluate soybean genotypes in infested fields or in artificially inoculated areas, and to avoid transportation and possible contamination of SCN free sites, the identification of molecular markers linked to the genes conferring resistance to SCN is highly desirable. Thus, crosses between resistant (BR 90-4722, BR 92-15440, BR 90-4617 and susceptible (FT-Cristalina) cultivars were made, and the BSA technique was used to identify RAPD markers linked to the genes conferring SCN (race 3) resistance. The marker OPR15220pb produced a band capable of distinguishing resistant and susceptible F2 plants, however further studies are necessary to develop a SCAR marker that can be efficiently used in marker-assisted selection.

D 10

Tracing the Origin of QTL Alleles for Resistance to *Sclerotinia sclerotiorum* in Soybean Ancestral Lines

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Pedigree analysis of the parents of a mapping population can be used to identify the potential progenitors of the parental alleles of putative QTLs. In prior research, six putative QTLs were detected on seven linkage groups in populations derived from matings of five resistant soybean cultivars, Corsoy79, NKS19-90, Vinton81, Dassel and DSR 173, to the susceptible Williams 82. Eighteen SSR markers, putatively linked to these QTLs for sclerotinia stem rot resistance, were used to screen 48 soybean lines in the pedigrees of the five resistant cultivars. The 48 soybean lines were also screened for sclerotinia stem rot resistance using a detached leaf assay. A marker- phenotype association was performed to track, if possible, the resistance allele in each of the five pedigrees. For three of the five cultivars, the favorable marker allele was consistently associated with smaller lesion size in each member of the three pedigrees, thus allowing an inference as to the probable progenitor of the QTL allele. These inferences were: 1) The resistance allele of the QTL linked to the Corsoy79 allele of Satt424 of linkage group (LG) A2 most likely originated from Mandarin, 2) The resistance allele of the QTL linked to the Vinton81 alleles of Sat_109 and Satt243 (LG O) was probably also of Mandarin origin, and 3) The resistance allele of the QTL linked to the NKS19-90 alleles of Satt114, Satt510 and Satt335 (LG F) probably originated from PI 257.435. For the Dassel and DSR pedigrees, the marker allele associated with smaller lesion size in the mapping population was not consistently associated with pedigree components having the smaller lesion sizes. Type I errors are possible in this pedigree analysis, and for moderate SSR-QTL linkages a recombination event can reverse the linkage phase. These results show that QTL alleles affecting a particular phenotype can be tracked in pedigrees. If so, such tracking is a "reverse" genetics approach to the confirmation of QTLs detected in mapping populations.

D 11

Identification of Early Induced Genes in Soybean-Soybean Cyst Nematode Interaction by Suppression Subtractive Hybridization

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The soybean plant introduction PI 437654 is resistant to all field soybean cyst nematode (SCN) populations in the US and has been extensively utilized in cultivar development. PCR select suppression subtractive hybridization technique was used to enrich for cDNAs induced or repressed in the roots of PI 437654, 10 hours after SCN infection. The cDNAs were cloned into the pGEM-T vector and 780 clones were randomly selected for single pass sequencing. In PI 437654, genes associated with cell wall modifications and defense responses were the two major classes of genes induced during early SCN-soybean interactions. Genes involved in protein synthesis were abundant in non-infected roots. Northern blot analysis using RNAs from SCN infected and non-infected roots of PI 437654 and a susceptible cultivar Essex were conducted. At present, twenty-one differentially expressed genes after nematode infection were confirmed. Southern analysis showed that all of the twenty-one genes were of soybean origin. Two clones exhibited root specific expression and were induced only during the soybean-SCN incompatible interaction.

D 13

Is Korea a New Source for Resistance Genes to *Phytophthora sojae*?

