SOY 2002

Program and Proceedings for

The 9th Biennial Conference of the Cellular and Molecular Biology of the Soybean

August 11-14, 2002

College of Agricultural, Consumer and Environmental Sciences
University of Illinois at Urbana-Champaign

P606

Involvement of Phenylpropanoid Metabolism in Soybean Root Response to Fsg

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The best genetic sources of soybean SDS resistance at present exhibit only decreased foliar symptoms but the roots are infected resulting in reduced plant health and yield losses. Our goal is to identify new ways to obtain higher levels of soybean resistance to *Fusarium solani glycines* sp., (FSG) that causes SDS by understanding the specific metabolic features of partially resistant genotypes and manipulating root levels of compounds that are toxic to fungi. To achieve this goal we will 1) determine the effect of FSG infection on various phenolic levels (isoflavones and other soluble and wall-bound phenolics) in roots of plants of genotypes that differ in their resistance to the pathogen; 2) manipulate the various phenolics in soybean hairy roots by transformation with sense and antisense constructs of important phenylpropanoid genes (isoflavone synthase, chalcone synthase, chalcone reductase) to learn if alterations in phenolic compounds reduce infection or colonization by FSG.

Our studies show that the phenylalanine ammonia-lyase activity increased and phenolics (isoflavones, glyceollin and lignin) accumulate in roots as a consequence of FSG infection to higher levels in the partially resistant genotypes. The capacity to produce the phytoalexin glyceollin rapidly and at high levels appears to be important to combat FSG infection. The dosage dependent inhibition of FSG growth by glyceollin was demonstrated using an *in vitro* system. The induction of the "defense" lignin synthesis and maintaining an increased rate of the lignin formation during infection progression is another biochemical response important for soybean resistance to FSG. Our data also indicate that FSG has lignolytic activity. Thus the genetic modification of lignin composition and levels could also be of importance to improve soybean SDS resistance.

This study was supported in part by the funds from the Illinois Soybean Program Operating Board, the United Soybean Board, NATO Collaborative Research Program (ref. LST.CLG.976259; JSTC.CLG.978212), the Illinois Agricultural Experiment Station, and the USDA Agricultural Research Service.

P607

Isolation of Two Soybean Sequences with Similarities to the *Arabidopsis NPR1* Gene

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NPR1 gene, also known as *NIM1*, is a key regulator of the salicylic acid-dependent signal transduction pathway that leads to the systemic acquired disease resistance in *Arabidopsis*. Over-expression of this gene in *Arabidopsis* and rice demonstrated broad-spectrum disease resistance against multiple pathogens. The objective of the present study is to isolate and characterize the orthologous *NPR1* gene sequences from soybean. Computer database search uncovered a soybean expressed sequence tag (EST) with high similarity to *NPR1.* PCR amplifications of the soybean genomic DNA using primers designed based on this EST sequence produced a 1.7-kb PCR product. Screening of a soybean BAC library using the PCR product as a probe identified eighteen BAC clones harboring *NPR1*-like sequences. *NPR*-like sequences in these BAC clones were classified into two genes based on their restriction digestion patterns and the PCR amplification products, and were tentatively termed as *GmNPR1* and *GmNPR2*. The *Eco*RI and *Xba*I fragments identified from BAC clones carrying *GmNPR1* and *GmNPR2,* respectively, were sub-cloned into a binary vector pTF101.1 to result in plasmids p143K5Xb1-2.1 and p101F23E1-2. Comparison of partial sequences from these two clones revealed that they include sequences with high identity to the *Arabidopsis* regulatory protein *NPR1*. Sufficient sequences to represent both 5'- and 3'-ends of these genes were found in these sub-clones. Therefore, we have transformed the *Arabidopsis npr1-1* genotype with these two binary plasmids p143K5Xb1-2.1 and p101F23E1-2. We expect that if one of these two genes is a true *NPR1* homolog, then most likely it should complement the *Arabidopsis npr1-1* mutant phenotypes. Future experiments include over-expression of the soybean *NPR1* homologue to confer broad-spectrum resistance in soybean.

P608

A Transient Assay for Analysis of Resistance/Avirulence Gene Interactions in Soybean

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Transient disease resistance assays have proved useful in several model systems for quickly analyzing plant resistance gene functions. One such assay that has been widely used in *Arabidopsis* is based on bombardment of leaves with separate DNA constructs encoding a pathogen avirulence (Avr) gene, a reporter gene, and in some instances a candidate resistance (R) gene. These assays are based on the observation that successful interaction of a plant R gene product with its cognate Avr gene product results in cell death. This cell death process quenches reporter gene expression in leaves containing a naturally occurring or introduced resistance gene. However, cell death is not initiated at a high level in leaves lacking the correct R gene, resulting in higher reporter gene expression as compared to leaves that do express the Avr-specific R gene. To aid in the functional complementation of the recently cloned soybean *Rpg1-b* gene, a bombardment assay originally developed for *Arabidopsis* was modified for use in soybean leaves. Leaves from soybean variety Flambeau, which lacks *Rpg1-b*, were bombarded with plasmids encoding the *Pseudomonas syringae* avirulence gene *AvrB* and the firely luciferase reporter gene, plus or minus the candidate *Rpg1-b* construct. *Rpg1-b* was shown to reduce luciferase expression in an *AvrB*-dependent manner in several independent experiments. These results demonstrate the utility of the transient assay and indicate that our *Rpg1-b* clone activates defense responses upon recognition of its corresponding avirulence gene *AvrB*.

SOY 2002

Posters
General Molecular Genetics

Abstracts P801-P806

P801

A Yeast Artificial Chromosome Library of Soybean

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Yeast artificitial chromosome (YAC) library is reported to be very successful in positional cloning of a number genes in tomato, barley, *Arabidopsis*, rice, pepper and beet. Currently no YAC library is available for soybean (*Glycine max* L). Here, we report the construction and characterization of a soybean YAC library. We have used high-molecular weight DNA isolated from leaf nuclei of the cultivar Conrad 94 that carries *Phytopthora* resistance genes *Rps1*-k and *Rps6*. Insert DNA was prepared by partial digestion with *Eco*RI using the *Eco*RI-*Eco*RI methylase competition assay followed by electrophoretic fractionation before in-gel ligation to the pJS97/pJS98 YAC vector system. Ligation products were also subjected to electrophoretic size selection before yeast spheroplast transformation. The library consists of 26,112 clones. The quality of this library has been accessed through analysis of 393 randomly selected YAC clones. Evaluation of these random YAC clones indicated that 54% of the clones in the library carry soybean DNA fragments with an average size of about 290 kb. Thus, the libarary represents approximately three haploid genome equivalents DNA. The clones were stored in 384 well micro-titer plates. A total of 272 pools each carrying 96 clones are being screened for molecular markers that are linked to: i) the *Phytopthora* root rot resistance gene *Rps6*, ii) a high protein content QTL and iii) super-nodulation gene *NTS2*. The library is also being screened for 40 random SSR markers representing all twenty linkage groups of the soybean genome.

P802

Promoter Capture Technology for Analyzing Soybean Regulatory Elements

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As a result of the soybean public EST project, there are currently over 250,000 soybean ESTs deposited into Genbank. These sequences are an excellent resource to further study control of gene transcription specifically at the level of regulatory elements in promoter regions. We present an adaptation of the Clontech Genome Walker technology to amplify unknown genomic regions flanking known sequences. Genome Walker libraries are constructed by cutting genomic soybean DNA with various restriction enzymes and ligating adaptors onto all of the fragments. PCR products are amplified using the Genome Walker libraries as template, the adaptor sequence as one primer, and a gene specific primer designed from the end of an EST contig sequence as the other primer. These PCR products are then sequenced directly. This technique will be used to obtain potential promoter sequences to identify soybean regulatory elements and to study the evolution of DNA sequences flanking coding regions.

P803

Pod Opening in Soybean - Isolation of Potential *IND*1 Orthologs

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In the course of the *Arabidopsis thaliana* genome project many transcription factors have been identified as paramount for normal development of the floral meristem and subsequent development of the silique. Many similarities exist between silique dehiscence in *Arabidopsis thaliana* and pod opening in soybean. Therefore we have chosen to address the question whether the knowledge of the genes controlling *Arabidopsis thaliana* silique development is directly applicable to soybean pod opening. Our efforts concentrate on *ind1*, a transcription factor necessary for differentiation of the dehiscence zone of siliques.

Amino acid alignments of IND1 and related sequences were used to design degenerate primers. Soybean genomic DNA from the cv. TGx1835-2E and RNA from valves and dehiscence zones from green fresh and yellow mature pods was prepared. cDNA synthesis on the isolated RNA was then carried out. Nested PCR with the degenarate primers was then performed directly on the genomic DNA and on the synthesized cDNA and the resulting DNA fragments were cloned and sequenced. Sequence analysis revealed that a number of fragments closely related to IND had been cloned. The sequences are now in use as probes to isolate the full-length genes

P804

SOYBASE 2002: One Decade of Soybean Genetics and Genomics: Genetic Maps

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SoyBase, the USDA-ARS soybean genetics database, was initially conceived as a central repository for soybean genetic data. In 1993 SoyBase offered the first publicly available RFLP maps of the soybean genome. Since that initial version, new data types have been continuously added that complement the genetic maps, including internal hyperlinks between mapped loci and metabolism, pathology and many other phenotypic traits. A major milestone occurred in 2000 when a set of composite genetic maps was released. The current versions of these maps include data from more than 30 populations and contain more than 3800 mapped classical and molecular loci, along with more than 900 QTL. Recent sequencing efforts have allowed the incorporation of physical mapping data into the genetic maps. The integrated genetic and physical maps, along with DNA sequence data, represent a significant increase in our understanding of the soybean genome and represent the foundation for future functional genomics projects.

P805

SOYBASE 2002: One Decade of Soybean Genetics and Genomics: Metabolism

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SoyBase, the USDA-ARS soybean genetics database, has been in existence for one decade. In 1993 SoyBase offered the first extensive collection of metabolic data in a plant genome database. Originally, this focused on nitrogen and fatty acid metabolism. Now, however, SoyBase has expanded to cover many enzymes and pathways common to all plants, including those of plant pigment and hormone synthesis, the Calvin cycle, glycolysis, and the TCA cycle. Over 75 interactive, multistep pathways are in SoyBase, with clickable links to the underlying enzymes and metabolites. SoyBase also includes information on isozymes that have been mapped in soybean as well as enzymes that have been purified or cloned from other plant sources. A wide variety of enzymatic data are provided, including E.C. number, isozymes, gene, sequence, physical properties, purification, clones, transgenics, chromosomal location, and cultivar. Reaction data include kinetics, regulation, activators, and inhibitors. Because these enzymatic data are directly related to important agronomic traits, they are of use to soybean scientists desiring to increase the nutritional value of soybean.

P806

SOYBASE 2002: One Decade of Soybean Genetics and Genomics: Transformation

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SoyBase, the USDA-ARS soybean genetics database, was conceived as a central repository where researchers could quickly find information on most aspects of soybean genetics, metabolism, and pathology. In 2002, Transformation was added to SoyBase as a separate topic. Although the perception exists that soybean transformation is extremely difficult, many laboratories have in fact successfully accomplished this technique. As of June, 2002, over 55 published papers on soybean transformation are detailed in SoyBase. The methodology includes transformation via Agrobacterium, particle bombardment, and other less frequently used procedures. Data include vector characteristics, selective agent, transgene incorporated, assay for transformation, cultivar, tissue, bacterium, and recovery of regenerated and fertile plants. Hyperlinks within SoyBase point to relevant enzyme, storage protein, pathology, and insect data. This latest SoyBase topic makes publicly available in one location the various data on soybean transformation. It will be of great value to soybean scientists wishing to manipulate important traits of seed quality or disease and insect resistance.

P505

Determination of Isoflavone Contents for Selected Soybean Lines by Fourier Transform Near Infrared Reflectance Spectroscopy

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Recently, soybean isoflavones are of considerable interest in relation to their possible health effects in human diets. Therefore, the rapid and economical determination of soybean isoflavone contents is essential for the investigation and development of soybean, health foods, as well as the selection of soybean seeds with optimal isoflavone levels for such foods. Fourier Transform Near Infrared Reflectance Spectroscopy (FT-NIRS) calibrations are here reported for the rapid and cost-effective analysis of isoflavones in soybean seeds. In our study, FT-NIRS measurements were carried out in quadruplicate for 50 soybean lines selected from the USDA National Germpalsm Collection at the National Soybean Research Center (Urbana, IL). The selected soybean seeds provided a wide range of isoflavone contents (from 0.3 mg/g to 6.0 mg/g) that is necessary for development of high-quality calibrations. Laboratory reference values of isoflavone composition were obtained by HPLC analysis@ of extracted soybean powders. Single soybean seeds were selected for each standard sample and were cut in half in order to avoid screening of the isoflavones NIR absorption bands by the seed coat. For comparison purposes, measurements were also made on soybean powders of the same samples. FT-NIR spectra were collected with a spectral range from 4000 to 12000 cm^{-1} at a resolution of 8 cm^{-1} on a Perkin-Elmer Co.'s SpectrumOne NTS spectrometer model. This spectrometer is optimized for high-sensitivity analysis of single seed composition, being equipped with an NIRA, integrating sphere accessory and an extended range InGaAs detector. FT-NIR spectra of half soybean seeds were preprocessed before applying a suitable Multiplicative Scattering Correction (MSC). Partial Least Squares multivariate regression analyses were employed for high-quality calibration model developments. Our isoflavone calibrations are characterized by low standard errors (<0.2%) and high degrees of correlation (>99%), made possible by the optimized FT-NIR instrument.

@: HPLC analyses of soybeans were kindly provided by Dr. J. Widholm's Laboratory at UIUC.

P506

Determination of Soy and Health Foods Contents by Fourier Transform Near Infrared Reflectance Spectroscopy

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Rapid, accurate, and cost-effective composition analyses of soy and health foods are essential for improving the efficiency and quality of health food production. This is the first attempt at developing Fourier Transform Near Infrared Reflectance Spectroscopy (FT-NIRS) calibrations for soy and health foods. FT-NIRS measurements were carried out in quadruplicate for 16 food samples, such as: soymilk powder and soymilk, soy crisps, dry roasted soy nuts, soy burgers, soy tofu, island black beans, rye cakes, rye bread, rye cocktail bread, dry tomato, popcorn minicakes, and lean ham. FT-NIR spectra were collected over a spectral range from 4000 to 12000 cm^{-1} at a resolution of 8 cm^{-1} with a PerkinElmer Co.'s FT-NIR spectrometer, model Spectrum One NTS. This spectrometer is optimized for high-sensitivity analysis of solid samples, being equipped with an NIRA, integrating sphere accessory and an extended range InGaAs detector. FT-NIR spectra were preprocessed before applying a suitable Multiplicative Scattering Correction (MSC). Partial Least Squares multivariate regression analyses were employed for high-quality calibration model developments. Major food components, such as: protein, fat, moisture, fiber, polysaccharides and sugars were quantitated. Composition changes of soy and health foods caused by microwave heating were also monitored. Our calibration for soy and health foods is characterized by low standard errors (~0.5%) and high degrees of correlation between NIR calculated values and laboratory reference values (~99%), made possible by the optimized FT-NIR instrument.

P507

Determination of Amino Acid Composition of Soybeans by ^{13}C High-Resolution NMR Techniques

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The nutritional quality of plant and animal proteins is primarily determined by their amino acid composition. We are reporting quantitative determinations of selected amino acid residues of soy proteins by C-13 NMR measurements on hydrated soy protein and soy flour gels. Our assignments of C_a peaks for the essential amino acid residues of soy proteins were consistent with those listed in the BioMagResBank, and are specified as follows: HIS (81.395ppm), ILE (77.231ppm), LEU (69.904ppm), LYS (42.628ppm), MET(47.127ppm), PHE (73.46ppm), THR (51.929ppm), TRP (47.127ppm) and VAL (51.929ppm). High-resolution C-13 NMR spectra of soy proteins were obtained by utilizing a Composite Pulse Decoupling Scheme WALTZ 16, on a Varian , Unity Inova TM 600 NB High-Resolution NMR spectrometer operating at 150MHz resonance frequency for C-13 NMR detection. The amino acid profiles of soy proteins and soy flours were calculated as ratios of the integral values of the C_a peaks listed above to the integrated area of the delta-C=O peak at ~172ppm. These amino acid profiles can be now utilized in conjunction with any protein data bank in order to evaluate the protein digestibility corrected amino acid scores (PDCAAS).

P508

Soybean Protein and Oil Content Measurements by ^{13}C NMR and Dual Diode Array NIR Spectroscopy. Novel Observations of Microstructure and Composition of Protein Bodies and Oil Droplets in Hydrated Soybean Seeds and Developing Soybean Embryos by High-Resolution, Transmission and Field Emission, Electron Microscopy Techniques

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We are reporting the first correlated protein and oil content determinations in intact soybean seeds and soybean flours by high-resolution ^{1}H , ^{13}C Solid State Nuclear Magnetic Resonance (^{1}H and C-13 SNMR, respectively), and by Dual Diode Array Near Infra Red (DDA-NIRS) (with a DA7000 spectrometer made by Perten Instruments Co., Springfield, IL, U.S.A) . SNMR measurements were carried out with a GN 300 WB (General Electric Co.) spectrometer, equipped with an ^{1}H / C-13 CP-MAS Solids Probe (Varian-Chemagnetics Co.), by employing optimized pulse sequences for high-resolution ^{1}H and ^{13}C SNMR monitoring of the oil and protein contents of soybean flour samples, as well as intact soybean seeds. The values obtained by integration of the resolved, sharp peaks present at, respectively, 130 ppm and ~172 ppm in the processed ^{13}C SNMR spectra of soybean flours were employed to monitor the oil and protein contents. 90% correlations were obtained between NMR and the corresponding DDA-NIRS protein and oil measurements on the same samples of well-defined soybean lines from the National Germplasm Collection at UIUC. *This suggests that both techniques are suitable for the non-destructive, practical determination of both protein an oil contents in soybean seeds and soy flours.* Novel, high-resolution microscopy observations of oil droplet formation and the microstructures of proteins bodies in soybean seeds are also reported. Transmission Electron Microscopy (TEM) and Environmental Scanning Electron Microscopy (ESEM) techniques revealed the soybean protein body microstructures in thin sections of soybean seeds and embryos. Our TEM studies of soybean seed and soybean embryo microstructures were carried out with a Hitachi 600 microscope. The new high-resolution results are consistent with previous reports of correlations between the size/ shape of oil droplets, or protein bodies, and their content in oil or protein, respectively, in both maturing soybean embryos and seeds. Field Emission Microscopy observations with a Phillips XL30 ESEM-FEG microscope confirmed the presence of such correlations also in fully-hydrated soybean seeds placed in a 98% R.H. atmosphere , therefore validating this novel technique for *in vivo* studies of soybean embryos in the near future.

P509

Novel Techniques for Microspectroscopy and Chemical Imaging Analysis of Mature and Developing Soybean Embryos

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We are reporting the first observations by Infrared Microspectroscopy on mature and developing soybean embryos under physiological conditions. Highly-resolved chemical images were obtained for mature soybean embryos that allow the quantitation of oil and protein components and their distribution throughout the entire embryo. Chemical Image analysis of soybean embryos and soybean seeds provides a new tool for in vivo experiments and genetic selection of improved single soybean seeds.

P510

Soybean Seed Coat Contains Higher Peroxidase than Other Parts of the Plant

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Peroxidases (PODs, EC 1.11.1.7) represent a class of ubiquitous enzymes widely distributed throughout the plant kingdom. These haemproteins catalyse the oxidation of a wide number of organic and inorganic substrates. Amony all types of peroxidase found in plants, soybean peroxidase (SBP) has attracted special attention because of its high thermal and pH staibility together with a great catalytic power. Soybean is a plant which is grown in many parts of the world with a wide range of use in human and animal food industry. Soybean is grown in different area of Iran where the temperature is moderatetly high (about 30 degree centigrade in summer) with a medium to high humidity. The aim of this research was to extract peroxidase from different parts of a special kind of peroxidase grown in north (near Caspian sea) and to establish a profile for the peroxidase content in different parts of the plant. The idea was to make use of soybean waste and extract their enzyme. After being extracted with water in a homogenizer, the peroxidase was separated and purified using high performance liquid chromatography (HPLC). It was found that the quantity of peroxidase was about 20% higher in the sead coat of the plant compared to other parts (roots, stems and leaves). The biological activity of the purified peroxidase was measured using UV spectrophotometry.

P512

Comparison the Peroxidase Content of Different Iranian Soybeans

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Peroxidase is a haemprotein whose main function is oxidation of a large variety of substances. One of the major limitation for its wide spread use, is the high cost of production of the enzyme. Peroxidase is found in different parts of many plants and often serves as a parameter of metabolic activity during growth alternations. It is shown that the enzymatic activity of peroxidase is decreased by gibberlin, but is increased during growth restricted states, e.g. in cold weather. Most of research work to present time have focused on the extraction of peroxidase from horse raddish. Peroxidase is also found highly in different parts of soybean. The aim of this research was to reduce the production cost of peroxidase by extracting from different soybean grown in Iran and compare the peroxidase content to find the most economic way of its high scale production. We found that the specific activity of different Iranian soybean peroxidases varied between 33-38 units/mg. This was about 1.4-1.5 time the activity of commercial Sigma soybean peroxidase.