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Genetic diversity is low among elite Northern American soybean breeding populations. Fewer than 20 soybean cultivars are responsible for 80% of the genes in public soybean cultivars released in recent years. Diversifying the soybean germplasm base could introduce new genes for traits from disease resistance to yield improvement. The objective of this study was to determine the genetic diversity present between South Korean soybean plant introductions (PI's), US cultivars, Chinese PI's, and Japanese PI's. The Korean soybean lines are of interest because they have been shown to be an excellent source of resistance to Phytophthora sojae. These lines may contain novel Rps genes or novel Rps gene combinations that account for this resistance. The relationship among the soybean lines was measured by evaluating 50 simple sequence repeat (SSR) primer pairs. The SSR data was used to compute Nei's distances. Clustering of the Nei's distances demonstrated that the South Korean PI's are most closely related to Japanese PI's and distantly related to US cultivars. These results indicate that in fact the South Korean germplasm may contain novel Rps genes that can be introduced into US lines to create new P. sojae resistant cultivars

D 14

Confirmation of the Minor QTL from PI96354 Conferring Root-knot Nematode Resistance

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Root-knot nematodes (*Meloidogyne* spp.) can cause severe yield loss of soybean in the southern production region of the U.S.A. Planting root-knot nematode resistant cultivars is the most effective method of preventing yield loss. Recently, DNA marker technology has been developed and integrated into soybean improvement programs. DNA marker-assisted breeding may accelerate the development of nematode resistant cultivars. Two quantitative trait loci (QTL) conferring resistance to the southern root-knot nematode [*Meloidogyne incognita* (Kofoid and White) Chitwood] (Mi) were previously identified in our laboratory (1) and the genomic regions near these QTL have been saturated with SSR markers (unpublished data). The minor QTL for Mi resistance is usually affected by environment and difficult to select in a practical breeding program. The objective of this research was to confirm the effect of minor QTL derived from PI96354, a major source of Mi resistance. A BC_2F_2 population was derived from the cross of 'Prichard'[3] x G93-9009. G93-9009 has PI96354 in its pedigree (2) and both G93-9009 and Prichard have 'Forrest' in their pedigrees. Forrest contains the *Rmi* gene for moderate resistance to Mi (3). Individual plants from the BC_2F_2 population along with parents, grandparents, and the susceptible check 'Bossier' were evaluated in a greenhouse nematode assay. These BC_2F_2 plants were classified with SSR markers on LG-G, the genomic region of the minor QTL. The results indicated that selection for the PI96354 allele with SSR marker Satt012 would enhance resistance of lines containing *Rmi*.

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D15

Genomic Analysis of Metabolic Pathways Conferring Partial Resistance to Fungi: *Fusarium solani* f. sp. *glycines*

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Sudden death syndrome of soybean is caused by an asexual fungus with almost no genetic variability. *F. solani* produces several phytoxic fractions. Resistance seems to operate by rate reduction of infection and/or colonization. Soybean (*Glycine max* [L.] Merr.) plants (cv Forrest and Essex) were inoculated by *Fusarium solani* f. sp. *glycines* (in the soil). The roots were harvested and used to identify differentially expressed genes in response to *Fusarium* infection in the soil. Differential display of mRNA, subtraction hybridization and EST sequencing were used to study the genetic response of soybean to the fungal infection. The differentially expressed sequences were cloned and used as probe to screen cDNA library constructed from Forrest roots infected by *Fusarium solani*. Fourteen positive clones were identified having sequence similarities with known soybean ESTs, ATP synthase, glyceraldehyde 3-phosphate dehydrogenase etc. The subtraction hybridization identified genes like Elicitor-induced cytochrome P450s of soybean (100% sequence identity), *Glycine max* chalcone synthase (99% sequence identity), cinnamyl alcohol dehydrogenase (CAD1) (93% sequence identity), mRNA for reductase involved in deoxychalcone synthesis (83% sequence identity), stress response genes and some carbon metabolism genes. Some of these genes have earlier been identified as being differentially expressed in soybean cell suspension culture in response to inoculation with *Pseudomonas syringae* pv. *glycinea*. Our results indicate that the genes involved include many of the hypersensitive (HR) genes. However, the genetic pathways involved seem more complicated than HR or the compatible reaction and there are 6 QTL involved in the resistance process. The results indicates that the processes of recognition of the pathogen, change in the metabolism rate, increased membrane transport, increased cell wall synthesis (differential expression of myo-inositol-Phosphate synthase), and induction of stress related genes are occur in response to fungal pathogen (*Fusarium*) infection. Genes capable of controlling these gene expression changes are sought within contiguous BAC clones covering the ~850 kbp region that contains 3 of the resistance QTL by hairy root transformation, EST hybridization and shotgun sequencing. This research was supported by the Illinois Soybean Program Board.

D 16

Propagation of the Soybean Cyst Nematode on Hairy Roots and Expression of Resistance in Transgenic Roots.

Hyeon-Je Cho, [1] G. R. Noel, [2] S. K. Farrand, [1] T. Eggett, [1] Yoshimi Inaba [1] and J. M. Widholm. [1]

[1]Department of Crop Sciences, and [2]USDA-ARS, University of Illinois, Urbana, IL 61801

Agrobacterium rhizogenes-mediated efficient transformation to produce transgenic hairy roots and expression of *gfp* and *gus* genes in various soybean cultivars, and the successful propagation of the soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) race 1, were accomplished (1). Hairy roots were produced from the wounded surface of cotyledon explants of SCN-susceptible cultivars, Lee 68, Mandarin, Maple Arrow and Williams 82, and SCN-resistant Cartter, Fayette, Hartwig, Jack, Peking and PI 437654 at rates of 54-95 % on selective medium containing 200 □g/ml kanamycin and 500 □g/ml carbenicillin. Eight weeks after inoculation with SCN eggs, the number of cysts formed on hairy roots of the resistant cultivars was 0 to 2, while the number on the susceptible cultivars was 0 to 175, indicating that the SCN resistance phenotype was preserved in transgenic hairy roots. We are also developing new sterilization methods for SCN eggs because this is one of the most important factors in these experiments. Furthermore, we have introduced several candidate genes into the SCN-susceptible cultivar, Williams 82, for evaluation of SCN-resistance imparted by the candidate genes. The expression of transgenes were confirmed by polymerase chain reaction (PCR), Southern, Northern, and Western blots, and the SCN resistance of these candidate genes is now being tested. This work was supported by funds from the Illinois Soybean Program Operating Board, the United Soybean Board and the Illinois Agricultural Experimental Station. (1) H-J Cho, SK Farrand, GR Noel, JM Widholm (2000) High-efficiency induction of soybean hairy roots and propagation of the soybean cyst nematode. Planta 210:195-204.

D 17

Isolation of Elicitor-inducible Cytochrome P450s from Tobacco Cell Suspension Cultures.

Lyle Ralston, Mark Schoenbeck, Jennifer Ralston, Soon Tae Kwon, and Joe Chappell

Capsidiol is an antimicrobial terpene produced by tobacco and other Solanaceous plants in response to infection by microorganisms. Biochemical studies suggest that the oxidation of the sesquiterpene precursor, 5-epi-aristolochene, to capsidiol is mediated by at least one elicitor-inducible cytochrome P450. This evidence includes the inhibition of capsidiol accumulation in elicitor-treated cells in the absence of NADPH or O_2 and in the presence of the P450 antagonists carbon monoxide, ancymidol, and ketoconazol. Since cytochrome P450s are especially hard to purify from plant tissue, a PCR-based strategy was employed to screen an elicited cDNA library for inducible cytochrome P450s, particularly the as aristolochene hydroxylase. The library was made using RNA extracted from tobacco cell suspension culture treated with the fungal elicitor, paraciticein. This strategy, which relied on vector-specific primers and degenerate primers complementary to amino acid sequence domains that are highly conserved among eukaryotic P450 families, resulted in the isolation of cDNA fragments coding for putative cytochrome P450s from four known families. These fragments were used to screen the same cDNA library to obtain the full-length clones. These clones were expressed in yeast under a galactose-inducible promoter, and microsomes were isolated in order to test for substrate specificity.