P513

Response of Embryogenic Cultures of Soybean to Chemical Mutagenesis

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Somatic embryogenic suspension cultures of soybean (*Glycine max* L. cv. Iroquois) were treated with varying concentrations (0, 1, 3, 10, and 30 mM) of the chemical mutagen ethyl methanesulfonate (EMS). Depending on the concentration of EMS used, the mean survival rate of embryogenic cultures ranged from 43 to 74%. Overall, the survival rate decreased with increasing EMS concentration with significant differences observed for cultures treated with 30 mM EMS compared to all other EMS concentrations used. Random amplified polymorphic DNA (RAPD) analysis was used to determine whether induction of genetic variability in embryogenic cultures in response to the different EMS treatments may result in identification of polymorphic markers. Two of 35 'core' primers tested revealed polymorphisms. One of the primers, OPO-01/1150, revealed polymorphism in tissue treated with 10 mM EMS, while the other primer, OPO-05/1200, revealed polymorphism in tissue treated with either 1 or 30 mM EMS. These results suggested that RAPD markers were useful in detecting mutations in embryogenic cultures of soybean.

P514

Characterization and Expression of the Gene Encoding β-Ketoacyl-acyl Carrier Protein Synthase (KAS) III in Soybean

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A cDNA clone, encoding soybean [*Glycine max* (L.) Merrill.] β-ketoacyl-acyl carrier synthase (KAS III), that might be involved in the regulation of fatty acid synthesis, was previously isolated using a soybean KAS III partial sequence obtained from RT-PCR using degenerate primers. The expression of this KAS III in different tissues of a soybean plant and during embryo development was investigated. A 1.9 kb transcript was detected by Northern analysis using this cDNA clone as a probe. The expression level of KAS III was highest when developing embryos were between 100 and 200 mg (approximately 30 DAF) in size. Multiple sequence alignment of the deduced amino acid sequence from the soybean cDNA clone revealed a high degree of sequence similarity with other previously reported KAS IIIs. A KAS III genomic clone was also isolated, and its sequence was used for structural analysis of the KAS III gene. The genomic clone contained 8 exons separated by 7 intervening sequences varying from 78 to 931 bp in length. This clone encoded a 397 amino acid polypeptide, including a putative signal peptide at the N-terminus. Southern analysis of total genomic DNA indicated that there were at least two KAS III genes present in soybean. Northern blot analysis revealed that the mRNA level for the KAS III gene in high-oil soybean lines was lower than that in low-oil soybean lines, suggesting that the KAS III gene expression appeared to be regulated by feedback inhibition of one of the end products through fatty acid synthesis. Expression profiles and sequence analysis of the KAS III gene with other genes involved in fatty acid synthesis will be presented.

P515

QTL Associated with Cell Wall Polysaccharides in Soybean Seed

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Cell wall polysaccharides (CWP) are a significant part of the total dry matter deposited during soybean seed development. Quantitative trait loci (QTL) analysis for CWP may reveal loci and perhaps genes useful in altering CWP in soybean seed. The objective of this study was to identify QTL associated with CWP variability in soybean seed using Minsoy x Archer RILs. CWP were hydrolyzed and quantified as their monosaccharides using the Uppsala total fiber assay. Three major locations for QTL were present for CWP in whole soybean seeds on linkage Groups U3-A2, U7-A1 and U24-K for fucose, galactose, and arabinose, respectively. Comparison of coatless embryos and whole seeds indicated that most QTL were due to CWP variation in coatless embryos. For coatless embryos there was a multi-trait locus present on U7-A1 for galactose, rhamnose, glucose and total CWP that seem to represent pectic polysaccharides. A smaller pair of QTL on U13-F for glucose and xylose may represent hemicellulose or cellulose polysaccharides.

SOY 2002

Posters
Molecular and Cellular Pathology

Abstracts P601-P608

P601

Resistance Loci Pyramids Alter Transcript Abundance in Soybean Roots Inoculated by *Fusarium solani* f. sp. *glycines*

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This study aimed to identify genes (ESTs) with altered mRNA abundance in soybean roots in response to Fsg inoculation. Unique sequences were enriched using suppression subtraction and differential display. The mRNA abundance was quantified in inoculated and non-inoculated roots by macroarray hibridizations. A unigene set of 135 ESTs was identified and used in a macroarray analysis. The mRNA abundance of 28 cDNA fragments was increased more than two-fold in inoculated compared to the non-inoculated roots of RIL 23. In Forrest and Essex, only one mRNA increased two-fold in inoculated roots compared to the non-inoculated roots. In Essex most mRNAs decreased in abundance (61/135 had a two-fold decrease) while in Forrest most mRNA abundances did not change. Among the 28 cDNAs with two-fold or higher increase in mRNA abundance in RIL 23, 14% encode for proteins known to be involved in plant defense, 21% in metabolism, 14% in cell structure and 4% in transport. Un-annotated ESTs accounted for 43% of the genes, 4% of the sequences were previously unknown. In Essex, genes involved in plant defense, cell wall synthesis, ethylene synthesis and metabolism were decreased in mRNA abundance in inoculated roots. The different response of the 2, 4 and 6 gene pyramids suggests SDS resistance QTL serve to delay symptoms or confer resistance by maintaining normal or increase in gene expression after inoculation/infection. This research was funded in part by grants from by ISPOB, United Soybean Board (USB), Illinois Council on Food and Agriculture Research (C-FAR) and the crop genome program of the NSF.

P602

Microscopic Study of White Mould Resistant (OxO Transgenic) Soybean Plants and the Wild Type Parental Line Infected by White Mould (*Sclerotinia sclerotiorum*)

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White mould, caused by the pathogen *Sclerotinia sclerotiorum*, devastates a broad range of crop plants, including soybean. This pathogen is unique as it releases oxalic acid which promotes infection by inhibiting the oxidative burst in the host which normally would signal plant defence response[1], by weakening the cell walls of the host plant by chelating calcium from wall calcium pectate[2], and by increasing the acidity of the environment which promotes the activity of fungal-derived plant cell wall degrading enzymes[3]. Transgenic soybean lines (20B7) have been developed that contain the transgene wheat germin (*gf-2.8*)[4] coding for an oxalate oxidase (OxO) that degrades oxalic acid to CO_2 and H_2O_2.

Assays show that OxO activity occurs in major plant organs of 20B7 including leaf, stem and floral tissue. New flowers *in planta* of the transgenic and the untransformed parental lines (X5) were inoculated with 5000 ascospores of *S. sclerotiorum* per 10 µl and ranked using a disease severity index (DSI). Within several days after infection, the DSI for both plant types was similar. By five days after infection the disease had progressed to the leaves and stems of the X5 plants, but not 20B7 plants. Microscopic observations of fungal growth on the floral, leaf and stem tissue of these plants will be used to determine where resistance occurs, at the tissue level, in the transformed line.

Ascospore germination, growth, and infection on petal tissue appears similar between both X5 and 20B7. Ascospores germinate on both hosts, the hyphae appear to grow at a similar rate, and infection in both lines occurs within 24 hours. Mycelia on excised leaves of X5 and 20B7 form similar looking infection cushions, and fungal growth patterns within the leaf tissue is also similar with fungi growing subcutaneously and intercellularly in the cortex. Comparisons of several infected leaf sections reveal that hyphae in the transgenic tissue have very large vacuoles, that sometimes occupy nearly the diameter of the fungi in cross section, while hyphae in X5 have small to medium sized vacuoles. This change in vacuole size may indicate stress to the fungi, and ultimately, the site of resistance in the transgenic host.

P603

Sequence Analysis of the I Locus in Williams 82

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A key enzyme of the flavonoid biosynthetic pathway is chalcone synthase (CHS). CHS is the last enzyme of a 4-step catabolic pathway that converts the amino acid phenylalanine into chalcone, a complex aromatic molecule that is the starting material for various plant secondary compounds including lignon, anthocyanidin pigments, phytoalexins, and flavones. In soybean, CHS is present as a gene family consisting of at least 7 members. We used a fragment of CHS4 to probe soybean BAC filters of Williams 82 genomic DNA. Several different BACs hybridized to the CHS probe and one clone, 104J7, was chosen to be sequenced. BAC 104J7 was restriction digested with either MunI, EcoRI, or HindIII and fragments were subcloned into the vector pGEM3Zf+. A diagnostic sequence was obtained by sequencing all subclones with universal primers off the vector. This sequence data was used to identify unique clones and to verify that soybean DNA was present. Unique clones were fully sequenced by primer walking and contigged using the software program Sequencher. Gaps were filled by either sequencing directly off the BAC, or by identification of new subclones using different restriction enzymes (either SstI, SstII, Sau3A, PstI, or AccI). BAC 104J7 is approximately 105,000 bases and gene rich. The I-locus, consisting of CHS4, CHS3, and CHS1 is present on 104J7 in duplication, which has complicated sequencing efforts because the individual family members are over 95% similar. Further annotation of this locus, as well as that of neighboring genes on this BAC clone, will be presented.

P604

Effect of *Fusarium solani* f. sp. *glycines*-cell free culture filtrates on the protein profiles of soybean cell suspensions.

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Fusarium solani f. sp. *glycines* (Fsg) has been reported to produce at least two phytotoxins. Li and her collaborator (1999) have shown that cell-free culture filtrates prepared from 12-day-old Fsg cultures develop sudden death syndrome (SDS) leaf symptoms in a susceptible soybean cultivar. They have also shown that the same cell-free Fsg-culture filtrates cause cell death in cell suspensions developed from a susceptible soybean cultivar. We have prepared cell free extracts from two 12-day-old Fsg isolates. One of these two isolates produced SDS symptoms in leaves of a susceptible cultivar Williams 82 three days following feeding the seedlings with the culture filtrates through cut ends. No symptoms were recorded in the medium control. Currently we are evaluating the responses of cell suspensions prepared from the cv. Williams 82 to the Fsg-culture filtrates. We will be testing the protein profiles of soybean cell suspensions and leaves following treatment with the Fsg cell-free culture filtrates. Long-term goal of the present study is to apply proteomics approaches for identifying those soybean proteins that are specifically degraded by the Fsg-specific toxins of the culture filtrates.

P605

Analysis of Paralogous *Rps*1-k Sequences in Soybean

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*Rps*1-k gene has been conferring race-specific resistance of soybean against most *Phytophthora sojae* races for the last two decades. It has been also the most widely used *Rps* gene among soybean cultivars grown in the North Central United States. We have mapped this gene to a locus physically spanned by 3 overlapping bacterial artificial chromosome (BAC) clones. Through sequencing and finger-print analyses, ten paralogous CC-NBS-LRR-type sequences were identified from these three BAC clones. These paralogous sequences share very high identity at both nucleic acid and amino acid levels. In fact two paralogs are identical. Comparison of sequences of these ten genes indicated that duplication followed by mutation, and then rounds of duplication and recombination between groups of genes resulted in the evolution of these paralogous sequences. Non-synonymous substitutions among these paralogs mainly occurred within the LRR region suggesting that the LRR region have been under diversifying selection. Two of these genes were expressed in stable transgenic soybeans. Both genes were shown to encode *Rps*1-k-mediated resistance to *P. sojae* race 4 in transgenic plants.

P304

Global Expression in Developing Seeds of Soybean (*Glycine max*) Transformed with a Lectin-CHS6 Construct

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Soybean (*Glycine max*, cv. Jack) somatic embryos were bombarded with a plasmid containing the soybean chalcone synthase 6 (CHS6) gene inserted in a lectin cassette to induce expression of the transgene in the seed. After selection, a transgenic line was identified by Southern hybridization. T0 plants were regenerated and the line was brought to the T3 generation. No homozygous CHS+/CHS+ has been recovered and the inheritance of the transgene has been lower than expected: 2 transgenic to 43 non-transgenic in the T1 generation, 11 to 19 in the T2 generation, 8 to 15 in the T3 generation. Northern hybridizations do not show significant changes in the expression of CHS in the seeds carrying the transgene. However, transformed plants have lower seed set and lower number of seeds per pod than wild-type plants. Various causes could be invoked for the observed phenotype, among which are somaclonal variation and disruption of an endogenous gene by the transgene. In an attempt to find differentially expressed genes in the transgenic and the wild-type line, we hybridized a soybean microarray with cDNA from developing seeds of heterozygous transgenic versus non-transformed plants. The array represents 9,276 unigenes selected from libraries constructed from cotyledons (2 stages), flowers (2 stages), seed coats and 1-2 cm whole pods. Preliminary results show that with a 2-fold threshold, only a handful of genes is differentially regulated in the transgenic line. Interestingly, lectin is down-regulated in the transgenic line while no other storage protein shows any difference in expression.

P305

Microarray Analysis of Gene Expression during the Induction of Soybean (*Glycine max*) Somatic Embryos

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Somatic embryos are the tissue of choice for soybean (*Glycine max*) transformation by particle bombardment. Embryos are induced from immature cotyledons on high-auxin medium. However, not all soybean cultivars respond positively to tissue culture. A genomics approach was used to profile gene expression during the reprogramming of cotyledon cells associated with induction. Microarrays were spotted with 9,000 soybean unigenes identified by sequencing cDNA libraries made from embryos (2 stages), seed coats, immature flowers, mature flowers, and 1-2-cm pods. Cotyledons of *Glycine max* cv. Jack were collected at four time points (every 7 days) during the induction of somatic embryos on 40 mg/L 2,4-dichloro-phenoxyacetic acid (2,4-D). The adaxial side of the cotyledon, where the somatic embryos appear was separated from the abaxial side, in contact with the medium. Total RNA was extracted from both parts of the explants at each time point. In a first series of experiments, the two sides of cotyledons at the same developmental stage were labeled with fluorescent dyes and competitively hybridized to the array. Only 10 genes showed differential expression at time points 1 and 2, suggesting de-differentiation of the cotyledon and callus formation. At time point 3, 71 genes were overexpressed in the adaxial side, many of which are involved in signal transduction and cell organization. In a second series of hybridizations, labeled cDNA from developing embryos (adaxial side) at adjacent time points were competitively hybridized to the same array in order to determine changes in gene expression overtime.

P306

Biolistic Transformation, Recovery, and Expression of Fertile Soybean Transgenics for Chitinase and *Pto* Genes

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Our long-term goal is to control soybean diseases through the use of genetic transformation. The diseases we have specifically targeted include charcoal rot, caused by the fungal pathogen *Macrophomina phaseolina* (Tass) and Soybean Cyst Nematode (SCN) caused by the nematode *Heterodera glycines* (Ichinohe). One of our approaches is to constitutively over-express plant and insect chitinase genes. The hypothesis is that overexpression these proteins will reduce the virulence or fitness of the fugal pathogen or SCN. Alternatively, we also have over-expressed the tomato gene (*Pto*) conferring resistance to *Pseudomonas syringae*. Over-expression of the *Pto* gene in tobacco has been demonstrated to confer broad resistance to bacterial, fungal, and viral pathogens. The experimental strategy was to biolistically transform soybean with a vector DNA containing a rice chitinase gene (*Chi11*), tobacco hornworm chitinase gene (*Msc*), and *Pto* gene, driven by the CaMV 35S promoter and linked to the *Hpt* gene as a selectable marker. Immature embryos of soybean cultivars 'Chapman', 'Jack', and 'Fayette' were bombarded, and several independent clones were selected on hygromycin-containing media and regenerated into plants. The majority of transgenic plants were morphologically normal and self-fertile. The integration, inheritance and expression of the genes have been confirmed by molecular analysis of T_1 and T_2 soybean transgenic plants. Independent transformants have been confirmed by the polymerase chain reaction (PCR) to contain our selectable (*Hpt*) marker gene and the gene of interest. The presence and estimated copy number of inserts were detected by Southern blot analysis. Northern blotting and Western blotting confirmed the expression of transgenes. Progeny from the *Pto* and chitinase-positive plants were tested for their resistance to the charcoal rot and soybean cyst nematode. The degree of resistance displayed by these transgenic plants was correlated with the level of the gene expression.

P307

Analysis of Expression of the Green Fluorescent Protein (*gfp*) Gene in Transgenic Soybean Obtained Using Sonication Assisted *Agrobacterium*-Mediated Transformation (SAAT)

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Proliferative embryogenic cultures of soybean (cv 'Jack') were transformed using Sonication-Assisted *Agrobacterium*-mediated Transformation (SAAT). *Agrobacterium* strain EHA 105 containing genes encoding hygromycin phosphotransferase and an ER-targeted jellyfish green fluorescent protein (GFP) were utilized in this study.

Expression profiles of gfp were examined in proliferative embryogenic cultures and in numerous tissues of regenerated plants and their progeny. Embryogenic cultures were examined using a fluorescent dissecting microscope while leaves, pulvini, stems, flowers, pods, immature zygotic embryos, ovules and roots were examined using laser scanning confocal microscopy. In proliferative embryogenic tissues, expression was somewhat variable with observation of both high and low expressing areas within a fairly homogeneous tissue type. In this tissue, high levels of gfp expression appeared to lead to tissue necrosis, but this was not always the case. In whole plant tissues, gfp expression was most apparent in epidermal and vascular tissues.

Tracking of GFP appears to be a very valuable tool for studying gene expression in soybean as well as other plants. Efforts are currently underway in the laboratory to introduce other promoter/gfp fusions into soybean to gain a better understanding of promoter control of gene expression in this plant.

Improving Plant Metabolism Using Trans-Acting Factors

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The standard approach for changing metabolic steps for plant improvement is to increase or decrease transcription of genes encoding key enzymes for such metabolism. Designing trans-acting factors or DNA-binding proteins to target these genes is another method for plant improvement. Among the most common and best-characterized DNA-binding proteins are the zinc finger proteins (ZFPs). ZFPs comprise a large class of DNA binding proteins characterized by tandem arrays (fingers) of related sequences containing conserved histidine and cysteine residues that chelate zinc ions. There are several families of ZFPs that differ in the length of the repeat units (fingers), the number of fingers, the number of zinc ions they chelate and the presence and positions of conserved cysteine and histidine residues. ZFPs are now well known in all higher organisms including plants and have been used to change gene expression in animals and yeast. We have utilized class I ZFPs, also known as Cys2/His2 or C2-H2 zinc fingers containing the modular KRAB domain, for repression experiments in plants. In this class, each finger is approximately 30 amino acids in length, binds one zinc atom and contains an antiparallel beta sheet followed by an alpha helix. We utilized three approaches for evaluating use of ZFPs for regulating plant gene expression: 1) transient expression studies; 2) chromosomally integrated targets; 3) an endogenous gene known to be changeable. The GUS gene in tobacco cells was the target for the transient expression and chromosomally integrated target studies. The endogenous targets were soybean and Arabidopsis *FAD2* genes. All three approaches were successful for repressing gene expression in plant cells. In the case of some matured soybean somatic embryos with introduced ZFPs targeting the *FAD2-1* gene we were able to reduce the linoleic (18:2) and linolenic acid (18:3) levels as much as 10-fold with a four-fold increase in oleic acid (18:1). Collectively these results demonstrate that trans-acting factors such as ZFPs can be utilized for improvement of crops including soybeans. This new crop genetic approach is likely to have greater specificity in some cases than standard techniques such as RNAi.

P309

Transformation of Soybean with the Tryptophan Biosynthetic Control Enzyme Gene

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Anthranilate synthase (AR) is the key biosynthetic control enzyme in the synthesis of tryptophan (Trp), indole-3-acetic acid, and indole alkaloids. The holoenzyme consists of two α-subunits and β-subunits. AS is feedback inhibited by the end product Trp, which binds to an allosteric site on the AS α-subunit. A cDNA clone that encodes a naturally occurring feedback-insensitive anthranilate synthase, ASA2, isolated from a 5-methyltryptophan (5-MT) – resistant tobacco cell line under the control of the constitutive cauliflower mosaic virus 35S promoter, was introduced into soybean [*Glycine max*] by both *Agrobacterium tumefaciens* and particle bombardment. Three lines were obtained from *Agrobacterium* mediated transformation with 5-MT selection using a mature seed embryo axis system. The ASA2 gene was expressed in leaf tissue as demonstrated by northern-blot hybridization analyses, the presence of feedback-insensitive AS and an increase in free Trp. The genes were inherited by progeny. Another 13 ASA2 transformed lines were taken from bombarded embyogenic tissue following hygromycin selection that have not been analyzed. However, two of these ASA2 transformed embryogenic lines grew in concentrations of 25uM α-MT, whereas the untransformed controls were inhibited. The results suggest that the ASA2 α-subunit can interact with the native soybean β-subunit to form an active enzyme to overproduce Trp and cause Trp analog resistance. Thus, the ASA2 gene has the potential to be a new selectable marker for plant transformation.