SOY 2000
8th Biennial Conference
of the Cellular and Molecular
Biology of the Soybean

PLENARY SESSION III

Genomics

Lila Vodkin and Randy Shoemaker, Presiding

8:30 am
Wednesday, August 16
Patterson Ballroom A/B/C

Poster Session 6:00–8:00 pm Monday
Patterson Ballroom D

PIII 01 **The NSF Functional Genomics Program for Soybean: An Update**

Lila O. Vodkin[1], Steve Clough[1], Reena Philip[1], Robin Shealy[1], Anupama Khanna[1], John Erplending[2], Margarita Paz[2], Randy Shoemaker[2], Virginia Coryell[3], James Schupp[3], Paul Keim[3], Alicia Rodriquez-Huete[4], Pei-yu Zeng[4], Joseph Polacco[4], Joann Mudge[5], Roxanne Denny[5], Nevin Young[5], Christina Raph[6], Libby Shoop[6], Ernest Retzel[6]

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An overview and update of the multi-university NSF-sponsored Functional Genomics project is presented. Over 80,000 ESTs have been entered in the public data bases (dbEST) from the Soybean Public EST project to date (see abstract by Shoemaker, et al., this conference). These represent the 5' sequence of cDNA clones from many different tissues, organs, and stages of development of the soybean plant. A major goal of the NSF-sponsored soybean project is to create a subset of 30,000 unique ESTs or cDNAs (unigenes) that have also been sequenced at the 3' ends. As a prototype, the first sequence-driven, non-redundant soybean gene set was developed from a cDNA library (Gm-c1004) prepared from mRNA from 8-day old seedling roots. A set of 6,392 5' sequences were subjected to cluster analysis and yielded 2952 singletons and 1,137 contigs. The singletons plus the 5' most read from the 1,137 contigs were selected and reracked by Incyte Genomics to yield library Gm-r1021 representing 4089 unique genes from the root library. High quality DNA templates (Qiagen preps) were prepared at the Keck Facility of the Biotechnology Center at the University of Illinois and the 3' ends were sequenced. The 3' data has been entered into dbEST and the average 3' read length is 731 bases. The Gm-1021 clone library is available from Incyte Genomics, St. Louis, MO. Presently, another 9,000 unique clones from cDNA libraries representing the cotyledons, seed coats, pods, and flowers have been selected by clustering the 5' sequences and are presently being sequenced at the 3' ends. High density arrays have also been used to reduce the presence of redundant sequences in some of the soybean cDNA libraries. Another focus of this project is to produce the genome sequences that mark over 1000 mapped positions on the soybean genetic map. This objective is accomplished by sequencing the ends of BAC clones that represent SSRs or RFLPs that have been placed on the genetic map. The objectives in functional genomics are to compare global gene expression methods including microarrays, and serial analysis of gene expression (SAGE) in soybean. Microarrays of 4000 unique genes from the Gm-r1021 "unigene" library have been constructed and are being used to ascertain baseline expression data for certain tissues and stages of soybean development as well as various treatments as pathogen challenge or stress (see abstracts by Clough, et al; Shealy, et al., this conference). A workshop on the soybean microarray development was held recently at the University of Illinois and 29 researchers from 15 different universities or locations attended. Supported by NSF (9872565).

PIII 02

The Public Soybean EST Project: An Update

Randy C. Shoemaker[1], John Erpelding[1], Sandra Clifton[1], Virginia Coryell[1], Marcia Imsande[1], Anupama Khanna[1], Paul S. Keim[2], Tina Ralph[3], Ernest Retzel[3], John Martin[3], James Schupp[3], and Lila Vodkin[4]

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The public soybean EST project is funded by the North Central Soybean Research Program and by the United Soybean Board. This project is a joint effort of the USDA-ARS, Iowa State University, Northern Arizona University, the University of Minnesota and the University of Illinois. The goal of this four-year project is to generate and deposit into a public database at least 200,000 ESTs representing mRNAs from a variety of soybean genotypes and organs at different stages of development and expressed in a wide range of environmental conditions. More than 65 cDNA libraries have been generated and submitted through Incyte Genomics for sequencing at the Washington University Genome Sequencing Center. To date, approximately 100,000 EST 5'-sequences have been generated and deposited into dbEST. The average size of the sequences is in excess of 400 bases. Fewer than 3% of the sequences are less than 100 bases long. A large number of clones already have been provided to government, academic and industry researchers. Sequences from each library are being analyzed for contig overlaps in an effort to develop sets of ESTs representing single genes. This project is scheduled to be completed March, 2002.