SOY 2002

Posters
Metabolic Engineering

Abstracts P501-P515

P501

Genetic Modification of Soybean Seed Isoflavone Content and Composition

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Soybean isoflavones (daidzein, genistein and glycitein and their glucosyl and malonylglucosyl conjugates) have significant beneficial effects on human health. Our goal is to improve the health promoting value of soybean via genetically manipulating (increasing or decreasing or altering composition) seed isoflavone and related phenolic levels.

We have performed soybean transformation via particle bombardment of embryogenic cultures (cv. Jack) with a construct pGLH-2 containing the phenylalanine ammonia-lyase (*PAL*), chalcone synthase (*CHS*) and isoflavone synthase (*IFS*) under the control of the seed-specific soybean lectin promoter. Presently we have a large number of soybean plants in the greenhouse transformed with the *PAL* gene in sense and antisense orientations. The *PAL5* gene is expressed in a number of lines and up to 30% increased isoflavone content was found in seeds of some plants and in another line a 50% reduction in seed isoflavone levels was detected. Gene combinations (*PAL5, CHS* and *IFS*) have been also used with the selectable marker gene *HPT* (hygromycin phosphotransferase) as a mix of plasmids or expression cassettes to bombard the embryogenic cultures. Hygromycin resistant lines have been obtained with all four genes. In all these cases we used the seed specific soybean lectin promoter. The plants will be regenerated soon.

This study was supported in part by the funds from the Illinois Soybean Program Operating Board, the United Soybean Board, NATO Collaborative Research Program (ref. LST.CLG.976259; JSTC.CLG.978212), the Illinois Agricultural Experiment Station, and the USDA Agricultural Research Service.

P502

Expression of genes regulating phenolic metabolism in soybean hairy roots

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Many important fungal pathogens reside in the soil and infect soybean roots leading to significant yield losses. Higher levels of crop resistance to fungi could be obtained by understanding and then genetically manipulating metabolic events that could lead to production of anti-microbial compounds or can strengthen the barriers of the plant cell to the pathogen entry.

Due to the difficulty of working with roots and root pathogens, hairy root cultures could simplify the study of soybean plant root-pathogen interactions as a model system to test the possibilities of metabolic engineering of plant disease resistance at the root level.

To engineer the resistance to root invading pathogens the target genes used for transformation should be driven by a root specific promoter. The present study aimed to use hairy root culture to investigate the regulation of phenylpropanoid metabolism via *Agrobacterium rhizogenes* transformation using the important pathway genes fused to rolD root specific promoter (from the Ri-plasmid of *A. rhizogenes*). We found that the rolD promoter is more active than the CaMV35S promoter in expressing the *GUS* gene in soybean hairy roots, which indicates that rolD could be useful for genetic engineering of root disease resistance. The constructs have been made that contain important genes for isoflavone synthesis: isoflavone synthase (*IFS*), chalcone synthase (*CHS*) and chalcone reductase (*CHR*) driven by either rolD or constitutive CsVMV promoters.

Both the individual genes and a mix of genes have been used for the *A. rhizogenes* mediated soybean hairy root transformation. The presence of target genes was confirmed in a number of lines obtained after kanamycin selection. The plasmids are under construction presently that would contain the genes important for lignin synthesis.

This study was supported in part by the funds from the Illinois Soybean Program Operating Board, the United Soybean Board, NATO Collaborative Research Program (ref. LST.CLG.976259; JSTC.CLG.978212), the Illinois Agricultural Experiment Station, and the USDA Agricultural Research Service.

P503

Real Time Expression Analysis of the Soybean Chalcone Synthase Gene Family Members

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Chalcone synthase, a key rate limiting enzyme in the flavonoid biosynthetic pathway is encoded by a single copy gene in some plants such as *Petroselinum crispum* to as many as 8 genes in bean. In *Glycine max*, at least 7 CHS genes have been previously reported. In our analysis, the Soybean EST database has indicated another potential candidate that has temporarily been named as CHS1H. Earlier studies on the structural organization of this gene family in soybean report the existence of a duplicated 10 KB region (*I* locus) comprising of *chs1*, *chs3* and *chs4*. In its dominant form the *I* locus exhibits a form of cosuppression leading to a lower level of CHS transcripts and absence of seed coat pigmentation. The existence of multiple copies, 5 of which are nearly 95% similar in the coding region at the nucleotide level, illustrate the difficulty in distinguishing the expression of gene family members in higher plants. Some techniques as fingerprinting based on conventional RT-PCR will give qualitative but not quantitative data on the individual family members.

Towards understanding the expression patterns of the multiple CHS genes, we have employed a powerful and sensitive technique, fluorescent real time RT-PCR, for the gel free detection and quantitation of gene expression. A variant of this technique, Taqman®-5'-nuclease assay, based on fluorescence resonance energy transfer of an oligonucleotide hybridization probe, was used to study the gene specific expression of the CHS family members. Gene-specific Taqman® primer-probe sets were designed using Primer Express. Total RNA was isolated from leaves, roots, seed coats and cotyledons, DNase treatment was optimized and cDNA generated using oligo dT primers. Relative transcript levels within each tissue were measured via amplification of the cDNA using the ABI Sequence Detection System. We have found expression of five of the different genes in both cotyledons and seed coats. Using these approaches we can profile the relative levels of transcripts of the family members to determine whether there is developmental, tissue, or organ specificity in the expression of the individual CHS genes and if they respond differently to environmental cues. Another application of the gene specific RT-PCR will be to determine the effect of gene silencing exhibited by the *I* locus on the expression of the individual CHS gene family members.

P504

Identification and Isolation of Genes from Soybean (*Glycine max*) Orthologous to Cold-Regulated Genes Found in *Arabidopsis thaliana*

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Some plants possess the ability to acclimate to cold temperatures if first exposed to a low non-freezing temperature. A number of the genes involved in cold acclimation and cold tolerance have been isolated from *Arabidopsis thaliana*. Among the genes that have been characterized are some that encode cold regulated proteins and transcription activators. It has been proposed that all plants possess similar genes and that difference in phenotype result from the regulation of these genes. Based on this premise, studies of major crop species have been undertaken to determine if they possess cold regulated genes, and to determine differences in gene expression that results in the inability of a plant to survive exposure to low temperature. Among the crop plants that are being investigated is soybean. To identify potential orthologs to the cold genes that have been isolated from Arabidopsis, the TIGR soybean EST database was searched using the Arabidopsis gene sequences as queries. Once potential orthologs had been identified, these sequences were then used to design primers to allow for the amplification of these genes from genomic soybean DNA using PCR. This process has successfully been used to isolate sequence encoding a putative CBF1 ortholog and putative orthologs to COR genes.

P201

Identification of Putative Yield Enhancing QTL from Exotic Soybean Germplasm

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This study evaluates exotic soybean germplasm as a source of genes for the improvement of seed yield in North American domestic soybean germplasm. A population was developed by crossing lines of domestic and exotic origin. Traits including days to flower, days to maturity, reproductive period, plant height, lodging, seed yield, and seed protein and oil content were evaluated in replicated field trials at multiple locations in 2000 and 2001. A genetic linkage map was constructed using simple sequence repeat markers that span the 20 linkage groups of the soybean genome. Significant quantitative trait loci (QTL) were detected using single marker-trait analysis and composite interval mapping methods. QTL introgressed from the exotic germplasm with positive effects on yield were identified. These results suggest that there may be useful genes for yield in exotic soybean germplasm.

P202

Assessment of Genetic Relationship among Soybean Cultivars Using DNA Amplification Fingerprinting (DAF)

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DNA amplification fingerprinting (DAF) with arbitrary oligonucleotide primers can identify cultivars and specify relationships between closely related plant accessions. Genetic relationships among 21 cultivars of soybean were investigated using DAF markers as discriminating characters. Resolved bands were scored for their presence or absence in a binary matrix and relationships among cultivars calculated using simple matching coefficient. The results have assisted in the development of a dendrogram suggesting genetic relationships among genotypes. Cluster analysis by unweighted pair group method using arithmetic averages showed that 21 cultivars can be placed in two main groups with a similarity ranging from 0.69 to 0.88. Variability observed had a narrow genetic base and it is necessary to expand the diversity of the soybean genetic base with exotic germplasm. The results also indicated that DAF technology can generates molecular markers that can be used reliably for DNA fingerprinting of important cultivars. This tool could be useful in determining relationships as well as and diversity and also in the identification of soybean varieties.

P203

Pedigree Analysis of a Major QTL for Soybean Resistance to Southern Root-knot Nematode

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In the USA, the southern root-knot nematode [Meloidogyne incognita (Kofoid and White) Chitwood] (Mi) is a serious pathogen of soybean [Glycine max (L.) Merr.]. Many soybean cultivars with Mi resistance and high productivity have been developed in the USA over the past few decades. RFLP and SSR markers have been used to identify a major QTL near the top of LG-O conferring resistance to Mi. This allowed a DNA molecular analysis of the source of resistance in elite soybean cultivars with the aid of pedigree information. The objective of this study was to determine the frequency of elite Mi-resistant cultivars that inherited the major LG-O resistance QTL from a single ancestral parent based on their consistent genotypic pattern at flanking SSR marker loci. Forty-eight soybean lines, including ancestral, Mi-susceptible, and Mi-resistant cultivars, were analyzed at six SSR loci that flank the major QTL for Mi resistance on LG-O. Codescent analysis of markers and phenotypes showed that Mi-resistant cultivars inherited a 200bp band at Satt358 and a 238bp band at Sat_132 from Palmetto. The results of this study indicate that marker-assisted selection for the major Mi-resistance QTL on LG-O should be highly effective.

P204

SNP on RFLP markers for Seed Protein Concentration in Soybean

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Breeding efforts have been directly toward the improvement of soybean seed protein, because soybean seed is a major source of protein for animal feed and human consumption. This study was performed to identify single nucleotide polymorphism (SNP) among four soybean genotypes associated with RFLP markers for soybean seed protein reported in SoyBase. Based on RFLP markers which were closely linked to soybean seed protein concentration in soybean, 12 sets of primers are designed to find SNPs in Sinpaldalkong2, SS2-2, Danbaekkong, and Taekwangkong. Clustal analysis revealed not only a plenty of SNPs but also indels. A total of 144 SNPs and 18 indels were found around genomic region (near 5,000bp). Of specific interest was Danbaekkong-specific SNP at A688 marker on LG I which were reported to be a major QTL for soybean seed protein concentration. These SNPs can be used in a marker-assisted selection for high soybean seed concentration by designing trait-specific selection kit.

P205

Identification of QTL Conditioning Fibrous Rooting in Soybean

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Fibrous rooting in soybean, *Glycine max* (L.) Merr., is a trait that has been associated with drought tolerance. Plant introduction, PI416937 was shown to possess fibrous rooting and has been used as a parent in breeding programs to improve drought tolerance. Little information is available on the number or genomic location of QTL conditioning fibrous rooting in soybean. The objective of this study was to identify QTL conditioning the fibrous rooting trait in a soybean population of 240 F6-derived lines from a cross of Benning (coarse) x PI416937 (fibrous). The 240 lines and parents were grown in the field near Athens GA in 2001. The lines were planted in 6-plant hill plots in nine replication of a randomized complete block design. At the R-5 stage of development, the roots were dug with a mechanical peanut inverter. Data on 177 SSR markers that spanned the soybean genome were collected on the 240 lines. Nineteen SSR markers on 10 linkage groups were identified to have significant association with fibrous rooting ($P<0.01$). Nine QTL detected by Map Manager QTX accounted for 55 % of the variation of fibrous rooting. A major QTL on LG I explained 10 % of the phenotypic variation and was located 1cM from Satt292. The genomic location and effect of this and the other eight QTL conditioning fibrous rooting will be presented.

P206

Molecular Mapping of Resistance Genes in Soybean Cultivars Showing a Broad Range of Resistance to SMV Strains

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Soybean Mosaic Virus (SMV) disease is one of the most important disease. SSR marker analysis was used to determine the chromosomal location of resistant gene loci using two recombinant inbred line (RIL) populations, Pureunkong x Jinpumkong 2 (NPJ) and Keunolkong x Sinpaldalkong (KS). Phenotypic data on resistance to SMV, SMV-G1 to G7H strains were obtained from parents and RILs. Resistance to SMV was evaluated 4 weeks after inoculation of each SMV strain on unifoliate leaves. For the genotypic data, SSR-PCR products were separated on 6.5% polyacrylamide gel. In KS populations, resistance to SMV-G5 was linked to SMV-G7. Recombinant line to two SMV strains was not detected, indicating that a resistant gene may be related with resistance to different SMV strains. For NPJ population, resistance genes to SMV-G4, G7, G7a, and G7H strains were placed into two linkage groups. There were a few recombinant lines to tested SMV strains. Two lines to SMV-G7 strains in linkage group B2 and five lines to SMV-G7H strains in linkage group F showed susceptible mosaic symptom, which was different from parent symptom to tested SMV strains. Comparison with previously reported molecular-map order suggested that reaction loci to SMV-G4 and G7 strains in NPJ populations were similar with Rsv1 and Rsv3 locus, respectively. In KS populations, resistance locus was similar to Rsv1 locus. Resistant loci to SMV-G7H on linkage group F and SMV-G7a on linkage group B2 in a NPJ population are thought to be putative new resistant gene loci.

P207

Mapping a Gene Associated with Phytic Acid Levels in Soybean Seed

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A gene associated with phytic acid (PA) levels in soybean seed was mapped in a 'Boggs' (high PA/low inorganic P) x CX1834-1-2 (low PA/high inorganic P) F2 population. Reduced PA is desirable because PA ties up much of the seed P in a form that nonruminant animals are unable to use. In addition, PA is a strong chelator of iron, zinc, calcium, and magnesium, thus reducing their nutritional availability. Soybean cultivars with reduced PA levels would therefore be desirable for both nutritional and environmental reasons. A locus with a stron influence on PA levels was mapped to linkage group N using simple sequence repeat (SSR) markers. DNA markers close to this gene can be used for marker-assisted selection to introgress the mutated form of the gene into elite cultivars, and localization of the gene may provide clues about its role in PA biosynthesis.

P208

SSR Band Variation Among Soybean Lines Selected from within Elite Cultivars for Seed Weight and Seed Protein

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Divergent honeycomb selection was used to investigate the genetic diversity within the elite soybean [*Glycine max* (L.) Merr.] cultivars Benning, Cook, and Haskell. This selection method, applied in the absence of competition, uncovered single plants with significant variation in seed weight and seed protein. To determine the origin of this variation, we compared the allele sizes of the selected plants, the source cultivar, and the source cultivar's parents using 31 simple sequence repeat (SSR) markers linked to previously reported QTL for seed weight and seed protein. While single-plant selections from Benning attributed 83% of the variant alleles to a parent, selections from Haskell and Cook derived only 67 and 55%, respectively, of the unique alleles from one of the parents. To verify whether these alleles existed in the source cultivar, we screened 372 plants of each line from the remnant of foundation seed using in the initial honeycomb selection. Variant alleles appeared at low levels for all markers tested among the 372 plants of Cook, for 94% of the markers in Haskell, and for 50% of the markers in Benning. As the oldest cultivar of the three, Cook showed greater *de novo* variation as a possible result of more cross-pollination and mutation events accumulating over time. The molecular analysis provides evidence that the within-cultivar variation found with honeycomb selection is due to latent as well as newly derived variation.

P209

Molecular Mapping of Genes Conditioning Oleic Acid Content in N00-3350 Soybean

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Oleic acid is a major fatty acid in soybean [*Glycine max* (L.) Merr.] oil with current cultivars possessing 18 – 22%. The fatty acid content of soybean is related to the flavor, stability, and nutritional value of the oil. Increasing oleic acid content would result in a decrease of the total saturated fatty acid content of soybean oil and increase the oil quality of soybean for human consumption. Several soybean genotypes with increased oleic acid content have been developed. The objective of this research was to map the genes conditioning increased oleic acid from N00-3350 (~ 55 % oleic acid) using simple sequence repeat (SSR) markers. The F2 generation of a population derived from the cross of G99-G725 x N00-3350 was used as a mapping population to locate the genes conditioning oleic acid. The results indicate that a major gene with an allele for increased oleic acid provided by N00-3350 is located on Linkage Group (LG) M. The SSR marker Satt540 on LG-M accounted for 20% of the variation in oleic acid content. To date, we have identified two additional QTL, one on LG-G near Satt394 which conditions 6% of the variation, and a second on LG-D2, near Satt154, conditioning 7% of the variation. At both QTL the N00-3350 allele increased oleic acid content.

P210

Analysis of QTL for Pod Dehiscence Based on Molecular Map in Soybean [*Glycine max* (L.) Merr.]

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Pod dehiscence (PD), sometimes referred to as shattering in soybean, causes serious yield loss in tropical and subtropical regions. However, breeding for resistance to PD is difficult due to the complicated genetic behavior and environmental interaction. The objective of this research was to improve breeding efficiency for resistance to PD based on SSR marker in soybean. To identify the genomic region associated with PD, QTL analysis was conducted using two F_2 derived F_{10} RIL populations, Keunolkong × Shinpaldalkong and Keunolkong × Iksan 10. Keunolkong is dehiscent, whereas Shinpaldalkong and Iksan 10 are non-dehiscent. PD was measured after oven drying the pod at 40 for 24hour. In Keunolkong × Shinpaldalkong, three QTLs were identified on the linkage group A, D1b+W, and J, accounting for 50% of the phenotypic variation. Especially, QTL at satt215 on LG J explaining 42.3% of the phenotypic variation was found to be a major QTL conferring PD. Using interval mapping method, QTL conditioning PD on LG J was also identified at satt215 showing 14.95 of maximum LOD (R^2 = 0.44). While in Keunolkong × Iksan10 three QTLs were identified on the linkage group D1b+W, F, and L, accounting for 26.4% of the phenotypic variation. Interval mapping revealed that QTLs conditioning PD on LG D1b+W and L. QTL linked to LG D1b+W was common in both populations. Compared with QTLs reported in SoyBase and other research results, QTLs for PD in LG A1, F, and L were newly identified. Results revealed that QTLs for PD were population-dependant. We concluded that a major QTL identified for PD may be used for minimizing soybean PD through effective marker-assisted selection.

P211

Molecular Beacons to Select for SCN Resistance at *rhg1* and *Rhg4*

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Resistant cultivars have long been used to control losses resulting from attack by the soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe). Molecular techniques to identify resistant genotypes increase the efficiency of programs focused on developing cultivars resistant to SCN. Single nucleotide polymorphisms (SNPs) were identified that are closely associated with *rhg*1 and *rhg*4, two important loci controlling resistance to SCN. In this study we examine two approaches for rapid identification of genotypes carrying alleles at *rhg*1 and *rhg*4 for resistance or susceptibility. Molecular beacons and locked nucleic acids (LNAs) were used to differentiate genotypes at these loci. Molecular beacons are hairpin shaped fluorescent oligonucleotide probes that can report the presence of a completely complimentary DNA target. Specificity is sufficient to distinguish targets whose nucleotide sequences vary by only a single base. The 5' end of a molecular beacon contains a fluorescent dye and the 3' end a fluorescence-quenching moiety. The hairpin structure places the fluorophore and the quencher in close proximity when the molecular beacon is not annealed to a complementary target. Under these circumstances fluorescence is inhibited. When the molecular beacon hybridizes to the target sequence, the fluorophore and the quencher are separated and fluorescence is observed. The second approach involves LNAs, which are a class of nucleic acid analogues developed to improve hybridization characteristics by increasing specificity and duplex stability with complementary nucleic acid targets. The LNA contains a methylene bridge that connects the 2'-O position to the 4'-C position, restricting the furanose ring conformation and increasing the specificity for the target sequence. In the application of LNAs for SNP detection, the LNA residue is placed at the 3' end of the allele specific PCR primer. The increased specificity of the 3' LNA residue decreases the occurrence of amplification in the presence of a 3' mismatch. The ability of these two approaches to identify genotypes carrying alleles for SCN race 3 resistance or susceptibility at the *rhg*1 and *rhg*4 loci will be presented.

P212

Soybean Repetitive Sequence Anchored to the Soybean Genetic Map

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More than 750 simple sequence repeat (SSR) and restriction fragment length polymorphism (RFLP) molecular markers have been used to identify bacterial artificial chromosome (BAC) clones in soybean. The markers anchor these BACs to the consensus molecular genetic map for soybean. Almost 60% of the end sequences from BACs identified by both types of markers produce significant similarity in BLAST database searches. Of the significant hits, the largest single category is repetitive sequence. Roughly half of the repetitive sequences have similarity to previously characterized long terminal repeat (LTR) and non-LTR retrotransposons. The remaining repetitive sequence represents novel soybean repetitive sequence which we are characterizing. Because the molecular markers used to identify the BAC sequences are spread across the soybean genome, we can look at the distribution of these novel repetitive sequences in the genome. This research was supported by the National Science Foundation Plant Genome Program.

P213

The Effect of the *rhg1* Gene on the Development and Reproduction of *Heterodera glycines* Race 3

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Since it first appeared in the U.S., Heterodera glycines has been a major cause of reduced soybean yield. The rhg1 gene is a major resistance gene that has been mapped to molecular linkage group G. A set of near-isogenic lines (NILs) was developed, allowing us to observe the effect of rhg1 on the development and reproduction of H. glycines. These NILs were derived from the F7 generation, differing only in the genomic region surrounding rhg1. To track nematode development, the NILs were inoculated with second stage juveniles (J2) and the number of the nematodes at different developmental stages was recorded in samples collected at 1, 4, 8, 12, 16, and 21 days after inoculation. NILs with the resistant rhg1 allele exhibited third and fourth stage juveniles (J3 and J4) at a peak 8 days after inoculation, compared to the susceptible NIL where the peak was reached 4 days after inoculation. In addition, development of mature females on the resistant NIL was reduced to just 33% of the number on the susceptible NIL. Mature females that developed on the resistant NIL were also smaller and contained fewer eggs. In a separate experiment, the NILs were examined for their potential to support egg production at 17 inoculum densities of H. glycines ranging from 50 to 1,638,400 eggs/plant. Mature females were harvested 40 days after inoculation to obtain eggs. At inoculum levels of 1,600 or lower, no more than 4,950 eggs were produced on either NIL. As the inoculum increased from 1,600 to 25,600 eggs/plant, eggs at harvest in the susceptible NIL increased to a peak of 111,062/plant, and then declined at higher inoculum levels. By contrast, a maximum of just 17,737 eggs was observed on the resistant NIL. Currently, histological observations are underway to compare H. glycines development between the NILs.

P214

Genetic Mapping of Genes Underlying Partial Resistance to Sclerotinia Stem Rot in Soybean Variety Asgrow A2506

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Sclerotinia stem rot is one of the most serious diseases in the U.S. and around the world. Sclerotinia stem rot can be found in most countries where environments are cool and moist and it infects a large host ranges. No sources of complete resistance to the disease have been identified to date. Soybean varieties Asgrow A2506 and NKS19-90 showed partial resistance to the disease. Kim and Diers (2000) studied the inheritance of partial resistance to the disease in NKS19-90 and identified three quantitative trait loci (QTL) conferring partial resistance to the disease in NKS19-90. The objective of this study was to identify putative QTL associated with sclerotinia stem rot resistance in the variety Asgrow A2506. A population of 140 of F_4 derived lines was developed from a cross Asgrow A2506 x NKS19-90. The population was tested at two locations where the disease pressure was induced by artificial inoculation. The tests were carried out with two replications at each location. The plots were rated for disease severity based on the rating system of Grau et al. (1982) at approximately the beginning of physiological maturity (R7). A disease severity index was calculated for each plot. The two parents of the mapping population were first screened with over 900 SSR DNA markers. Fifty five SSR markers that were polymorphic between the two parents were used to genotype the entire population. The computer program JoinMap was used to determine the linkage relationships among polymorphic SSR markers and the program QTL-Cartographer was used to test associations of the DNA markers with resistance to the disease. Both single marker analysis and composite interval mapping (CIM) methods were used in the analysis. No significant QTL were found with the CIM method. However, single marker analysis revealed that marker Sat_327 on linkage group C1 was significantly ($P<0.001$) associated with resistance to the disease.

P215

Genetic Mapping of Genes Underlying Agronomic and Seed Composition Traits in Wild and Cultivated Soybean

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Five BC_2F_4-derived populations were developed as the mapping populations using the *G. max* cultivar IA2008 as the recurrent parent and the *G. soja* plant introduction (PI) 468916 as the donor parent. There are from 57 to 112 BC_2F_4-derived lines in each population and a total of 468 lines for the five populations. All the lines in the five populations were evaluated in field tests for yield, maturity, plant height, and lodging during the summers of 1999, 2000, and 2001. Two replicates of the lines were grown at Lincoln in Nebraska and at Urbana in Illinois in each summer. Soybean seeds of all the lines were evaluated for contents of protein, oil, and fatty acids including palmitic, stearic, oleic, linoleic, and linolenic acids. The lines from each population were genotyped with a set of simple sequence repeat (SSR) markers that were determined to be polymorphic in the population from a preliminary screen of over 600 mapped SSR markers. Marker and trait data of each population were analyzed independently for both linkage and QTL analysis. The computer program JoinMap was used for linkage analysis and QTL Cartographer was used for QTL analysis. Composite interval mapping method was employed in the QTL analysis. The threshold of the LOD score for declaring a putative QTL significant at 5% experimentwise error rate level was determined by 1000 permutations. Four QTL were located for yield on linkage groups C2, E, K and M. Five QTL were identified for plant height on linkage groups, C2, E, K, M and O. Four QTL were located for maturity on linkage groups C2, L, M and O. One QTL was identified for lodging on linkage group K. Four QTL were detected for protein content on linkage groups C2, E, I and L and four QTL were found for oil content on linkage groups E, K, I, and L. One QTL was located for palmitic acid content on linkage group K and one QTL was detected for stearic acid content on linkage group C2. Two QTL, one on linkage group E and one on linkage group L, were found to affect both oleic acid and linolenic acid contents. A QTL on linkage group A1 were found to affect both oleic acid and linoleic acid contents. Favorable alleles affecting the agronomic and seed composition traits were identified from both the wild and the cultivated soybean parents of the mapping populations.

P216

Co-Inheritance of Brown Stem Rot and Soybean Cyst Nematode Resistance from PI88788

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Soybean lines carrying genetic resistance to soybean cyst nematode (SCN) from PI 88788 frequently carry resistance to brown stem rot (BSR), caused by the soil-born fungus *Phialophora gregata*. A SCN resistance quantative trait locus (QTL) and the BSR resistance genes *Rbs1*, *Rbs2* and *Rbs3* have all been mapped to the same region on linkage group (LG) J. The objective of this research was to map the location of BSR resistance QTL in 'Bell', a cultivar that carries SCN resistance from PI 88788. Ninety three F4- derived lines were developed from a cross between Bell and 'Colfax'. These lines were tested with molecular markers from LG J and for BSR resistance in the greenhouse and in two field locations. All polymorphic markers were significantly associated with BSR resistance. Marker alleles from Bell were associated with significantly ($p<0.001$) greater BSR resistance than Colfax alleles in all environments. Segregation of the simple sequence repeat (SSR) marker Satt547 explained 75% of the genetic variability in the field while the marker 21E22.sp2 explained 51% of the genetic variability for BSR resistance in the greenhouse environment.

P217

Examining SSR Markers for Iron Chlorosis

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Iron deficiency chlorosis (IDC) results in yield losses for certain soybean varieties, including some IDC-resistant varieties, grown on calcareous soils in the Midwestern United States. Because the environment plays an important role in IDC expression, breeders need environment-independent tools, such as marker-assisted selection, to expedite the development of varieties with improved IDC resistance. Resistance to IDC is controlled genetically, and quantitative trait loci (QTLs) previously have been identified using restriction fragment length polymorphisms (RFLP). Unfortunately, RFLP markers are impractical for breeding programs, but simple sequence repeat (SSR) markers may be a potential alternative to RFLP markers. We present preliminary data on the identification of SSR markers associated with IDC resistance in soybean. A breeding population was developed from the artificial-cross pollination of parents differing in both IDC resistance and yield potential. The F_1 seed harvested from the cross were advanced to the F_2 and $F_{2:4}$ generations. Chlorosis evaluation of the parents and $F_{2:4}$ lines was conducted in replicated field trials at two Iowa locations, Ames and Humboldt, on calcareous soils in 2001. Foliar chlorosis was measured four-weeks after planting using a 1.0 to 5.0 rating scale (1.0= no chlorosis to 5.0=severe chlorosis and plant death). Chlorosis data were subjected to analysis of variance (ANOVA). Although the mean chlorosis scores were not significantly different (P 0.05) between the parents at either location, the scores among $F_{2:4}$ lines were significantly different (P 0.05) at each location. In addition, the mean chlorosis score for each parent and the $F_{2:4}$ lines was approximately 2-fold greater at Humboldt than Ames. For SSR marker analysis, F_2 lines were genotyped with 16 polymorphic SSR markers genetically linked to IDC QTLs. The association of the SSR marker (i.e., allele segregation) and mean chlorosis scores was tested with single-locus ANOVA and regression analysis. Three SSR markers were associated (P 0.1) with chlorosis scores at each location; however, the identity of these markers was different between locations. The R^2-values ranged from 3.9% to 8.3%. We are conducting additional field evaluations in order to determine the reproducibility of the association between the SSR markers and IDC. Our preliminary results demonstrate the importance of environment on the expression of this disease and further supports the need for environment-independent tools in soybean breeding for IDC resistance.

P218

SSR Analysis of Soybean Protein QTLs in High Protein Plant Introductions

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Many soybean germplasm accessions have high levels of seed protein. To date, various soybean protein QTLs have been identified. Our objective was to use selective or extreme genotyping to identify genomic segments (i.e., QTLs) influencing seed protein content in F_2 populations of high-protein germplasm accessions mated to high-yielding soybean cultivars. Fifty-two Plant Introductions (PIs) with very high seed protein content (43.1 to 50.5%) were selected from seven maturity groups (000 - IV) to mate as females to seven recently released, high-yielding public cultivars, one from each maturity group. For 44 of the 52 matings, populations of 150 to 200 F_2 plants were grown in the summer of 2002. The parents of each mating have been screened for parental polymorphisms with 100 SSR markers distributed across all Linkage Groups (LGs). For each population, F_2 plants representing the lowest 10% and highest 10% of the range in F_3 seed protein will be identified by near-infrared reflectance (NIR). DNA of these 10% extremes will be assayed with SSRs. Parentally polymorphic SSRs bracketing genomic segments known to contain major protein QTLs on LG-I and LG-E have now been identified. These QTLs were chosen due to their statistical significance, multiple independent reports, and strong effects of seed protein as reported in the literature. Presence of QTLs in or near the bracketed region will be tested by chi-square deviation of the two parental SSR alleles from the expected 1:1 ratio in the low and high protein F_2 extremes. A single-marker ANOVA to assess marker-phenotype associations will also be conducted.

P220

Exploration of Origin and Evolution of Soybean (*Glycine max*) Based on Molecular Evolution of Internal Transcribed Spacer 1 Region of Nuclear Ribosome DNA

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Soybean (*Glycine max*) is one of important economic crops in the world crop production. It was domesticated from its nearest annual wild species *Glycine soja*. Both of them behave like one species. While their ancestor is still a question. The problem is explored from molecular evolution of the internal transcribed spacer 1(ITS1) region of nuclear ribosome DNA (nrDNA). Two special regions in ITS1 sequences of nrDNA from species in Glycine Willd. were identified. Length variation of the identified homologous region is stable between the two subgenera Glycine and Soja. But the identified most variable region is not stable among species and even individuals within a species in Glycine. The length variation of the two regions is produced by unequal crossover. If the identified two regions are excluded from the sequences the rest of them will be the same in length. This fact indicates that Soja and Glycine would come from a same ancestor. The data investigated also showed that mutation and unequal crossover occurred in the direction from Soja to Glycine. In combination with the fact that species of subgenera Glycine and Soja are geographically isolated in the temporary distributions and the fact that the base number in all tribes of Phaseoleae is x=11, a hypothesis has arisen. A putative common diploid ancestor would grow in the late of Mesozoic Era around 300 million ago while there was only one continent on the Earth. As the continent drifted and climate on the Earth surface changed, the possible ancestor growing in different environments could diverge, generating ancestor of annual wild soybean and ancestor of perennial wild soybean species respectively due to natural selection or genetic drift. Meanwhile their chromosome number would be duplicated, or then diploidized, forming Soja and Glycine in the East Asia and in Australia respectively as we see the geographic distribution character today. Key words: Glycine max - Soja (Moench) F.J.Herm. - Glycine - Glycine Willd. - ITS1 - mutation - unequal crossover - evolution - secondary structure.

P221

Efficiency of Microsatellite Markers for Resistance to Soybean Cyst Nematode (Race 3)

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Four microsatellite sequences were tested using DNA from soybean segregating genotypes developed at UNESP/FCAVJ. Three of them are close to the resistance locus rhg1 on molecular linkage group G (Satt309, Sat-168, Sat-163) and one is close to Rhg4 locus on group A2 (Sat-162). Progenies previously classified as resistant in field tests and others with unknown reaction were tested, using as a control the resistant cultivars Liderança and Renascença, and the susceptible cultivars OCEPAR-4 and Cristalina. The best primer for resistance to SCN was Sat-162, that produced bands for resistant genotypes (150 pb) and for susceptible genotypes (200 pb). Sixty resistant segregant progenies were submitted to tests with Sat-162, presenting the same band (150 pb) observed on resistant cultivars. Other 39 F3 and F4 progenies, with unknown reaction to the pathogen, were also analyzed and produced a segregation for 150 and 200 pb bands, allowing classification of the genotypes as resistant or susceptible. Previous studies have shown that this microsatellite marker is efficient for selection of genotypes carrying Peking-derivative resistance. All segregant progenies evaluated in this study had Peking in their genealogy. Thus, the obtained results with the Sat-162 showed that it can separate resistant and susceptible soybean genotypes, however further studies are necessary to confirm its efficiency.

SOY 2002

Posters
Transformation

Abstracts P301-P309

P301

Can TAIO (Targeting Agrobacteria Into Ovaries) be a New Approach for Crop Transformation?

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Arabidopsis thaliana transformation via *Agrobacterium* tumefaciens is an interesting and academic example of in planta transformation. A simple infiltration of *Arabidopsis* flowers with an *Agrobacterium* suspension (Bechtold et al. 1993) allows us to obtain a good number of transformants without a tissue culture step. The selection is done directly on the progeny with an appropriate selectable marker. Ye et al. (1999) showed that the *Agrobacterium* transformation target is the ovule and Bechtold et al. (2000) that the chromosome set of the female gametophyte is the main target for the T-DNA. Furthermore, in planta *Agrobacterium* transformation is an interesting tool because of the simple integration profile of the transgene in the plant genome compared to naked DNA technologies. We wondered if such a technology could also be developed for crops. We have evaluated an in planta approach for soybean called TAIO (Targeting Agrobacteries Into Ovaries). The idea is to deliver an *Agrobacterium* suspension in planta directly into the target organ ie the ovaries, passing through the physical barriers (membranes). *Agrobacterium* is then directly in contact with the ovules to transfer the T-DNA. In practice we used a micro-injection system involving the injection of the bacterium suspension into the ovaries via a micro-capillary connected to a micro-injector. This was done using a binocular microscope to control the dissection of the flower and to target the ovaries. In this poster we discuss the possibility of using this technology to routinely transform soybean.

P302

Gene Silencing in Transgenic Soybean Plants Transformed via Particle Bombardment

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Transgenes are susceptible to silencing in plants especially when multiple copies of the gene of interest are introduced. Transgenic plants derived by particle bombardment, the preferred method for soybean transformation soybean, have a tendency to result in multiple integration events. Three independent transgenic soybean plants obtained via particle bombardment were analyzed for transgene silencing. A GUS transgenic soybean line had at least ten copies of the GUS gene while there were approximately eighty copies of the transgene in the two soybean lines transformed with a 15 kDa zein storage protein gene from maize. Soybean plants transformed with the GUS gene showed variable GUS expression. The coding region and promoter of the GUS gene in the plants with low expression of GUS were heavily methylated. Variability in GUS expression was observed in the progeny of the high expressors in the T2 and T3 generations as well. Variable expression levels of the 15 kDa zein gene in transgenic soybean plants exhibited an inverse correlation with the level of transgene methylation. Transgenic plants were inoculated with the soybean mosaic potyvirus (SMV) to test the silencing mechanism since the SMV helper component proteinase is known to suppress post-transcriptional gene silencing. No suppression of transgene silencing in the SMV infected plants was observed. These results suggest that the silencing in the transgenic plants was not due to post-transcriptional gene silencing and also that SMV infection cannot suppress transcriptional gene silencing.

P303

Resistance to Bean Pod Mottle Virus in Transgenic Soybean Lines

Venkata Tavva, Randy Dinkins, Srinivasa Reddy, Said Ghabrial, and Glenn Collins

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Resistance to Bean pod mottle virus (BPMV) in soybean (*Glycine max*) cv. Jack was developed by transforming the capsid polyprotein (pCP) gene via particle bombardment of the somatic embryos. The binary vector (pHIG/BPMV-pCP) used in these experiments contained the BPMV-pCP coding sequence, an intron-containing GUS gene, and the hygromycin phosphotransferase gene. Southern blot hybridization analysis showed that 19 transgenic soybean plants selected for resistance to hygromycin contained the genes for GUS and BPMV-pCP. The progeny of five of these transgenic soybean plants were characterized in detail. An additional transgenic plant that contained the intron-GUS and hygromycin resistance genes, but lacked the BPMV-pCP gene was used as a negative control. Western and northern blot analyses showed that the expression levels of BPMV-pCP and pCP transcript were high in the five pCP transgenic plants. Infectivity assays with detached leaves demonstrated that all five pCP plants exhibited resistance to virus infection because they accumulated lower levels of BPMV compared with vector transformed control and nontransformed plants. Progeny of a homozygous transgenic line exhibited systemic resistance and this could potentially be useful in generating commercial cultivars resistant to BPMV.

SOY 2002

Plenary Session 3
Genomics

Wednesday, August 14
8:00 am

Abstracts 701-707

Medicago truncatula: Can crop species benefit from model organisms?

G.D. May

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Medicago truncatula (also known as "barrel medic" because of the shape of its seed pods) is a forage legume commonly grown in Australia, and is closely related to the world's major forage legume: alfalfa (*Medicago sativa*). However, whereas alfalfa has a complex genome consisting of four copies of each of its eight chromosomes and is an out-crossing plant species, *M. truncatula* has a simple diploid genome (2 x 8 chromosomes) and can be self-pollinated, greatly facilitating genetic analysis.

M. truncatula has been chosen by a large community of researchers in the United States, Europe and Australia as a model legume species for genomic studies. Its advantages are a small genome, fast generation time (from seed-to-seed) and high genetic transformation efficiency, features that are not shared with more agronomically important legume species. Genes from *M. truncatula* share high sequence identity to the corresponding genes from alfalfa and are arranged in a similar order on the chromosomes to those of other legumes. These features make *M. truncatula* an excellent model for understanding the genetics and molecular biology of agronomically important legumes with more complex genomes. As a legume, and unlike the most studied genetic model plant, *Arabidopsis*, *M. truncatula* established symbiotic relationships with nitrogen fixing organisms. Furthermore, the complex interactions of legumes with microorganisms have resulted in the evolution of a rich variety of natural product biosynthetic pathways impacting both mutualistic and disease/defense interactions.

More than 175,000 EST from 24 *M. truncatula* cDNA libraries have been generated. In addition, a genome sequencing project focusing on the gene-rich regions of the *M. truncatula* genome began at the University of Oklahoma earlier this year. Adding to this the expression, protein and metabolite profiling programs already in place, allows researchers to ask complex questions specific to legume biology. Sequence comparisons between *M. truncatula* and soybean will be discussed in addition to progress on transcript profiling, proteomics and metabolomics in *M. truncatula*. Collectively, these resources will allow us to begin to address the question: Can crop species benefit from model organisms?

Plant Genome Sequencing: Strategies and Expectations

Chris Town

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Two plant genomes have now been "completely" sequenced. The first, *Arabidopsis thaliana* (~120 Mb), was pronounced complete in December, 2000. The strategy adopted was a BAC by BAC approach that relied upon a physical map derived from filter hybridization, BAC end sequencing and BAC fingerprinting. The second genome, that of *Oryza sativa* (rice, ~400 Mb) has been the subject of several efforts in both the public and private sectors. Like Arabidopsis, the public rice effort is BAC based, which permits the relatively facile distribution of effort among continents, nations and sequencing centers. Currently, ~364 Mb of sequence have been finished by this effort. Other efforts have been both BAC-based (Monsanto) and whole genome shotgun (Syngenta Corporation, Bejing Genomics Institute) and have resulted in draft versions of the rice genome.

The challenges facing plant whole genome sequencing are manifold. There are numerous deserving candidate genomes, and the genomes of the most important crop plants in the US are relatively large, often due to ancestral polyploidy and relatively large amounts of repetitive (non-genic) DNA. Unlike the case with Arabidopsis (and possibly rice), the main goal of new plant genome efforts focuses on maximizing gene discovery at a minimal cost rather than completing the genome. In *Medicago truncatula*, cytogenetic evidence suggests that the euchromatic gene-rich region may occupy as little as 20% of the genome. Based upon this evidence, Bruce Roe (U. of Oklahoma) switched from a whole genome shotgun to a BAC-based approach using seed BACs known to be derived from gene-containing regions. In corn (*Zea mays*) over 50% of the genome is derived from transposable elements which are heavily methylated. A recently-funded NSF Plant Genome Project proposes to use a combination of techniques (methylation restriction, self-annealing) to enrich for non-methylated and low copy number sequences that are relatively gene-rich. The merits, accomplishments and expectations of the various approaches will be discussed.