PIII 03

Development and Use of Soybean Microarrays for Analysis of Global Gene Expression

Steven J. Clough, Reena Philip, Robin T. Shealy, Anupama Khanna, and Lila O. Vodkin

Department of Crop Sciences
University of Illinois, Urbana, IL

We have constructed microarrays of spotted PCR products corresponding to 4,089 different soybean ESTs (expressed sequence tags) from a young seedling root library. This root "unigene set" is part of the larger public EST and NSF projects (see also abstracts by Shoemaker, et al., and Vodkin et al., this conference). PCR products from the clones were suspended at approximately 100 ng/ul in spotting solution. Approximately 1 nl of PCR product was spotted in duplicate on aldehyde or amine coated glass slides within a 20 x 40 mm set of grids. Additional sets of other genes as controls, or genes from additional soybean organs, were also spotted repetitively within the array. These microarrays can be used to study global gene expression changes that occur under various plant developmental stages, environments, genetic backgrounds, disease stresses, and abiotic stresses. As one of our initial studies, we are examining expression differences between tissues of healthy plants grown under standard greenhouse conditions. Analyses of gene expression from various tissues (such as roots vs leaves or seed coats vs cotyledons) allows us to ascertain basal soybean tissue expression profiles which will be continually updated as ESTs from more tissues become available for microarray construction. Technical and troubleshooting aspects of microarray construction and use will be discussed.

PIII 04

Soybean Genomic Survey: What We are Learning About the Soybean Genome from BACs Identified by RFLP and SSR Markers.

Laura Fredrick Marek1, Joann Mudge2, Laura Darnielle3, David Grant3, Nadja Hanson3, Margie Paz1, Yan Huihuang2, Roxanne Denny2, Karin Larson2, Dawn Foster-Hartnett2, Anne Cooper2, Dariush Danesh2, Tina Raph4, Rod Staggs4, John A. Crow4, Ernie Retzel4, Nevin Young2 and Randy Shoemaker1,2

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3 USDA-ARS, Corn Insects and Field Crop Research, Iowa State University, Ames, IA 50011
4 Computational Biology Centers, University of Minnesota, Minneapolis, MN 55455

We are building a framework physical infrastructure across the soybean genome by using SSR (simple sequence repeat) and RFLP (restriction fregment length polymorphism) markers to identify BACs from two soybean BAC libraries. The two libraries are prepared from two different genotypes digested with different restriction enzymes. We are obtaining end sequence from the BACs. The sequences are analyzed by using BLAST algorithms to search nucleotide and protein databases maintained by the University of Minnesota Computational Biology Centers (CBC). The SSR identified BACs have a higher percentage of significant BLAST hits then do the RFLP identified BACs. This difference is largely accounted for by a higher percentage of hits to repetitive type sequences for the SSR identified BACs. Both types of library screens have a similar percentage of BAC-end sequences with significant hits to known genes (6-8% of total sequences). These genes represent a wide range of metabolic functions. The BAC-end sequences have also allowed us to identify microsynteny between soybean and the model plants *Arabidopsis* and *Medicago truncatula*. This map-based approach to genome sampling provides a means to assay soybean genome structure and organization.

PIII 05

Development Of Physical Maps Integrated with Genetic Markers and EST: Prelude to Genome Sequencing

Khalid Meksem1, Jeffry Shulz, Javed Iqbal, Eliza Ruben1, Kanokporn "Tik" Triwitakorn1, Kim Zobrist1, Hong Bin Zhang2, David A. Lightfoot1.