Global Gene Expression Analysis of Soybean Using Serial Analysis of Gene Expression (SAGE)

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Understanding global gene expression patterns of Soybean may facilitate improvements in existing cultivars as well as cultivation techniques with great economic consequences. Serial Analysis of Gene Expression (SAGE) is a high throughput technique that can provide rapid and highly quantitative gene expression data on thousands of genes simultaneously. Unlike microarray analysis, SAGE has the potential for novel gene discovery as well. SAGE was utilized to study the gene expression profiles of a variety of Soybean tissues various stages of development. SAGE libraries were constructed from the following tissues and cultivars: seedling root tissue (Williams, Bragg cultivars) post-rhizobium inoculated seedling root tissue (supernodulating Bragg mutant), mature stems, developing cotyledons, etiolated and non-etiolated degenerating cotyledons, vegetative leaf buds, expanding trifoliates, mature leaves, mature white and purple flowers, and mature root nodules. Approximately 5000 tags from each library were analyzed for differential expression among salient libraries, except for the Bragg seedling root and the post-rhizobium inoculated supernodulating Bragg mutant seedling root libraries, from which ca. 20,000 tags were analyzed. From 20 diverse tissue libraries, 132,992 total 13bp tags were sequenced, resulting in 40,121 unique tags. These data along with SAGE tag matches with Soy EST sequences are available at the University of Minnesota website http://soybean.ccgb.umn.edu/. Notable identified messages were auxin-down-regulated genes and channel proteins for the root tissue, lipoxygenases and protease inhibitors for the immature cotyledon tissue, photosynthesis related genes from the stem and leaf tissues, and numerous nodulins from the root nodule tissue. Cluster analysis on the gene expression profiles reveals expected grouping of photosynthetic tissues, root tissues and flower tissues. The developing cotyledon and mature root nodule tissues were quite distinct from the other tissues as well as from themselves.

705

Building Version 3 of The Soybean Integrated Physical and Genetic Map: Progress Toward Functional, High Density Gene Maps

Jeffry Shultz[1], Chengcang Wu[2], Quanzhou Tao[2], Padmavathi Nimmakayala[2], Filip Santos[2], Javed Iqbal, Kanokporn "Tik" Triwitayakorn, Chad LaMontagne[1], Kimbely Zobrist[1], Christopher Town[3] Hongbin Zhang[2], Khalid Meksem[1], David A. Lightfoot[1]

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A marker anchored physical map derived from bacterial artificial chromosomes (BACs) was constructed. We fingerprinted 95,322 BACs from cv. Forrest. Editing provided 86,386 useful fingerprints. There are 30,720 clones from the soybean Forrest *Hind* III BIBAC library (first posted on www in January 2000), 20,736 clones from the Forrest *Bam*HI BIBAC library (released May 2001), 30,720 clones from the large insert (157 kbp) Forrest *Eco*RI BAC library (released January 2002) and 3,384 clones from the Williams 82 *Hind* III and Fairbault *Eco* RI BAC libraries (released February 2002). The BAC fingerprints created three versions of an FPC database for investigator driven contig assembly (see http:// hbz.tamu.edu/ - Physical Mapping/Soy Map). About 5,488 automatic contigs (contigs assembled without manual editing) were posted (March 2002), The longest contig contained 320 clones and encompassed 5.86 Mbp. The contigs consisted of a total of 396,843 unique bands, estimated to span 1,667 Mb (Table 2) more than the genome size of soybean (1,100 Mb/haploid). Many of the contigs may overlap even though the common bands were not identified under the contig assembly conditions used however some misassembly is inevitable before manual editing to remove multiplets and multi-band artefacts. About 10% of contigs contained false merges due to clone contamination. Progress in editing and merging contigs on linkage group G suggests that a 500 contig map encompassing more than 95% of the soybean genome will be built by the end of 2002. We used 370 micro-satellite markers to anchor individual Forrest BACs. In addition we have integrated the 3,384 BACs identified with 267 SSRs and 105 RFLPs by Dr. Shoemaker laboratory at Iowa State University and Dr. N. Young laboratory at University of Minnesota. False positives, homeologs and paralogs were frequent among anchored clones but were readily detected by fingerprinting. Physical distance did not correspond well with genetic distance. About 6% of marker pair orders are inverted. The physical-genetic map location for all markers and plate addresses for all clones can be viewed at www.siu.edu/~pbgc. The Forrest BAC libraries are available from TAMU-BAC Center, SIUC-PBGC and Invitrogen. We have used the database to build a minimally overlapping clone tile of 7,241 large insert clones for high-throughput gene (EST) mapping, large-scale genome sequencing and targeted DNA marker development in phase 4 of this project. Integrating ESTs (60% of the genes) and predicted paralogs (40% of the genes) with the soybean physical map identified several intervals as gene rich islands, four are present on contigs anchored to linkage group G. Genome sequencing on linkage group G has begun at two neighboring gene rich seed-points using the physical map. From 512 Kbp of sequence we observed mean predicted gene density of 1 gene per 7.2 kbp, gene length of 2.5 kbp, intragenic distance of 4.7 kbp, intron number 3.5 (0-14). This work was supported by NSF project #9872635.

Soybean Gene and Genome Evolution

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Molecular genetic mapping of soybean has revealed evidence of genome duplication. Using RFLP mapping data, homeologous segments have been identified between soybean linkage groups. Comparative mapping with other legumes (Phaseolus and Vigna) and with Arabidopsis has provided further evidence of polyploidy in soybean's evolutionary past. Additional evidence suggests other, more ancient duplications also may have occurred, thus complicating an analysis of genome structure. The Public Soybean EST Project has produced approximately 250,000 ESTs representing about 80 different cDNA libraries. An analysis of the sequences of ESTs from the same genotype has provided much information on the prevalence of duplicated, triplicated, and quadruplicated genes, in addition to the prevalence of `singletons.' An evaluation of synonymous mutations between pairs of duplicated genes suggests a coalescence time of approximately 8.7 mya. This date would correspond to the time of whole-genome duplication if soybean is an autopolyploid. If soybean is an allopolyploid, this date would correspond to the time of divergence of the genomes of each parent.

Global Gene Expression in Soybean Using Microarrays

Lila Vodkin[1], Robin Shealy[1], Anupama Khanna[1], Francoise Thibaud-Nissen[1], Steve Clough[1], Reena Philip[1], Elizabeth Shoop[2], Christina Schmidt[2], Martina Stromvik[2], Ernest Retzel[2]

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DNA microarrays are powerful tools to analyze the expression patterns of thousands of genes simultaneously. As part of the NSF-sponsored "Soybean Functional Genomics Program", we are accumulating a set of unique genes from a larger collection of soybean 5' ESTs developed by the "Public EST Project for Soybean" (in collaboration with the laboratories of Randy Shoemaker and Paul Keim). To date, we have prepared a "unigene" set of over 18,000 genes from cDNA libraries made to mRNA extracted from developing cotyledons, seed coats, young pods, immature and mature flowers, 7-day seedling roots, mature roots, and roots challenged with *Bradyrhizobium japonicum*. After cluster analysis, we selected each singleton and the most 5' representative of each contig for additional sequencing at the 3' end. Functional assignments of clones were inferred by matching the blastx hits of the 5' and 3' sequences to the best MetaFam superset of proteins. The inserts were amplified from each clone by PCR and were spotted onto glass slides for microarray analysis. Two arrays have been produced, each with approximately 9,216. Several projects with microarrays will be summarized including their use to profile tissue-specific and developmental changes in gene expression. In another project, we have examined gene expression during the reprogramming of cotyledon cells associated with induction of somatic embryos in soybean tissue culture and in transgenic plants (see abstracts by Thibaud-Nissen, et al.). Web sites for the project are the following: http://soybeangenomics.cropsci.uiuc.edu/ and http://web.ahc.umn.edu/biodata

SOY 2002

Posters
Molecular Breeding and Genetic Mapping

Abstracts P201-P221

SOY 2002

Concurrent Session
Metabolic Engineering

Tuesday, August 13
1:30 pm

Abstracts 501-508

Genetic Modification of Soybean for Enhanced Industrial Value

Edgar Cahoon[1] and Anthony Kinney[2]

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The industrial value of soybean is limited by the fatty acid composition of its seed oil. Efforts have been undertaken to increase the industrial potential of soybeans by the introduction of biosynthetic pathways for novel fatty acid structures. The production targets of this work have included epoxy fatty acids, fatty acids with conjugated double bonds, and very long chain monounsaturated fatty acids. Vegetable oils enriched in these fatty acids are highly desired for industrial applications including coatings, adhesives, and hydraulic lubricants. Species that accumulate novel fatty acids in their seed oils are widely distributed in the plant kingdom and typically have limited agronomic potential. Using an EST approach, we have identified cDNAs for a number of novel fatty acid biosynthetic enzymes from species such as *Calendula officinalis* (pot marigold) and *Momordica charantia* (bitter melon). These cDNAs have been successfully introduced into soybean to generate seeds that accumulate moderate amounts of novel fatty acids. The technical challenges that lie ahead include the identification of biochemical factors that limit the accumulation and proper metabolism of novel fatty acids in soybeans.

502

Engineering of Essential Amino Acids In Soybean for Animal Feed

Tim Oulmassov

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Soybeans represent a valuable source of protein for both human and animal nutrition. Although present in large amounts, the protein in soybeans does not contain a balanced proportion of dietary essential amino acids. As part of an effort to create soybeans with more favorable amino acid compositions, we are developing technologies to generate transgenic soybeans that are enriched in selected essential amino acids. Two approaches are being used to increase the levels of amino acids. One involves modifying a particular biosynthetic pathway to cause an accumulation of free amino acid in the seed. The second approach is relying on the knowledge of crystal structure and computer modeling technology to modify existing soybean seed storage proteins to improve their essential amino acid composition, and expressing such modified proteins at high levels in soybean endosperm. A combination of these approaches should improve the amino acid composition of the soybean, and increase its value as a source of protein to meet human dietary needs and for livestock feed formulations.

503

Production of Gamma Linolenic Acid in Seeds of Transgenic Soybean

Tom Clemente[1,2,3], Aiqiu Xing[1], Xinguo Ye[2], Shirley Sato[1], Bruce Schweiger[4], and Anthony Kinney[4]

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Hundreds of fatty acids species have been identified in the plant kingdom and many of them may have important commercial applications. Most of these fatty acids are only available from native plant sources in which cost-effective production is not possible. The ability to introduce novel traits into soybean makes it technically feasible to alter oil metabolism for the production of high value/low volume fatty acids in a cost effective manner. Two examples of high value fatty acids that can be cost effectively produced in soybean are gamma linolenic acid (GLA) and stearidonic acid (STA). GLA has pharmacological applications in treatment of skin conditions such as eczema and is known to possess some antiviral and anticancer properties. Like GLA, STA is of interest for the pharmaceutical and nutraceutical industry. To produce GLA and STA, marker gene free, in the seed storage lipids of soybean we assembled a two T-DNA binary vector that harbored a *bar* cassette in T-DNA one for plant selection and the delta-6 desaturase gene from *Borago officinalis* under the control of the soybean embryo specific promoter beta-conglycinin in T-DNA two. The two T-DNA binary vector was mobilized into *Agrobacterium tumefaciens* strain EHA101 and the derived transconjugant was used for soybean transformations implementing the cotyledonary-node transformation protocol. Progeny from 29 soybean lines have been characterized from the transformation experiments. Seventeen of the lines produced GLA and STA in the seed storage lipids. T_1 individuals were identified that were free of the marker-gene T-DNA element in four of the 17 lines GLA/STA producing lines. Among the 17 lines producing GLA and STA average GLA content ranged from 5.8% to 31.2%, while average STA levels within these lines ranged from 0.9% to 3.8%.

Accumulation of Soybean Seed Isoflavones: Synthesis within the Seed or Transport from Maternal Tissues?

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Isoflavones are biologically active natural products that accumulate in soybean seeds during development. The amount of isoflavones present in soybean seed is variable, depending on genetic and environmental factors that are not fully understood. We are studying whether isoflavones are synthesized within the seed during the development, or made in maternal plant tissues and transported to the seeds where they accumulate. Embryos excised from developing soybean seeds accumulated isoflavone from the culture medium. The rate of uptake varied with the type of isoflavone fed but displayed saturation kinetics for all isoflavones tested. An analysis of isoflavones by HPLC detected the compounds in all organs of soybean plant, but the amount of isoflavones present varied depending on the tissue and developmental stage. The greatest concentrations were found in mature leaves and embryos. The 2-hydroxyisoflavone synthase genes (IFS1 and IFS2) were studied to determine their pattern of expression in different tissues and developmental stages. The highest level of expression of IFS1 was observed in the seed coat and root while IFS2 was most highly expressed in embryo and pod tissues. These results indicate that 2-hydroxyisoflavone synthase gene expression does not necessarily correlate with the level of isoflavones present in different tissues. Feeding soybean plant parts with [^{14}C]phenylalanine, a precursor molecule for the synthesis of isoflavones, showed incorporation of radioactivity in isoflavones in leaf tissues but not in pod and seed tissues. From these results, we propose that transport from maternal tissues, at least in part, may contribute to the accumulation of isoflavones in soybean seeds.

Cloning and Characterization of APS Reductase from Soybean

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The nutritional quality of soybean (*Glycine max* [L.] Merr.), as human and animal feed, is often limited by a low amount of sulfur-rich amino acids, cysteine and methionine. We are interested in improving the sulfur amino acid content of soybean proteins by manipulating the enzymes involved in sulfur metabolism. The initial reduction of the activated form of sulfate, adenosine phosphate sulfate (APS), is mediated by APS reductase (E.C.452450). By screening a soybean seedling cDNA library with a ^{32}P-labelled EST, we isolated three APS reductase clones. Nucleotide sequence analysis of these clones indicated that one of them contained the entire coding region (1414 bp) and encoded a 51.9 kD protein. We have investigated the expression of the APS reductase gene during seed development and in response to nutrient stress. We have also overexpressed soybean APS reductase in *Escherichia coli* and purified the protein by affinity chromatography. Western blot analysis using antibodies raised against the recombinant APS reductase showed that this protein accumulated during early stages and declined gradually during the later stages of seed development. We have also isolated a genomic clone by screening a cosmid library with radiolabeled APS reductase cDNA clone. The gene has four introns interspersed between the exons. Southern blot analysis indicated that a small gene family encoded APS reductase in soybeans.

The Pleitropic Tawny Pubescence Locus (*T*) Encodes a Flavonoid 3' Hydroxylase. Molecular Characterization of Gray Pubescence Mutations (*t*)

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Secondary metabolites derived from the flavonoid pathway such as proanthocyanidins and anthocyanins play a relevant role in plant pathogen defense and protection from UV light exposure in addition to their nutritional value due to their antioxidant properties. In soybeans (*Glycine max*) there are three independent loci (*I*, *R* and *T*) that control pigmentation of the seed coats and are distinct from those controlling flower color. *I* encodes a chalcone synthase (CHS) and in its dominant form inhibits pigment formation. No functional assignment has been proposed to date for the *R* locus. The *T* (tawny pubescence) locus also controls pubescent hair color and a leaf wavy phenotype. In addition, an epistatic effect of the *t* allele results in damaged seed-coat structure. We report the identification and isolation of a flavonoid 3' hydroxylase gene from *Glycine max* (GmF3'H) and the linkage of this gene to the *T* locus. This GmF3'H gene was highly expressed in early stages of seed coat development and very low or not at all in other tissues. Evidence that the GmF3'H gene is linked to the *T* locus came from the occurrence of multiple RFLPs in lines with varying alleles of the *T* locus, as well as in a population of plants segregating at that locus. GmF3'H genomic and cDNA sequence analysis of color mutant lines with varying *t* alleles revealed a frame shift mutation in one of the alleles. In another line derived from a mutable genetic stock, the abundance of the mRNAs for GmF3'H was dramatically reduced. Isolation of the GmF3'H gene and its identification as the *T* locus will enable investigation of the pleiotropic effects of the *T* locus on cell wall integrity and the wavy leaf phenotype as well as characterization of its role in plant pathogen defense. It also becomes a crucial marker to study the regulation of the anthocyanins differential tissue expression as well as the channeling through the multiple branches of the flavonoid pathway in soybeans.

507

Differentially Expressed Genes in Hypernodulation Soybean Mutant

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SS2-2 is a hypernodulating soybean mutant induced by EMS mutagenesis with Sinpaldalkong 2. It produces greater nodules and smaller plant growth than Sinpaldalkong 2. The cDNA-AFLP was used to identify regulating gene for greater nodulation which was discriminating hypernodulating soybean mutant SS2-2 from Sinpaldalkong 2. To compare differentially expressed genes between Shipaldalkong 2 and SS2-2, RNA was extracted from soybean root one week after inoculation with *Bradyrhizobium*. AFLP was performed with cDNA synthesized from RNA. AFLP was analyzed using 5% Urea/acrylamide gel electrophoresis and silver staining. The unique bands in each variety were eluted from the gel, re-amplificated by PCR, cloned in T-vector, and sequenced with ABI377. The sequencing data were analysed with BLAST search program in NCBI to compare with published soybean ESTs. Out of 712 bands which were differentially expressed from SS2-2 and Sinpaldalkong 2, 78 bands expressed in Sinpaldalkong 2 and 53 bands in SS2-2 were eluted and sequenced. DNA homology searches against NCBI database using the Blast tools revealed that several important genes associated with carbon and nitrogen metabolism were identified.

Calibration of Dual Diode Array and Fourier Transform NIR Spectrometers for Composition Analysis of Single Soybean Seeds in Genetic Selection, Cross-Breeding Experiments

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Accurate, reliable, and cost-effective composition analyses of single soybean seeds are essential to improving the efficiency of soybean breeding and soybean genetic selection. We are presenting the first report of extensive efforts aimed at developing single soybean seed calibrations for both dispersive (Dual Diode Array) and Fourier Transform Near Infrared Reflectance Spectroscopy (NIRS) instruments. Single soybean seed calibrations were developed for six, different NIRS spectrometer models that are commercially available. Accurate measurements can be therefore reliably performed on single soybean seeds for: protein, oil, moisture, sugars and fiber. Random variations and light scattering effects were eliminated by the Multiplicative Scattering Correction (MSC) prior to carrying out the Partial Least Square regressions with either the PLS-1 or PLS-2 model. Such corrections as well as the pre-processing of NIR spectra through baseline correction and normalization are essential for the development of high quality calibrations of such instruments. Our novel calibrations are characterized by low standard errors (<0.2%) and a high degree of correlation for all major soybean constituents (>99%) in the NIR spectra range from 4,000 to 12,000 cm^{-1} at 8 cm^{-1} resolution. Seed-to-seed variations in protein and oil content can thus be monitored for the first time on single soybeans of selected soybean accessions. These analytical capabilities may prove to be important in wide scale, molecular breeding experiments concerned with single soybean seeds.

SOY 2002

Concurrent Session
Molecular and Cellular Pathology

Tuesday, August 13
1:30 pm

Abstracts 601-606

Soybean Phenolics (Isoflavones and Lignin) Affecting Seed Quality and Root Resistance

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In leguminous plants different classes of phenylpropanoid compounds play very important roles in defense reactions. On the other hand the intense studies are focused at the specifi flavonoid and isoflavonoid compounds due to their antioxidant activity and potential benefit for human health.

We are interested in three main aspects related to soybean phenolic metabolism: 1) evaluation of the role of phenylpropanoid metabolism in the soybean root defense response to FSG infection; 2) determination of the effect of genetic and various environmental factors on the alteration in phenolic metabolism and accumulation of isoflavones in soybean seeds; 3) genetic manipulation of phenolic metabolism to enhance health promoting value of soybean seeds and soybean plant disease resistance.

The results will be presented indicating the alterations in phenolic metabolism that are critical for soybean resistance to sudden death syndrome (SDS), caused by the soil-born fungal pathogen *Fusarium solani P.* sp. glycines (FSG). Considerable changes were found both in compounds of isoflavone and lignin branches of phenylpropanoid pathway with the phytoalexin glyceollin levels being much higher in partially resistant genotypes. Activation of lignin synthesis as a response to FSG infection was found in resistant but not in susceptible plant roots. Genetic modifications that could be effective in enhancing SDS resistance will be discussed.

We found (based on the analysis of more than 1000 samples from the USDA soybean Germplasm Collection) that the diversity of isoflavone concentration and composition in plant introductions exists to be used either for increasing or decreasing the current amount or changing the composition of isoflavones in the U.S. cultivars. Information on the critical combinations of genetic and environmental factors that will allow for the production of soybean seeds with uniformly high or low isoflavone levels will be provided.

The approaches used and the progress achieved in our genetic manipulation of isoflavones and cell wall phenolics will be presented.

This study was supported in part by the funds from the Illinois Soybean Program Operating Board, the United Soybean Board, NATO Collaborative Research Program (ref. LST.CLG.976259; JSTC.CLG.978212), the Illinois Agricultural Experiment Station, and the USDA Agricultural Research Service.

Molecular analysis of isoflavones in relation to pathogen attack of soybean, *Glycine max* L. Merr.)

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One hundred recombinant inbred lines (RILs) of soybean (*Glycine max* L.) derived from the cross between Harovinton and OAC Arthur were evaluated for tolerance to *Sclerotinia sclerotiorum*, isolate 38, and *Phytophthora sojae*, race 4. The objective of this study is to determine if isoflavone levels in root, leaf and stem (non-seed) tissues are associated with tolerance to two fungal pathogens of soybean. Evaluations were conducted in the growth room using the stem inoculation technique and inoculum layer test, respectively. Significant differences in tolerance were observed among the RILs to both fungi. Levels of the isoflavones, daidzein, genistein and glycitein, in non-seed tissues were determined by gas chromatography before and one week after inoculation. The relationship between isoflavone levels post inoculation and levels of tolerance to both fungal pathogens will be determined in non-seed tissues. The phenotypic data will be used to identify quantitative trait loci (QTLs) associated with isoflavone-dependent tolerance to each fungus.

Identification of SSR Markers Associated with Rhizoctonia Root Rot Resistance in Soybean (*Glycine max* L. Merr.)

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Rhizoctonia root rot, caused by *Rhizoctonia solani* Kühn, is a damaging soybean disease that appears to be on the rise in most soybean producing areas of North America. Very little is known about possible sources of resistance to this disease that could be utilized to control it using the genetic means. The objective of this study was to genetically map resistance to rhizoctonia root rot in a plant introduction, PI 442031. Three simple sequence repeat (SSR) markers, Satt281, Satt177 and Satt245, were found significantly associated with resistance to rhizoctonia root rot in an F_5 population derived from the cross of PI442031 x Sterling. The three markers together explained 69% of the phenotypic variation and marker satt281 alone explained 58%. The association between satt281 and the resistance trait was confirmed in another F_5 population, OAC 9608 x PI442031, in which Satt281 explained 29% of the phenotypic variation. One significant applications of this study is that Satt281 could be used as a molecular marker to assist in selection for resistance to rhizoctonia root rot in soybean breeding programs.

The *Rps1*-k Locus Carries Multiple Functional Phytophthora Disease Resistance Genes in Soybean

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Phytophthora root and stem rot disease of soybean caused by the oomycete pathogen *Phytophthora sojae* results in millions of dollars of crop loss annually. *Rps1*-k, the most widely used monogenic resistance gene, has been providing stable resistance in cultivars grown in the North-Central United States for the last two decades. Isolation of this gene will allow us to investigate the mechanism of stable resistance encoded by this most extensively used gene. We have applied a positional cloning strategy for isolating this gene. Previously we identified AFLP markers that encompass the *Rps1*-k gene in a 0.13 cM interval. Analysis of a cosmid and several bacterial artificial chromosome (BAC) libraries indicated that the *Rps1* region is highly underrepresented in these libraries. Furthermore, the recombination in the *Rps1*-k region is about 2.5 fold less frequent than that in the adjoining region. A sequence with high identity to the LRR-domain of disease resistance genes was isolated from a BAC that carries an *Rps1*-k-linked AFLP marker. This LRR sequence was applied to identify BAC clones for the *Rps1*-k region. This sequence is highly repetitive, and there are at least 38 copies of the sequence in the soybean genome. Most polymorphic copies of the *Rps1*-k haplotype were mapped to an about 0.7 cM introgressed region. Sequencing of about 300 kb DNA carrying the *Rps1*-k gene revealed ten coiled-coil NBS-LRR type disease resistance genes. Three of these genes were expressed in stable transgenic soybean plants, and all three genes have shown to confer resistance against *P. sojae* race 4 in transgenic plants.

Instability in a Resistance Gene-like Sequence Caused by Mitotic Recombination in Soybean

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A series of single dominant *Rps* genes confer resistance to soybean against root and stem rot disease caused by *Phytophthora sojae*. We have recently cloned NBS disease resistance gene-like sequences NBS-Rps4 that co-segregated with the *Rps4* gene in a small F_2 population between Williams (*rps4rps4*) and HARO4272 (*Rps4Rps4*). *Rps4* was genetically mapped to the molecular linkage group G. For fine mapping, a total of 1000 F_2 plants was screened with *P. sojae* race 1, of which 197 were susceptible. High-resolution genetic linkage map was constructed using susceptible F_2 plants. DNA gel blot analyses of F_1s between Williams (*rps4rps4*) x HARO4272 (*Rps4Rps4*) indicated that about 5% of the F_1s were rearranged for NBS-Rps4 sequences, whereas the parents were normal. The pattern of rearrangements among F_1s obtained from a single cross was unique suggesting that rearrangement occurred before meiosis. Randomly selected 201 HARO4272 plants were analyzed with the NBS-Rps4 probe, and 6 aberrant plants were identified. One of these aberrant plants showed two different types of rearrangements involving two different loci detected by the NBS-Rps4 probe. We conclude from these data that these rearrangements were developed through an unusual mitotic recombination event just prior to differentiation of the whole flower, and may have implication in the evolution of resistance gene like sequences in soybean.

606

Molecular Analysis of QTL Associated with Partial Resistance to Sclerotinia Stem Rot

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Sclerotinia sclerotiorum , causing sclerotinia stem rot, is a major pathogen of soybean (*Glycine max* (L.) Merr.) that results in major yield losses in many soybean growing areas around the world. Understanding the heritability of partial resistance observed in certain cultivars is key to developing new soybean cultivars having elevated partial resistance to the disease. The objective of this study was to map the quantitative trait loci (QTL) for partial resistance to sclerotinia stem rot. A segregating population consisting of 200 F_2 plants derived from the cross S 08-80 x OAC Shire was evaluated using the straw inoculation technique based on fungal mycelia. More than 400 SSR markers have been screened and analyzed to identify regions of the soybean genome harboring QTLs for partial resistance to sclerotinia stem rot. In addition, recombinant inbred line populations were developed also from crosses involving partially resistant and susceptible soybean lines to map QTLs associated with plants' response to natural infection and a field-based inoculation method.

Agrobacterium-Mediated Transformation of Soybean Using the Cotyledonary-Node Method

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An essential component in investigating the functions of plant genes is the production and utilization of transgenic plants. Therefore, to meet the demands of future soybean genomics programs in the production of large numbers of transgenic plants, we have recently improved the transformation efficiency of soybean to an average of 16% (number Southern+ plants per total explants inoculated) using a modified Agrobacterium-mediated cotyledonary-node method. Increased transformation efficiency resulted when combinations of thiol-compounds, such as L-cysteine, dithiothreitol, and sodium thiosulfate, were added to the solid co-cultivation medium in conjunction with a stringent selection regime using hygromycin B during shoot regeneration. Increases in both transient and stable GUS expression of explants treated with various thiol compounds, and the copper and iron chelators, bathocuproline and bathophenanthroline, suggest that these compounds reduce the plants' defense response pathways that are stimulated by wounding and Agrobacterium infection. Unexpectedly, mixtures of thiol compounds during co-cultivation with Agrobacterium resulted in significant increases in transgenic plants that were greater than treatments with a single thiol compound. In addition to high transformation efficiencies, the hygromycin B selection protocol rapidly produced healthy, fertile plants with no non-transformed 'escapes'. Southern analysis of T0 and T1 plants showed that the majority of transformed plants contained simple T-DNA hybridization patterns, the T-DNA was heritable, and no correlation existed between the complexity of integration and thiol treatment applied at co-cultivation. The low escape rate of non-transformed shoots, rapid culture time, and repeatability of this method substantially decreases the resources needed to achieve the goal of producing and screening large numbers of transgenic plants necessary for soybean functional genomics programs.

Fatty Acid and Triglyceride Changes in Transgenic Soybeans

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Soybean seeds have ~ 20% oil and 40% protein. Soybean seed oil is mainly composed of five fatty acids, palmitic (16:0) (12-15%), stearic (18:0) (2-4%), oleic (18:1) (11-14%), linoleic (18:2) (50 – 60%) and linolenic (18:3) (6-10%) acids. The quality and quantity of the soybean seed oil provides a good target for improvement. Traditional breeding methods to increase TAG content of soybean have had limited success and usually result in a reduction of seed protein levels. Recent studies indicate transforming plants with the acyltransferase genes such as plant GPAT, plant DGAT and yeast LPAT (SLC1 and SLC1-1) result in increased triglyceride levels in different plants. However no specific reports are available for TAG increase in soybeans by gene transfer methods. The major saturated fatty acid in soy oil is palmitic acid. Reduction of 16:0 levels with simultaneous increase in 16:1 levels will result in nutritionally superior soybean oil. Plant Δ9 desaturases desaturate palmitic or stearic acids esterified to acyl carrier proteins. The soybean Δ9 desaturase is primarily an 18:0 desaturase with little activity with 16:0. However the mammalian Δ9 desaturase used here can desaturate both 16:0 and 18:0 fatty acids providing a means for 16:1 synthesis in soybeans and other plant tissues. In regenerated Δ9 desaturase transgenics examined so far the 16:1 levels in the seeds only increased to ~2%. Interestingly compared to controls there is an increase in average triglyceride values also observed with ~ a 20% increase seen in some cases. In case of SLC1 transformants the triglyceride level increased in the seeds as well as matured somatic embryos. Compared to controls the average increase in triglyceride values went up 12% in zygotic seeds and 25% in matured somatic embryos.

Acetolactate Synthase as a Selectable Marker for Soybean Transformation

Ted Klein, Allison Finch, Christine Hazel, Cheryl Caster, and Zhongsen Li

Pioneer Hi-Bred International, Inc., DuPont Stine-Haskell Research Center, Newark, DE 19714

Soybean transformation is hindered by the relative lack of effective selectable markers. We show that the acetolactate synthase (ALS) gene can be used to select transformants following bombardment of embryogenic cultures. The ALS enzyme catalyzes a step in branched chain amino acid biosynthesis and is the target for several commercial herbicides including the sulfonylureas. We have used ALS coding regions from either tobacco, arabidopsis or soybean under the control of various promoters to confer resistance to the SU, chlorsulfuron. Selection parameters have been optimized so that transgenic events can be consistently recovered from bombarded cultures. Transgenic plants are fertile and resistant to the application of chlorsulfuron. Our results demonstrate that a plant gene under the control of a plant promoter can be used to efficiently recover transgenic soybean.

Automated Image Collection and Analysis for Studies of Gene Expression

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Robotics systems are often used to perform tasks that may be hazardous, time consuming, highly repetitious or impossible to perform by humans. In the area of plant developmental biology, robotics is used to gather information on gene expression profiles in different tissues or in similar tissues, grown under different environmental conditions.

Digital imaging permits the non-destructive evaluation of plant growth and response to different environments. Images are either analyzed as they are acquired (real time) or stored for subsequent analysis. Digital images can also be used to make time-lapse animations, which can show details of growth and development, previously imperceptible with single time point determinations.

In this study, automated image collection and analysis was used to track gene expression using GFP. Expression profiles and levels were determined in both transiently- and stably-transformed tissues. Transient expression studies yielded general information on the levels and timing of gfp expression while time-lapse animations showed the differential appearance and disappearance of individual gfp expressing foci. In addition, movement of GFP within bombarded cells and into adjacent cells was observed. In stably transformed tissues, tracking of GFP expression was used to quantify promoter strength and specificity in developing soybean embryos. The lectin promoter displayed cotyledon-specific expression, late in embryo development while the 35S promoter gave higher expression levels, over the whole length of the developing embryo.

Optimization of Somatic Embryogenesis in Soybean [*Glycine max* (L.) Merrill]

Sharad Tiwari

J.N. Agricultural University

SOY 2002

Plenary Session 2

Tuesday, August 13
8:00 am

Abstracts 401-407

Adapting soybean to current and future change in atmospheric composition. Do we need more than field selection under current conditions?

Steve Long[1,2], Randall Nelson[1,3], Lisa Ainsworth[1], Kevin Hollis[2], Tim Mies[1], Patrick Morgan[2], Shawna Naidu[1], Don Ort[2,3], Richard Webster[2], Xin-guang Zhu[2].

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Carbon dioxide is rising at about 0.4% per year. In 1900, levels were about 290 parts per million (ppm), in 2000 they were 370 ppm, and by 2050 they will be about 550 ppm. Carbon dioxide has the potential to increase plant production by inhibiting the wasteful process of photorespiration. By decreasing photorespiration, the increase in CO_2 to date theoretically has had the potential to increase production of C3 crops, including soybean, by 20%. However, there is little evidence that plant breeding has selected lines capable of realizing this potential. Our review of studies on soybean responses to elevated CO_2 show, that while significant increases in biomass production are limited by a loss of photosynthetic capacity. Increase in grain yield is yet further limited by a decrease in harvest index. Furthermore, analysis of Rubisco suggests that it is optimized to the pre-industrial CO_2 concentration, and is becoming increasingly inefficient as CO_2 levels rise. Engineering a form optimized to current and future conditions would significantly improve nitrogen use.

Ozone is a secondary pollutant that is formed a few to 1000 miles downwind of release of nitrogen oxides (NO_x), mainly from fossil fuel combustion. Ozone has risen by about 1% per year in the northern temperate zone. Although ozone pollution in cities has received much attention, concentrations are often higher in the surrounding rural areas. Of the major crop plants, soybean is the most vulnerable to ozone. A peak daily level of 40 ppb (parts per billion) is sufficient to significantly decrease yield, yet average summer levels in central Illinois are 60 ppb and can exceed 100 ppb. Depending on weather conditions, ozone concentrations fluctuate greatly from year to year, impairing the potential for selecting more tolerant cultivars in simple field selection. This may explain the lack of any apparent difference in the tolerance of old and recent cultivars. Further, plants grown in greenhouse and other sheltered conditions often respond differently to plants grown in the open. Selection and mechanistic understanding therefore requires a facility where elevated levels can be maintained every year in the open.

SoyFACE, an open-air computer-controlled field-exposure system has been developed at the University of Illinois. This releases CO_2 or/and ozone according to wind speed and direction to maintain elevation of these gases within 12 replicated 1200 ft^2 plots. Within these, the effects of these gases on a range of genotypes are being assessed. Variation in responses of genotypes and insights into mechanisms of response, observed to date, will be presented.

Soy as a Functional Food: Implications for Women's Health

C. M. Hasler

Functional Foods for Health Program and Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL 61801

Functional foods, defined by the International Food Information Council as "foods that provide a health benefit beyond basic nutrition," is one of the leading trends in the food industry today. Soy can clearly be viewed as a functional food due to the numerous health benefits attributed to the consumption of this legume.

Numerous clinical trials have documented the cholesterol lowering benefits of soy, and in 1999, the Food and Drug Administration approved a health claim for the relationship between consumption of soy protein (25 grams per day) and reduced risk of coronary heart disease. This significant regulatory event has had a dramatic, positive effect on the market for soyfoods and soy supplements, as well as on consumer awareness of soy's health benefits. A recent survey from the United Soybean Board found that the number of consumers who are aware that soy may lower cholesterol rose from 27% in 1999 to 42% in 2001.

Additional research ongoing in academic and private-sector research centers around the world have highlighted additional health benefits for women related to the consumption of soy protein and/or isoflavones, including the improvement of bone density and amelioration of the vasomotor symptoms associated with menopause. This presentation will summarize current research on the benefits of soy or soy isoflavones for women with an emphasis on cardiovascular and menopausal health issues.

Psychology of Soy: Increasing the Consumer Acceptance of Soy

Brian Wansink

College of Commerce and Business Administration, University of Illinois, Urbana, IL 61801

Given the many health benefits of cost benefits of soy, what is it (other than it's unfamiliar taste) that is slowing down the widespread acceptance of soy? A series of 5 field studies addresses issues such as does a soy label influence taste, what do soy fanatics have in common, what is the best way to introduce soy to countries like Russia vs. countries like Columbia, and others. These studies are part of a forthcoming book titled, "Marketing Nutrition: Functional Foods, Soy, and Biotechnology."

Genomics of Plant Pathogen Interactions

Andrew F. Bent

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A broad overview of disease resistance engineering strategies will be presented with respect to genomics methods that are now available or needed in soybean. Disease resistance traits can be recruited from single antimicrobial gene products, or from R genes and other genes that control endogenous soybean defense responses. How can these genes be identified and manipulated? Soybean disease resistance traits are often manipulated most effectively through classical breeding, but what will be the exceptions? Expression profiling is yielding extensive new information about defense responses. How can this information be used? Facile technologies for reverse genetics (silencing, transposon tagging, tilling) would strongly facilitate progress, as would improved methods for genetic transformation of soybean. Work from our laboratory and other laboratories will be covered in a discussion of the above technologies.

Positional Cloning of the Soybean *Rpg1-b* Disease Resistance Gene

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RPM1 and *Rpg1-b* are functionally analogous disease resistance genes (R-genes) from *Arabidopsis thaliana* and soybean, respectively. Both confer resistance to *Pseudomonas syringae* strains that express the avirulence gene *avrB*. *RPM1* is a CC-NBS-LRR type R-gene and is unusual in that it also recognizes a second pseudomonal avirulence gene, *avrRpm1*. In contrast, the soybean *Rpg1-b* gene is specific for *avrB* with *avrRpm1* being detected by a second tightly linked gene, *Rpg1-r*. It is not known whether such functionally analogous R-genes typically result from the conservation of an ancestral specificity through speciation or whether the same specificity evolves independently in different plant lineages.

We have mapped *Rpg1-b* to a cluster of resistance genes in molecular linkage group F tightly linked to the marker R45. Also present in the cluster are R-genes or QTLs effective against viral, fungal, nematode and insect pathogens. A positional approach was used to clone the *Rpg1-b* gene. *Rpg1-b* is present in a complex locus containing several families of tightly linked NBS-LRR type genes. We have confirmed the identity of *Rpg1-b* both by sequencing an EMS-generated mutant allele and by functional complementation using a particle bombardment-based transient assay. In common with the Arabidopsis *RPM1* gene, *Rpg1-b* is a CC-NBS-LRR type R-gene. *Rpg1-b* and *RPM1* share 28% amino acid identity when aligned using the BLASTP algorithm. Significantly, phylogenic analysis suggests that *RPM1* and *Rpg1-b* are not orthologous, consistent with an independent origin for these functionally analogous R-genes. Our observations suggest that R-genes specific for *avrB* have evolved at least twice during the evolution of land plants.

Acknowledgements
We thank David Lightfoot for the provision of RILs and Laura Marek, Dariush Danesh, Khalid Meksem, David Lightfoot, Nevin Young and Randy Shoemaker for BAC library screening.

406

Improvement of Soybean Seed Quality for Food Uses

Anthony Kinney

Pioneer, a Dupont Company

Many people have increased their consumption of soy-based foods in recent years in response to the perceived health benefits from consuming soybean protein, although not all consumers care for the flavor of soy-based food products. Improving the oxidative stability of soybean oil results in better tasting soy protein. We have increased the oxidative stability of soybean oil by reducing polyunsaturated fatty acid content to less than 5% (compared with over 60% in commodity lines). Modifying other oil fractions, such as the tocopherol content and profile, further increases the stability of soybean oils. Additional flavor improvements may be achieved by removing certain secondary metabolites such as saponins. Combining these traits with genes that increase the digestibility of soy flour and improve the functionality of soy proteins has led to the development of superior milk and meat substitutes. The presentation will cover these and other genetic modifications of soybeans that are resulting in healthier, better tasting soy-products with improved functional properties.

Genetic Modification Removes an Immunodominant Allergen from Soybean

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The increasing use of soybean products in processed foods poses a potential threat to soybean-sensitive food-allergic individuals and reported incidents of soybean allergies, including suspected cases of anaphylaxis, are on the rise. In vitro assays on soybean seed proteins with sera from soybean-sensitive individuals have shown that, in addition to abundant storage proteins, also a few minor seed proteins account for most IgE-binding, implicating these proteins in eliciting allergic reactions. One such protein, Gly m Bd 30K, a member of the papain superfamily of cysteine proteases also referred to as P34, has been identified as a major (immunodominant) soybean allergen. We utilized transgene-induced gene silencing to prevent the accumulation of this allergen in seed. The resulting P34/Gly m Bd 30 K -suppressed plants (and their seeds) lacked any obvious compositional, developmental or structural phenotypic differences. Together these data provide evidence for substantial equivalence of composition of transgenic and non-transgenic seed. Sera from soy-sensitive individuals showed significantly less reactivity towards protein extracts of P34/Gly m Bd 30 K -suppressed seeds when compared with reactivity toward extracts from commodity soybean lines. Thus the production of a P34/Gly m Bd 30 K/ -suppressed line eliminates one of the primary allergens present in soybean seeds.