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The development of robust techniques for physical mapping of entire complex genomes provides an new strategy for gene isolation from eukaryotes. Using a proprietary PAGE fingerprinting method and enzyme kit we are developing large insert clones libraries (BAC) and physical maps of several plant (egs. soybean, rice, Arabidopsis, Lotus, Pea, Moss) and fungal (*Ustilago, Fusarium*) genomes. We will integrate the physical maps with genetic maps and portions of EST libraries. The soybean physical map currently consists of 90,786 fingerprinted BACs covering 11.1 x soybean haploid genomes generated by PAGE after digestion with two restriction endonucleases. The soybean BACs are generated with *Eco*RI, *Hin*dII or *Bam*HI from cultivar 'Forrest'.The developing physical map was integrated with 77 genetic markers on 14 linkage groups (see www.siu.edu/~pbgc), 313 microsatellites are in the later stages of integration. We are integrating unique ESTs in groups of 512 by multiplexed hybridization methods. The physical map can thereby be confirmed. A robust physical map can be used to increase the efficiency of whole genome sequencing by providing a minimum tile path for DNA sequencing, identifying gaps in a genomic clone libraries and identifying regions with high gene content for selective sequencing. The physical map has been used to develop new genetic markers (micro-satellites InDels and SNPs) in regions of the genome targeted for a lack of conventional genetic markers. The Genomics laboratory in the Center for Excellence in Soybean Research, Teaching and Outreach has increased technical capabilities in genomics that are available to researchers on a fee for use basis. There are two dedicated DNA sequencers, a PE377 and an automated Beckman CEQ2000 that use visible and infrared fluorescence detection respectively. There is an arraying robot (Genomics Solutions) capable of selectively picking white colonies into 384 well plates and transferring 1 pl-500 nl of DNA to filters or microarray slides. There is a hybridization unit and array scanner. There is a liquid handling robot for DNA preparation and reaction set up. We will describe automated, multiplexed methods based on fluorescent tags for physical map generation, minimum tile sequencing and the identification of ESTs with BAC clones and contigs. This research was supported by the National Science Foundation and the Illinois Soybean Program Board.

PIII 06

Development of the Universal Soybean Genetic Map.

Jeongran Lee[1], Jijun Zhou[1], Shujun Xu[1], Krishna P. Kollipara[2], Perry B. Cregan[3], Ram J, Singh[1], and Theodore Hymowitz[1].

[1] Department of Crop Sciences, University of Illinois, Urbana IL
[2] Pioneer Hi-Bred International, Inc., Johnston, IA
[3] USDA-ARS, Beltsville, MD

At least 4 molecular linkage maps of soybean based on RFLP, RAPD, AFLP and SSR markers have been developed and available for soybean geneticists. None of these, however, have been associated with specific chromosomes of the soybean. This study has been conducted with the objective of integrating all available classical genetic (pathological, morphological, and biochemical) and molecular (RFLP, RAPD, AFLP, SSR) linkage maps to specific chromosomes for the purposes of developing the universal soybean map. The overall goal of this study is to assign the 20 soybean linkage groups to each of the 20 soybean primary trisomics (2n = 41), which have been developed at the University of Illinois. Thus far, 8 out of the 20 soybean chromosomes have been associated to molecular linkage groups (MLG). D1a+q, N, A1, A2, K, F, D2 and L have been associated to soybean chromosomes 1, 3, 5, 8, 9, 13, 17 and 19, respectively. The universal soybean genetic map will be extremely usefu!
I for establishing the genetic position of economic traits for improving soybean yields, for use in plant variety protection, for detection of quantitative trait loci (QTLs), and for map-based cloning of economically important genes.

PIII 07

Genomics Applications for Quality Traits.

Rita Varagona

Monsanto Corporation,
St. Louis MO

PIII 10

SOYBASE 2000: Creation of Composite Genetic and QTL Maps.