Molecular Markers Associated with Seed Protein and Fatty Acid Content in an Interspecific Soybean Population

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The modification of seed quality components in soybean [*Glycine max* (L.) Merr.] has application for both soy food and industrial markets. Wild soybean [*Glycine soja* Sieb. *et* Zucc.] offers a source of genetic diversity which can be utilized by breeders to make desired changes in seed quality traits. The objective of this study was to identify molecular markers linked to quantitative trait loci (QTL) controlling seed protein and fatty acid content. An interspecific hybridization was made between a soybean cultivar, NK S08-80, and a wild soybean plant introduction, PI 458536. The F_2 seed was space-planted 50 cm apart at the Woodstock Research Station, Woodstock, ON. The population segregated for seed protein content, oil content and fatty acid profile. Two hundred and eighty six F_2 plants were genotyped using 150 simple sequence repeat markers that were polymorphic among the parents. A genetic linkage map was constructed that covered an approximate distance of 1850 cM. One-way analysis of variance was used to identify markers associated with seed quality traits. QTL were localised to intervals on the genetic linkage map. Individual QTL explained 4.7 to 25.6 % of the observed variance in protein. A major QTL for protein was confirmed on linkage group (LG) I, while additional major QTL were located on LG A2 and LG L. Two QTL explained 8.9 and 20.4 % of the observed variance in seed oil content and were located on LG D1a+Q and LG I, respectively. Major QTL for linolenic acid content were identified on LG C2 and LG I and explained 9.1 to 27.6 % of the observed variance in the fatty acid. Molecular markers will be confirmed in the F_4 generation and subsequently used to modify seed quality traits through a marker assisted selection program.

208

Genetically Determined Polymorphism of Enzymes at Some Varieties of Soybean (*Glycine max*) and Wild Soybean (*Glycine soja*)

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Evolution of genetic structure, gene ensembles in domestication process isn't enough investigated. Receiving such information might have made easier using the representatives of wild varieties for improving cultivated plants and also searching for genetic structures, stimulating domestication of wild forms. The aim of present work was the comparative analysis of protein polymorphism in cultivated soybean (G. max) varieties and of representatives of wild soybean (G. soja), and also estimation of genetic interrelations between them, based on comparative analysis of allelic variants of different enzymes distribution. There were discussed the 18 varieties of domestic soybean (G. max) of different countries and three populations of wild soybean (G. soja).

Analysis of 22 enzymatic systems in grains was carried out, polymorphism of 42 loci with the use of starch gel electrophoresis was examined (four loci of phosphoglucoisomerase – PG1, four loci of 6-phosphogluconate dehydrogenase – ICD, one locus of sorbitol dehydrogenase – SOR DH, one locus of phosphoglucomutase – PGM, two loci of shikimate dehydrogenase – SH DH, five loci of esterase – EST, three loci of malate dehydrogenase – MDH, one locus of hexokinase HK, one locus of kreatin kinase – KK, one locus of superoxide dismutase – SOD, one locus of glucose-6-phosphate dehydrogenase – G 6PD, two loci of alpha-glycerophosphate dehydrogenase – alpha-GPD, two loci of alcohol dehydrogenase – ADH, one locus of xanthine dehydrogenase – KDH, two loci of leicine amino peptidase – LAR, three loci of diaphorase – DP, two loci of lactate dehydrogenase – LDH, two loci of aspartate amino transferase - AAT, one locus of peptidase A – PEPA, one locus of alkaline phosphatase-1 – AP1). It was detected monomorphism at 21 loci in all investigated groups of plants. Five loci (two loci were presumably of plasmon – PG1-C1 and PGI-C2, and nuclear genes 6PGD-3, SHDH-1, DP-1) had three allelic variants, in 16 loci there were observed two allelic variants (PGI-1, 6PGDX, ICD, PGM, SHDH-2, ESTD-1, ESTD-2, MDH-1, MDH3, G-6-PD, alpa-GPD, ADH-2, LAP-2, DP-2, EST-1, EST-2). At the three loci one of two-three allelic variants were presented only on representatives of wild type and wasn't detected at cultivated varieties (MDH-1, DP-1, EST-2); these loci were monomorphous at cultivated varieties except DP-1. Generally, in three investigated populations of wild soybean there were detected only 7 polymorphous loci from 42 (PGI-C1, PgI-C2, PGI-1, ICD, ESTD-1, MDH-3, ADH-2), but 19 loci in 18 varieties of cultivated soybean. >From these 19 loci at ESTD-1, ESTD-2, LAP-2, DP-1, DP-2 alternative allelic variants were detected only in one or two varieties. Received data are the evidence about expressed polymorphism between variety in domestic soybean, detected at 14 loci from 42 investigated, it's substantially higher (in two times), than quantity of polymorphous loci, detected in wild soybean. Early we obtained the data that these domesticated and wild forms didn't essentially differ at polymorphism of anonymous DNA sequences, flanking by microsatellite loci [1]. As dividing genetic-biochemical systems into enzymes, taking part in glucose metabolism – G and all the rest – not taking part in ones – NG, 7 polymorphous loci of wild species included only one locus NG (ESTD-1) and six loci of G, domestic varieties - 11 loci G and 8 loci NG. It permit to suppose the existing of "subgenome", marked by enzyme loci of NG group, increased variation of which is necessary condition of domestication. The genetic distances between wild populations (0,059-0,129) and between varieties (0,038-0,264) indicated on the similarity in intraspecies differentiation in both species.

SOY 2002

Concurrent Session
Transformation

Monday, August 12
1:20 pm

Abstracts 301-306

Endogenous Retroviruses as Transgenic Vectors - The Ubiquitous SIRE1 Family

Howard Laten[1], Ericka R. Havecker[2], Lisa M. Farmer[1], and Daniel F. Voytas[2]

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SIRE1 is a family of soybean retroelements that in addition to gag, protease, integrase and reverse transcriptase domains - the standard features of retrotransposons - encodes an envelope-like protein. Homologs of SIRE1 have been detected in Glycine soja, other Glycine species, lotus, favabean, Arabidopsis, tomato, and maize. Unlike most plant retroelements, members of the SIRE1 family contain intact ORF's with full coding capacity. A single stop codon separates the gag-pol ORF from the env ORF. The possibility of a readthrough mechanism is supported by a conserved stop-codon context which matches a consensus sequence (UAGCARYYA) found in several ssRNA plant viruses that is required for translational readthrough. Readthrough assays with GUS constructs support this suggestion. Ten members of the family have been sequenced. These members are remarkably homogeneous. An analysis of base-pair substitutions between members supports the inference that the small degree of divergence has occurred under selection. In addition, since the LTR's of each element are identical at the time of integration, the divergences between pairs of LTR's (<0.0009 for SIRE1-4 and SIRE1-7) have been used to date their insertions to less than 30,000 years ago. Two of the ten SIRE-1 copies are adjacent to novel LTR retrotransposons suggesting that, like Z. mays, some LTR retroelements may be clustered in the G. max genome. SIRE1 has successfully colonized the genomes of a wide variety of plants, and the sequenced copies from G. max appear to be functional and of recent origin. The presence of an envelope-like ORF strongly suggests that SIRE1 is an endogenous retrovirus capable of infectious transfer. Selective maintenance of the envelope ORF suggests that the protein it encodes is functional. Harnessing these qualities for the development of novel transformation vectors is underway. An infectious agent that has a long and possibly recent history of integrating into the genomes of monocots and dicots would be a welcome addition to the existing collection of gene transfer strategies.

Mining for Yield Genes in Wild Soybeans

Vergel C. Concibido, Bradley LaVallee, Jennifer Meyer, Paul McLaird, Liesa Hummel, Kunsheng Wu, and Xavier Delannay

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The value of exotic germplasm in broadening the genetic base of most crops has been demonstrated many times. However, the difficulties involved in working with exotic germplasm have limited their utility in plant breeding. Unwanted linkages often thwart the successful incorporation of beneficial exotic genes into commercial lines. Thus, the use of exotics in traditional breeding makes the process of crop improvement a tedious, time-consuming, and expensive endeavor. The availability of molecular markers makes it possible to isolate specific genomic regions and transfer them into commercial varieties with minimal linkage drag. Here, we report the discovery, characterization, and introgression into elite soybean lines of a yield-enhancing quantitative trait locus from *Glycine soja* (Siebold & Zucc.).

Progress in Mapping for SCN Resistance in Soybean

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Genetic mapping and QTL analysis has been used to elucidate genetics of complex traits including resistance in soybean to soybean cyst nematode. Recently, DNA markers have contributed greatly to identification of QTLs and can be used for MAS of resistant soybeans. Three resistance QTLs were mapped from PI 209332 to LGs A, G and J. The QTL on LG A was in proximity to Rhg_4 and a QTL on G was confirmed to be rhg_1, and presumably Rhg_5 corresponds to QTL on LG J. Two of the major resistance QTLs from PI 437654 were also mapped to LGs A and G and a third was mapped to LG M and probably corresponds to rhg_2. Most resistance from cv. Hartwig was mapped to primarily LG B. Several QTLs were mapped from Peking and LGs included A, F, C, B, H and I. Resistance in *G. soja* was mapped to LGs E and G, where no resistance was previously mapped. Recently, several QTLs were also mapped in two novel sources of resistance, PIs 438489B and 89772. A novel QTL on LG D1a was uncovered in PI 438489B and resistance to Race 14 (PI 88788 type) did not include QTLs on both LGs A_2 and G. This is unique type of resistance. LG E appears to be novel from PI 89772. There are some discrepancies in the results reported by several authors and these can be explained based on, differences in the nematode populations, differences in the seed sources and lack of specific markers segregating for the region or the markers used to map QTLs to LGs may not have been completely anchored. However, three markers Satt 309, Satt 583 and A006 can be highly useful in MAS for increased selection efficiency to breeding cultivars with improved resistance.

A Comparison of Recombination Rates between Random Lines and Adapted Cultivars

Thomas R. Stefaniak, David Hyten, Vince Pantalone, and Todd Pfeiffer

University of Kentucky

It has been estimated that in the period between 1930 and 1980 soybean yields in America increased 93% due to the introduction of superior cultivars. However, it is unclear what source of genetic variation has been utilized for this improvement. The objective of this study was to investigate the possible contribution of genetic variation from meiotic recombination in the improvement of soybean cultivars. Crossing-over events were detected using 88 SSR markers spanning 13 of soybeans linkage groups. The two populations consisted of 10 adapted high yielding cultivars and 156 **R**andom **R**ecombinant **I**nbred **L**ines from the cross Williams x Essex. The calculated recombination rates were standardized for potential crossovers. For total recombination the rates for the 10 cultivars were significantly greater than those of the RRILs. These rates were 0.24 and 0.36 for the RRILs and the cultivars respectively. The population by linkage group interaction was non-significant. These results indicate that breeding progress has been accomplished in part utilizing the genetic variation resulting from recombination.

Location of SSR Markers to the Soybean Chromosomes

Jijun Zou[1], Ram Singh[1], Perry Cregan[2], Theodore Hymowitz[1]

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

Primary trisomics (2n=41) were used to associate molecular (MLGs) and classical (CLGs) linkage groups to the eleven soybean chromosomes. The relationships among chromosomes, MLGs and CLGs are: Chromosome 1 = D1a+q = 3; Chromosome 3 = N = 10; Chromosome 5 = A1; Chromosome 8 = A2 = 7 and 9; Chromosome 9 = K = 2 and 12; Chromosome 13 = F = 8 and 13; Chromosome 14 = C1 = 21; Chromosome 17 = D2 = 20; Chromosome 18 = G = 18; Chromosome 19 = L = 5; and Chromosome 20 = I = 4. We encountered reverse segregation ratios at three chromosome regions (chromosome 13, 14, and 17), and could not associate presently available SSR markers with specific chromosomes.

Commodity Agriculture AND / OR Global Food Chains: Why? Why Not?

Steve Sonka

University of Illinois at Urbana-Champaign

During the last 50 years, the soybean has become one of the major agricultural crops produced and traded in the world. First, this paper briefly reviews the market forces fueling that transformation. Then the roles of income and population growth are examined relative to the global needs for protein in the future. The third segment of the paper focuses on the commodity marketing system, whose characteristics and functions have served as the primary platform by which soybeans have been delivered in the food industry. Today societal forces are challenging the nature of the commodity marketing system, leading some to predict that future soybean production and marketing will be conducted with global food chains. These forces, and implications for research, are analyzed.

Better Bean Initiative (BBI) - A Tool to Enhance Competitiveness for the U.S. Soybean Producer

J.R. Sallstrom

United Soybean Board Director, Production Committee Chairman, Winthrop, MN 55396

U.S. soybean producers enjoy a strong reputation for providing quality and as reliable suppliers of soybeans. However, soybean producers face increasing competition from both global soybean producers and other crops such as canola, corn and sunflower, as well as from synthetic meal displacing additives. During the past decade, competitive production pressures from Brazil and Argentina have increased. USDA projections are for U.S. exports of soybean, soybean meal, and soy oil to increase 9 million metric tons from 1995 through 2010. South American exports are projected to increase by 30 million metric tons during the same time period. This increase is due in part to the significant expansion of production acres in Brazil during the past 5 years. It is estimated that Brazil can increase soybean production acres by 25% to 50% during the next decade. The U.S. cannot 'out-compete' Brazil by out producing them. Improving the composition of U.S. soybeans is a strong compliment to increasing overall productivity to maintain our international competitiveness.

In anticipation of health issues, market functionality improvements and global competitive forces, the United Soybean Board (USB) began its efforts to accelerate the development and availability of compositionally enhanced soybean varieties in the mid-1990s. These efforts led to the Better Bean Initiative (BBI), which represented USB's objective of investing the U.S. soybean farmer checkoff to meet the evolving needs of end-users in the food and feed industries more effectively than competing producers and products. USB assembled a team of experts including farmer leaders, agribusiness veterans and soybean breeders to assess the status of oil and meal composition targets, provide input into USB breeding programs and develop an initial strategic plan to accelerate the development and commercialization of compositionally enhanced varieties. Key quality targets include altering soybean fatty acid profile, increasing metabolizble energy, and reducing the environmental impact from phytate-phosphorus. The BBI is a major initiative for USB and serves as an industry platform to address the competitiveness of U.S. soybeans.

USB's strong commitment to furthering the aims of the BBI demonstrates to domestic and international customers that the U.S. soybean grower strives to be the best possible business partner now and into the future.

The Soy Processing Industry Perspective on Soybean Attributes

Parry Dixon

Archer Daniels Midland Company

What Pigs Need from Soybeans

R. A. Easter, Maria Palacios, and Kevin Soltwedel

Department of Animal Sciences and College of Agricultural, Consumer and Environmental Sciences, University of Illinois, Urbana 61801

Soybeans are clearly superior to other oilseeds and most animal-protein ingredients as a source of protein to offset the amino acid deficiencies of the cereal grains commonly used in the formulation of swine diets. But, the soybean isn't perfect. Heat treatment to destroy digestive inhibitors continues to be necessary despite promising genetic advances in this area. In regions of the world where swine diets are typically energy-deficient, the potential value of full-fat, inhibitor-free soybeans is substantial. Lysine continues to be the first limiting amino acid and tryptophan and/or threonine the second in cereal-soybean meal-based diets. Although the industrial production of lysine in recent years has reduced the economic value of genetic modifications to increase the concentration of that amino acid in soybean protein, additional threonine and tryptophan would be very useful. Dietary excesses of amino acids depress pig performance and contribute significantly to environmental nitrogen pollution. The opportunity to improve the nutritional value of soybean protein by reducing amino acids that contribute to dietary excesses should not be ignored. Soybean proteins are well digested by pigs with a mature gastrointestinal tract; however, the large hydrophobic protein molecules are not easily broken down by young pigs, and this places a constraint on the use of soybean protein in weanling pig diets. Recent work has shown the potential that some of the bioactive compounds in soybeans may positively influence both muscling and immune system responsiveness. If this work is verified, it may be desirable to increase the concentration of these molecules in soybeans destined to produce soybean meal for inclusion in finishing pig diets. Finally, pigs lack capacity to efficiently digest the complex carbohydrates found in soybean products. This results in a lost opportunity to capture metabolically useful energy and increases the undigested residues that contribute to waste handling problems at the farm level.

Advances in Transformation

J.J. Finer

Department of Horticulture and Crop Science, OARDC/The Ohio State University, Wooster, OH 44691

Two main methods exist for transformation of soybean. *Agrobacterium*-mediated transformation of cotyledonary nodes and particle bombardment-mediated transformation of proliferative embryogenic tissues were first reported over ten years ago, and there have since been substantial improvements in the efficiency of these approaches. Other transformation systems have also been described, with varying levels of success and repeatability.

For *Agrobacterium*-mediated transformation of cotyledonary node tissue, evaluation of alternate selectable markers as well as use of reducing agents have led to significant improvements in transformation and regeneration efficiency. For particle bombardment-mediated transformation of proliferative embryogenic tissues, improvements in growth of the target tissues and selective conditions have also led to significant increases in transformation rates. Both of these systems are repeatable and each has advantages and disadvantages. Often, the area of expertise of the researcher (shoot morphogenesis or embryogenesis) determines which protocol is preferred.

Other transformation systems in soybean include particle bombardment of shoot apical tissues, pollen tube pathway, *Agrobacterium*-mediated transformation of seeds, *Agrobacterium* floral dip, meristem electroporation and *Agrobacterium*-mediated transformation of proliferative embryogenic tissue. All of these procedures show limited repeatability and need to be further evaluated.

Linkage Disequilibrium and Association Analysis for QTL Discovery

P .B. Cregan[1], Y.L. Zhu[1], Q.J. Song[1], N. E. Hofmann[1], R. W. Yaklich[1], J. E. Specht[2], and R. L. Nelson[3]

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[2] Dept. of Agronomy and Horticulture, Univ. Nebraska, Lincoln, NE 68583-0915
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Sequence and haplotype diversity as well as the level of linkage disequilibrium (LD) are important considerations for assessing the likelihood of successfully applying association analysis for QTL or gene discovery. In its simplest form association analysis (originally proposed by Risch and Merikangas, *Science* 273:1516) compares DNA marker allele frequencies across the genomes of two groups of individuals with contrasting phenotypes. Those regions in the genome at which significant marker allele frequency differences are detected between the two groups would putatively contain genes controlling phenotype. The virtue of such an approach to QTL or gene discovery is that a formal population structure is not required. In addition, extensive sets of phenotypic data are already available on large numbers of genotypes in germplasm collections. In an attempt to define sequence and haplotype variation in soybean 46607 bp of DNA (17010 bp coding, 6247 bp UTR, 16586 bp intron, 6764 bp genomic sequence) was sequenced in each of 18 N. Am ancestral cultivars; 52 diverse *G. max* accessions from Asia; 24 cultivars representative of currently grown N. Am. soybean; as well as 24 diverse *G. soja* genotypes from Asia. Eighty-four fragments averaging 555 bp in length and derived from 73 genes and 11 genomic fragments were included in the analysis that discovered 471 SNPs. The mean nucleotide diversity ($\hat{\theta}$) was very similar in the N. Am. ancestral cultivars and Asian *G. max* genotypes ($\hat{\theta}$ = 0.00135 and 0.00130, respectively). Somewhat less variability was present in modern cultivars ($\hat{\theta}$ = 0.00092). In contrast, the corresponding value in *G. soja* indicated a level of diversity ($\hat{\theta}$ = 0.00246) which was approximately twice as high as that of cultivated soybean. An analysis of LD in a 10 centiMorgan region on linkage group G showed a very significant decline suggesting that like in *Arabidopsis*, another autogamous species, LD decays over distances that are sufficiently short to permit successful association analysis. In order to test this possibility we have undertaken a study to compare association analysis with classical QTL analysis. Seed size which is an easily measured quantitative variable will be determined in a set of large (>25 g/100 seeds) vs. small (<8 g/100 seeds) seeded germplasm accessions and will also be determined in progeny of a large x small seeded genotype. The genomes of all individuals will be scanned using SNPs. SNP loci with significant allele frequency differences between the large vs. small germplasm lines will suggest the presence of seed size QTL. These will be compared with QTL discovered by standard QTL analysis of progeny from the large x small seeded cross.

Advances in Molecular Breeding

H. Roger Boerma

University of Georgia, Center for Applied Genetic Technologies, Athens, GA, 30602

In soybean, *Glycine max* (L.) Merr., researchers have developed robust sets of DNA markers (RFLP, RAPD, AFLP, and SSR) that span the soybean genome. These markers have been used to map major genes and QTL for many agronomic, physiological, pest resistance, and seed composition traits. Based on a review of existing literature, there are at least 319 unique QTL reported for various quantitative soybean traits. Of these, approximately 50% (162 QTL) condition 10% or more of the phenotypic variation in the trait. Studies attempting to confirm previously reported QTL have met with mixed success. Generally, the QTL conditioning large amounts of the phenotypic variation (e.g., pest resistance QTL) have been confirmed while QTL conditioning less than 10% of the phenotypic variation have been difficult to confirm. Presently, SSR markers are the marker of choice in most public soybean breeding programs and QTL mapping studies. Single nucleotide polymorphism (SNP) markers are currently being developed in the public sector and are already extensively employed by commercial breeding organizations for marker-assisted selection (e.g., selection for major disease resistance genes). In the public sector, three backcross-derived soybean cultivars have been developed by the application of SSR markers for the recovery of recurrent parent genome. DNA markers are also being employed for the improvement of previously intractable traits such as drought tolerance, resistance to foliar-feeding insects, and the combination of high protein/high yield. These applications will be discussed in the presentation.