Marcia Imsande, David Grant, and Randy Shoemaker
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Joint mapping efforts of several universities and of USDA-ARS have identified many SSR and RFLP loci which aligned into 20 homologous linkage groups. The presence of many loci in common between the three populations studied has allowed us to construct a composite genetic map for soybean. The 1999 USDA/Iowa State University maps were used as the foundation, with markers not mapped in this population interpolated onto each linkage group in one of two ways. 1) If a marker had two surrounding anchor loci, it was placed onto the map using a proportional relationship based on the distance between common loci. Thus, genetic distances and relative positions of closely spaced markers are only approximate. 2) Markers that fell between the most distal anchor locus and the end of the map are displayed on a bar to the left of the map, indicating that the locus could be anywhere in this region. By using these methods, over 1870 mapped loci (RFLP, SSR, RAPD, AFLP, and classical genes) from over a dozen published soybean molecular maps have been placed onto the 20 linkage groups of the composite map. Quantitative trait loci (QTL) are indicated on the composite map as well. The composite map has direct links to the original mapping and QTL data in SoyBase. This represents the first unification of all soybean linkage and QTL data onto a single composite map, and it will be of value to soybean geneticists, breeders, and molecular biologists. The composite map has been incorporated into SoyBase, a soybean genome database. SoyBase can be accessed over the WWW (http://genome.cornell.edu/cgi-bin/WebAce?db=soybase) or from the SoyBase home page (http://soybase.agron.iastate.edu).

PIII 11

Soybean Genomic Survey: BAC-end Sequence and Contig Building Near SSR and RFLP Markers

Laura Fredrick Marek1, Joann Mudge2, Laura Darnielle3, Nadja Hanson3, Margie Paz1, Roxanne Denny2, Karin Larson2, Dawn Foster-Hartnett2, Anne Cooper2, Dariush Danesh2, Tina Raph4, Rod Staggs4, John A. Crow4, Ernie Retzel4, Nevin Young2 and Randy Shoemaker1, 2

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We are building a physical infrastructure of BAC contigs across the soybean genome using SSR (simple sequence repeat) and RFLP (restriction fragment length polymorphism) markers to identify BACs from two soybean BAC libraries. One of the BAC libraries was constructed with "Williams82" DNA in the HindIII site of the pBeloBAC 11 vector. The second library was constructed from "Faribault" DNA in the EcoRI site of the pECSBAC4 vector. The BACs identified from each marker are grouped into contigs. Because the RFLP probes usually hybridize to more than one genomic region, the BACs identified from each RFLP usually sort into more than one contig. The SSRs are usually single copy markers. We are obtaining end sequence from the BACs within each contig. The sequences are analyzed using the Blast algorithms to search nucleotide and protein databases maintained by the University of Minnesota Computational Biology Centers (CBC). In addition, the sequences are run through a gene predictor program under development by the CBC. The SSR identified BACs have a higher percentage of significant Blast hits (51% of total sequences) then do the RFLP identified BACs (31% of total sequences). The difference is due to a higher percentage of hits to repetitive type sequence for the SSR identified BACs. Both types of library screens have a similar percentage of BAC-end sequences with significant hits to known genes (6-8% of total sequences). These genes represent a wide range of metabolic functions.

PIII 12

Detection of a LG-I Protein/Oil QTL in RIL and F2 Populations.

Heather Olsen, James Specht, George Graef, and Paul Staswick

Department of Agronomy, University of Nebraska, Lincoln, NE

Using a population of 76 F5-derived RILs derived from the mating of a high yielding elite soybean cultivar (Asgrow A3733) with a high protein plant introduction (PI437088A), we previously identified a QTL on linkage group I that strongly affects soybean seed protein, oil, and yield. Based on a 12-replicate 2-year trial of these RILs, the PI allele enhanced seed protein by almost one percentage point, but also reduced seed oil by a half percentage point and seed yield by about 150 kg/ha (2.3 bu/ac). RIL "AA" and "BB" genotypes at this QTL thus differed, on average, by 2% in protein, 1% in oil, and about 300 kg/ha (4.6 bu/ac) in yield. We are obviously interested in eventually cloning this QTL to better understand how its alleles exert these three correlative effects. To do that, we need a more precise map, and thus a larger number of progeny, so we re-mated the same two parents and obtained ca. 650 F2 seed. Those F2 plants were selfed in 1998 and individually harvested to obtain F2 plant-derived seed progenies, which were evaluated for seed protein/oil content. In 1999, those progenies were grown in F2.3 rows of about 25 F3 plants per row. In mid-summer, a balanced bulk of 20-25 leaves collected from each progeny row (and two parent rows), lyophilized, and subjected to DNA extraction. The 650 DNA samples were then screened for SSR and RAPD markers on LG-I. The rows were bulk harvested to obtain 650 F2-derived F3 seed progenies. The one-year single-replicate F2 and F2.3 phenotypic data were subjected to a QTL analysis using Mapmaker and QTL Cartographer software. The analyses confirmed the QTL's presence and position on LG-I, and revealed that the alleles of the QTL act in a mostly additive manner (little dominance). The population has been advanced, via single plant descent, to the F5 generation (growing this year). The pleiotropic effects of this QTL will be critically assessed examined by evaluating the yield/protein/oil performance of the 600 F5-derived RILs in paired irrigated/rainfed production trials over the course of the next two years.

PIII 13

Statistical Analysis of Tissue-Specific Expression Data Using Soybean Microarrays

Robin T. Shealy, Reena Philip, Steve J. Clough, and Lila O. Vodkin

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Microarray and chip technology allows the monitoring of expression of thousands of genes simultaneously. The resulting data sets have thousands of variables, and often only a few replications. Statistical analysis of this kind of data requires exploratory multivariate data approaches in clustering similar data into groups or extracting major factors via principal components analysis. As part of the larger NSF Functional Genomics Soybean Project (see abstracts by Vodkin et al., Clough, et al., this conference), we have constructed arrays of a unigene set of over 4000 sequences from a cDNA library derived from entire roots of 'Williams' 8-day old seedlings and carried out analyses of gene expression in roots, shoots tips, leaves, and cotyledons. The data from these experiments was normalized and clustered using several methods, and diagnostics computed on spot quality. Genes significant for organ and tissue specific expression are reported in the results.

PIII 14

QTL for Reproductive Traits in Soybean

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This study used molecular markers to identify and locate chromosomal regions that control traits for flowering time, maturity and photoperiod sensitivity in soybean. Two single-cross populations, IX132 (PI 317.336 X 'Corsoy') and IX136 (PI 317.334B X 'Corsoy'), were analyzed. Days to R1 were measured among F6:7 RI lines in the field during 1991 and 1992 and in the growth chamber using both 12 h and 20 h photoperiods with a combination of fluorescent and incandescent lamps. Days to R3 were measured in the field during 1991 and in the growth chamber with a 12 h photoperiod. Days to R7 were measured in the field in 1991. A total of 139 markers (88 RFLPs and 51 SSRs) in the IX132 population and 125 markers (73 RFLPs and 52 SSRs) in the IX136 population were used to map quantitative trait loci (QTL) affecting these traits. Our results show that large-effect QTL for days to R1, R3, and R7, and photoperiod sensitivity were found at similar locations on linkage group C2. These QTL were detected in all environments tested. In both populations, the QTL accounted for 35.4-45.7 %, 29.7-43.2%, 24.7-25.6 %, and 22.4-25.7% of total phenotypic variances for days to R1, R3, R7, and photoperiod sensitivity, respectively. This result suggests that traits for photoperiod sensitivity, flowering time, and maturity may be controlled by the same gene(s) or by tightly clustered genes in the same chromosomal region. Minor effect QTL controlling these four traits also were observed in these populations. These QTL account for as much as 18.1% and 17.9% of the phenotypic variance in populations IX132 and IX136, respectively. Thus, we propose that time of flowering, maturity, and photoperiod sensitivity in soybean are controlled by major QTL with a large effect and modified by several minor QTL.

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