SOY 2002

Concurrent Session
Molecular Breeding and Genetic Mapping

Monday, August 12
1:20 pm

Abstracts 201-208

SOY 2002

Poster Session, *Illini Room C*

All posters will be available for viewing during the entire meeting

Abstract	Title
Molecular breeding and genetic mapping	
P201	Eileen Kabelka (University of Illinois) Identification of putative yield enhancing QTL from exotic soybean germplasm
P202	Cyrus Abdmishani (Penn State University) Assessment of genetic relationship among soybean cultivars using DNA amplification fingerprinting (DAF)
P203	Bo-Keun Ha (University of Georgia) Pedigree Analysis of a Major QTL for soybean resistance to southern root-knot nematode
P204	Eun Young Hwang (Seoul National University) SNP on RFLP markers for seed protein concentration in soybean
P205	Geungjoo Lee (University of Georgia) Identification of QTL conditioning fibrous rooting in soybean
P206	J.K. Moon (Seoul National University) Molecular mapping of resistance genes in soybean cultivars showing a broad range of resistance to SMV strains
P207	David Walker (University of Georgia) Mapping a gene associated with phytic acid levels in soybean seed
P208	Jennifer Yates (University of Georgia) SSR band variation among soybean lines selected from within elite cultivars for seed weight and seed protein
P209	Maria Monteros (University of Georgia) Molecular mapping of genes conditioning oleic acid content in N00-3350 soybean
P210	S. T. Kang (Seoul National University) Analysis of QTL for pod dehiscence based on molecular map in soybean (*Glycine max* L. Merr.)
P211	Nicolle Hofmann (USDA-ARS) Molecular beacons to select for SCN resistance at *rhg1* and *Rhg4*
P212	Laura Marek (Iowa State University) Soybean repetitive sequence anchored to the soybean genetic map
P213	Yuhong Li (University of Minnesota) The effect of *rhg1* gene on the development and reproduction of *Heterodera glycines* Race 3
P214	X. Guo (Michigan State University) Genetic mapping of genes underlying partial resistance to Sclerotinia stem rot in soybean variety Asgrow A2506
P215	Dechun Wang (Michigan State University) Genetic mapping of genes underlying agronomic and seed composition traits in wild and cultivated soybean
P216	Megan Kirsch (University of Illinois) Co-Inheritance of Brown stem rot and soybean cyst nematode resistance from PI88788
P217	Dirk Charlson (Iowa State University) Examining SSR markers for iron chlorosis
P218	Renee Ritchie (University of Nebraska) SSR analysis of soybean protein QTLs in high protein plant introductions
P219	Julian Chaky (University of Nebraska) Advanced backcross QTL analysis in a mating between *Glycine max* by *Glycine soja*
P220	Yantong Wang (Chinese Academy of Agricultural Sciences) Exploration of origin and evolution of soybean (*Glycine max*) based on molecular evolution of internal transcribed spacer 1 region of nuclear ribosome DNA
P221	Antonio Di Mauro (UNESP/FCA) Efficiency of microsatellite markers for resistance to soybean cyst nematode (Race 3)

SOY 2002

Abstract **Title**

Transformation

P301 Sophie Ducerf (Aventis) Can TAIO (Targeting Agrobacteria Into Ovaries) be a new approach for crop transformation?

P302 Randy Dinkins (University of Kentucky) Gene silencing in transgenic soybean plants transformed via particle bombardment

P303 Venkata Tavva (University of Kentucky) Resistance to bean pod mottle virus in transgenic soybean lines

P304 Francoise Thibaud-Nissen (University of Illinois) Global expression in developing seeds of soybean (*Glycine max*) transformed with a lectin-CHS6 construct

P305 Francoise Thibaud-Nissen (University of Illinois) Microarray analysis of gene expression during the induction of soybean (*Glycine max*) somatic embryos

P306 Wojciech Ornatowski (Kansas State University) Biolistic transformation, recovery, and expression of fertile soybean transgenics for chitinase and *pto* genes

P307 Paulo Mello-Farias (Ohio State University) Analysis of expression of the green fluorescent protein (*gfp*) gene in transgenic soybean obtained using sonication assisted Agrobacterium-mediated trasformation (SAAT)

P308 David Hildebrand (University of Kentucky) Improving plant metabolism using trans-acting factors

P309 Yoshimi Inaba (University of Illinois) Transformation of soybean with the tryptophan biosynthetic control enzyme gene

Metabolic engineering

P501 Olga Zernova (University of Illinois) Genetic modification of soybean seed isoflavone content and composition

P502 Aline Andres (University of Illinois) Expression of genes regulating phenolic metabolism in soybean hairy roots

P503 Jigyasa Tuteja (University of Illinois) Real time expression analysis of the soybean chalcone synthase gene family members

P504 Stephanie Roberts (Ohio State University) Identification and isolation of genes from soybean (*Glycine max*) orthologous to cold-regulated genes found in *Arabidopsis thaliana*

P505 Tiefeng You (University of Illinois) Determination of isoflavone contents for selected soybeans by fourier transform near infrared reflectance spectroscopy

P506 Jun Guo (University of Illinois) Determination of soy and health foods contents by fourier transform near infrared reflectance spectroscopy

P507 Doina M. Costescu (University of Illinois) Determination of amino acid composition of soybeans by ^{13}C NMR techniques

P508 Doina M. Costescu (University of Illinois) Soybean oil and protein content measurements by high-resolution ^{13}C NMR and NIR spectroscopy. Novel observations of oil droplets and protein body ultrastructure in hydrated soybeans by TEM and ESEM electron microscopy techniques

P509 Doina M. Costescu (University of Illinois) Novel techniques for microspectroscopy and chemical imaging analysis of mature and developing soybean embryos.

P510 Reyhaneh Sariri (Gilan University) Soybean seed coat contains higher peroxidase than other parts of the plant

P511 Randy Nelson (University of Illinois) Genetic and environmental control of soybean seed isoflavone concentration and composition

Abstract	Title
P512	Ali Reza Rezazadeh (Gilan University) Comparison the peroxidase content of different Iranian soybeans
P513	Schuyler Korban (University of Illinois) Response of embryogenic cultures of soybean to chemical mutagenesis
P514	Schuyler Korban (University of Illinois) Characterization and expression of the gene encoding β-ketoacyl-acyl carrier protein synthase (KAS) III in soybean
P515	Susan Stombaugh (University of Minnesota) QTL associated with cell wall polysaccharides in soybean seed

Molecular and cellular pathology

P601	M. Iqbal (Southern Illinois University) Resistance loci pyramids alter transcript abundance in soybean roots inoculated by *Fusarium solani* f. sp. *glycines*
P602	Andrea Davidson (Agriculture and Agri-Food Canada) Microscopic study of white mould resistant (oxox transgenic) soybean plants and the wild type parental line infected by white mould (*Sclerotinia sclerotiorum*)
P603	Steve Clough (University of Illinois) Sequence analysis of the I locus in Williams 82
P604	Junli Ji (Iowa State University) Effect of cell free *Fusarium solani* f. sp. *glycines*-culture filtrates on the protein profiles of soybean cell suspensions
P605	Hongyu Gao (Iowa State University) Analysis of paralogous *Rps1-k* sequences in soybean
P606	Anatoliy Lygin (University of Illinois) Involvement of phenylpropanoid metabolism in soybean root response to Fsg
P607	Made Tasma (Iowa State University) Isolation of two soybean sequences with similarities to the Arabidopsis *NPR1* gene
P608	Laura Miller (Indiana University) A transient assay for analysis of resistance/avirulence gene interactions in soybean

General molecular genetics

P801	Dipak Santra (Iowa State University) A yeast artificial chromosome library of soybean
P802	Jessica Schlueter (Iowa State University) Promoter capture technology for analyzing soybean regulatory elements
P803	Lynge Christiansen (DIAS) Pod opening in soybean - isolation of potential *IND1* orthologs
P804	David Grant (Iowa State University) SOYBASE 2002: One decade of soybean genetics and genomics - Genetics maps
P805	Marcia Imsande (Iowa State University) SOYBASE 2002: One decade of soybean genetics and genomics - Metabolism
P806	Marcia Imsande (Iowa State University) SOYBASE 2002: One decade of soybean genetics and genomics - Transformation

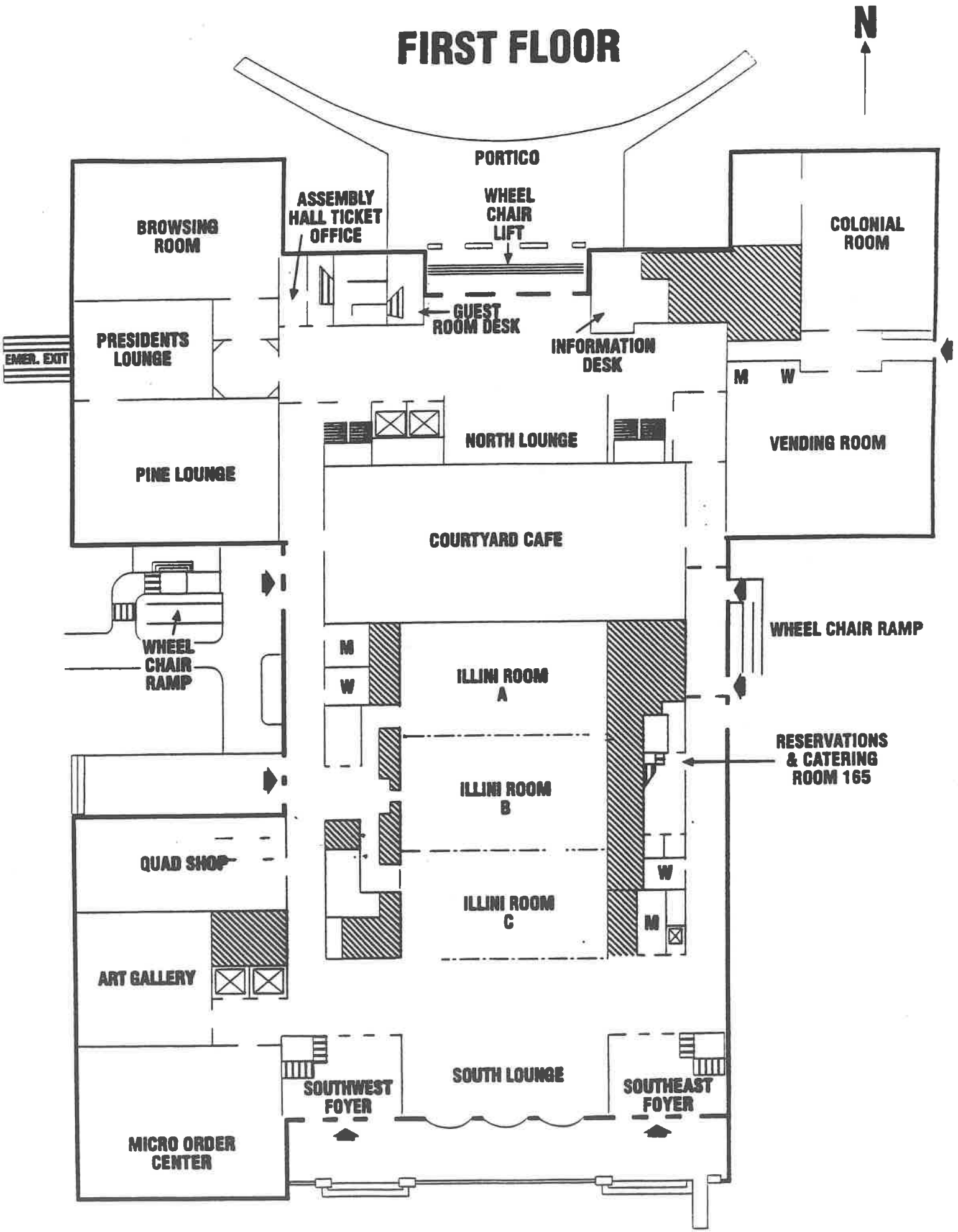
SOY 2002

Plenary Session 1

Monday, August 12
8:00 am

Abstracts P101-P107

FIRST FLOOR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PINE LOUNGE
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RESERVATIONS & CATERING ROOM 165
ILLINI ROOM B
QUAD SHOP
W
ILLINI ROOM C
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ART GALLERY
SOUTHWEST FOYER
SOUTH LOUNGE
SOUTHEAST FOYER
MICRO ORDER CENTER

SOY 2002

Time	Abstract	Title
Sunday, August 11, 2002		
4:00-7:00		Registration and Poster Setup in the Illini Union, *South Lounge*
5:00-7:00		Welcome Reception, *South Lounge*
Monday, August 12, 2002		
7:00		Breakfast, Registration, and Poster Setup, *South Lounge & Illini Room C*
Plenary session (Gary Heichel, Presiding), *Illini Rooms A & B*		
8:00		Welcome, Steve Pueppke (College of Agriculture, Consumer and Enivronmental Sciences), Lyle Roberts (Illinois Soybean Checkoff Board)
8:10	101	Steve Sonka (University of Illinois) Commodity agriculture and / or global food chains: why? why not?
8:40	102	Jim Sallstrom (USB Production Committee Chairman) Better Bean Initiative (BBI) - A tool to enhance competitiveness for the U.S. soybean producer
9:10	103	Parry Dixon (ADM) The soy processing industry perspective on soybean attributes
9:40	104	Bob Easter (University of Illinois) What pigs need from soybeans
10:10		Break, *South Lounge*
10:30	105	John Finer (The Ohio State University) Advances in transformation
11:00	106	Perry Cregan (USDA-ARS) Linkage disequilibrium and association analysis for QTL discovery
11:30	107	Roger Boerma (University of Georgia) Advances in molecular breeding
12:00		Lunch, *Illini Union Ballroom*
Concurrent Sessions		
Molecular breeding and genetic mapping (Brian Diers, Presiding), *Illini Room A*		
1:20	201	Vergel Concibido (Monsanto) Mining for yield genes in wild soybeans
1:50	202	James Specht (University of Nebraska) A genomic perspective on the soybean protein(+)/oil(-)/yield(-) enigma
2:10	203	Brian Diers (University of Illinois) Fine mapping of a protein QTL on linkage group I from *Glycine soja*
2:30	204	Prakash Arelli (USDA-ARS) Progress in mapping for SCN resistance in soybean
2:50	205	Thomas Stefaniak (University of Kentucky) A comparison of recombination rates between random lines and adapted cultivars
3:10		Break, *South Lounge*
3:40	206	Jijun Zou (University of Illinois) Location of SSR markers to the soybean chromosomes
4:00	207	Aron Weir (University of Guelph) Molecular markers associated with seed protein and fatty acid content in an interspecific soybean population
4:20	208	Valerii Glazko (Ukrainian Academy of Agricultural Science) Genetically determined polymorphism of enzymes at some varieties of soybean (*Glycine max*) and wild soybean (*Glycine soja*)

SOY 2002

Time	Abstract	Title
Transformation (Ted Klein, Presiding), *Illini Room B*		
1:30	301	Howard Laten (Loyola University Chicago) Endogenous retroviruses as transgenic vectors - The ubiquitous SIRE1 family
1:50	302	Paula Olhoft (University of Minnesota) Agrobacterium-mediated transformation of soybean using the cotyledonary-node method
2:10	303	David Hildebrand (University of Kentucky) Fatty acid and triglyceride changes in transgenic soybeans
2:30	304	Ted Klein (DuPont) Acetolactate synthase as a selectable marker for soybean transformation
2:50	305	John Finer (Ohio State University) Automated image collection and analysis for studies of gene expression
3:10		Break, *South Lounge*
3:40	306	Sharad Tiwari (J.N. Agricultural University) Optimization of somatic embryogenesis in soybean [*Glycine max* (L.) Merrill]
4:00-5:00		Panel discussion on soybean transformation (David Somers, University of Minnesota, Presiding)
5:00-7:00		**Poster Reception**, *Illini Room C & South Lounge*
7:00		**Banquet**, *Illini Union Ballroom*

Tuesday, August 13, 2002

Plenary session (Steve Pueppke, Presiding), *Illini Rooms A & B*		
7:00		Breakfast, *South Lounge*
8:00	401	Steve Long (University of Illinois) Adapting soybean to current and future change in atmospheric composition. Do we need more than field selection under current conditions?
8:30	402	Susan Kundrat (University of Illinois) Soy as a functional food: implications for women's health
9:00	403	Brian Wansink (University of Illinois) The psychology of soy: increasing the consumer acceptance of soy
9:30		Break, *South Lounge*
10:00	404	Andrew Bent (University of Wisconsin) Genomics of plant pathogen interactions
10:30	405	Tom Ashfield (Indiana University) Positional cloning of the soybean *Rpg1* disease resistance gene
11:00	406	Tony Kinney (DuPont) Improvement of soybean seed quality for food uses
11:30	407	Eliot Herman (USDA-ARS) Genetic modification removes an immunodominant allergen from soybean
12:00		Lunch, *Illini Union Ballroom*

SOY 2002

Time	Abstract	Title

Concurrent Sessions

Metabolic engineering (Tara Vantoai, Presiding), *Illini Room A*

1:30	501	Edgar Cahoon (USDA-ARS) Genetic modification of soybean for enhanced industrial value
1:50	502	Tim Oulmassov (Monsanto) Engineering of essential amino acids in soybean for animal feed
2:10	503	Tom Clemente (University of Nebraska) Production of gamma linolenic acid in seeds of transgenic soybean
2:30	504	Sangeeta Dhaubhadel (Agriculture and Agri-Food Canada) Accumulation of soybean seed isoflavones: synthesis within the seed or transport from maternal tissues?
2:50		Break, *South Lounge*
3:20	505	Hari Krishnan (USDA-ARS) Cloning and characterization of APS reductase from soybean
3:40	506	Gracia Zabala (University of Illinois) The pleitropic tawny pubescence locus (*T*) encodes a flavonoid 3' hydroxylase. Molecular characterization of gray pubescence mutations (*t*)
4:00	507	Eun Young Hwang (Seoul National University) Differentially expressed genes in hypernodulation soybean mutant
4:20	508	Tiefeng You (University of Illinois) Calibration of dual-diode array and fourier transform near infrared reflectance spectrometers for composition analysis of single soybean seeds in genetic selection, cross-breeding experiments

Molecular and cellular pathology (Andrew Bent, Presiding), *Illini Room B*

1:30	601	Vera Lozovaya (University of Illinois) Soybean phenolics (isoflavones and lignin) affecting seed quality and root resistance
1:50	602	Elizabeth Trebovac (University of Guelph) Molecular analysis of isoflavones in relation to pathogen attack of soybean (*Glycine max* L. Merr.)
2:10	603	Guiying Zhao (University of Guelph) Identification of SSR markers associated with Rhizoctonia root rot resistance in soybean (*Glycine max* L. Merr.)
2:30	604	Madan Bhattacharyya (Iowa State University) The *Rps1-k* locus carries multiple functional Phytophthora disease resistance genes in soybean
2:50		Break, *South Lounge*
3:20	605	Devinder Sandhu (Iowa State University) Instability in a resistance gene-like sequence caused by mitotic recombination in soybean
3:40	606	Jerome Auclair (University of Guelph) Molecular analysis of QTL associated with partial resistance to sclerotinia stem rot

6:30-8:30 Reception, *ACES Library Information & Alumni Center (LIAC)*

SOY 2002

Time	Abstract	Title

Wednesday, August 14, 2002

Plenary session - Genomics (Jack Widholm, Presiding), *Illini Rooms A & B*

7:00		Breakfast, *South Lounge*
8:00	701	Greg May (Noble Foundation) *Medicago truncatula*: Can crop species benefit from model organisms?
8:30	702	Chris Town (TIGR) Plant genome sequencing: strategies and expectations
9:00	703	Jim Schupp (Northern Arizona University) Global gene expression analysis of soybean using serial analysis of gene expression (SAGE)
9:30		Break, *South Lounge*
10:00	704	Gary Stacey (University of Missouri) Soybean genome: survey sequencing and microsynteny
10:30	705	David Lightfoot (Southern Illinois University) Building version 3 of the soybean integrated physical and genetic map: Progress toward functional, high density gene maps.
11:00	706	Randy Shoemaker (Iowa State University) Soybean gene and genome evolution
11:30	707	Lila Vodkin (University of Illinois) Global gene expression in soybean using microarrays
		Adjourn

SOY 2002

9th Biennial Conference of the Cellular and Molecular Biology of the Soybean

Illini Union
August 11-14, 2002

Hosted by:

College of Agricultural, Consumer and Environmental Sciences
University of Illinois at Urbana-Champaign

Organizing Committee:
Brian Diers
Steve Pueppke
Steve Sonka
Jack Widholm

Conference Coordinators:
Linda Kull
Megan Puzey

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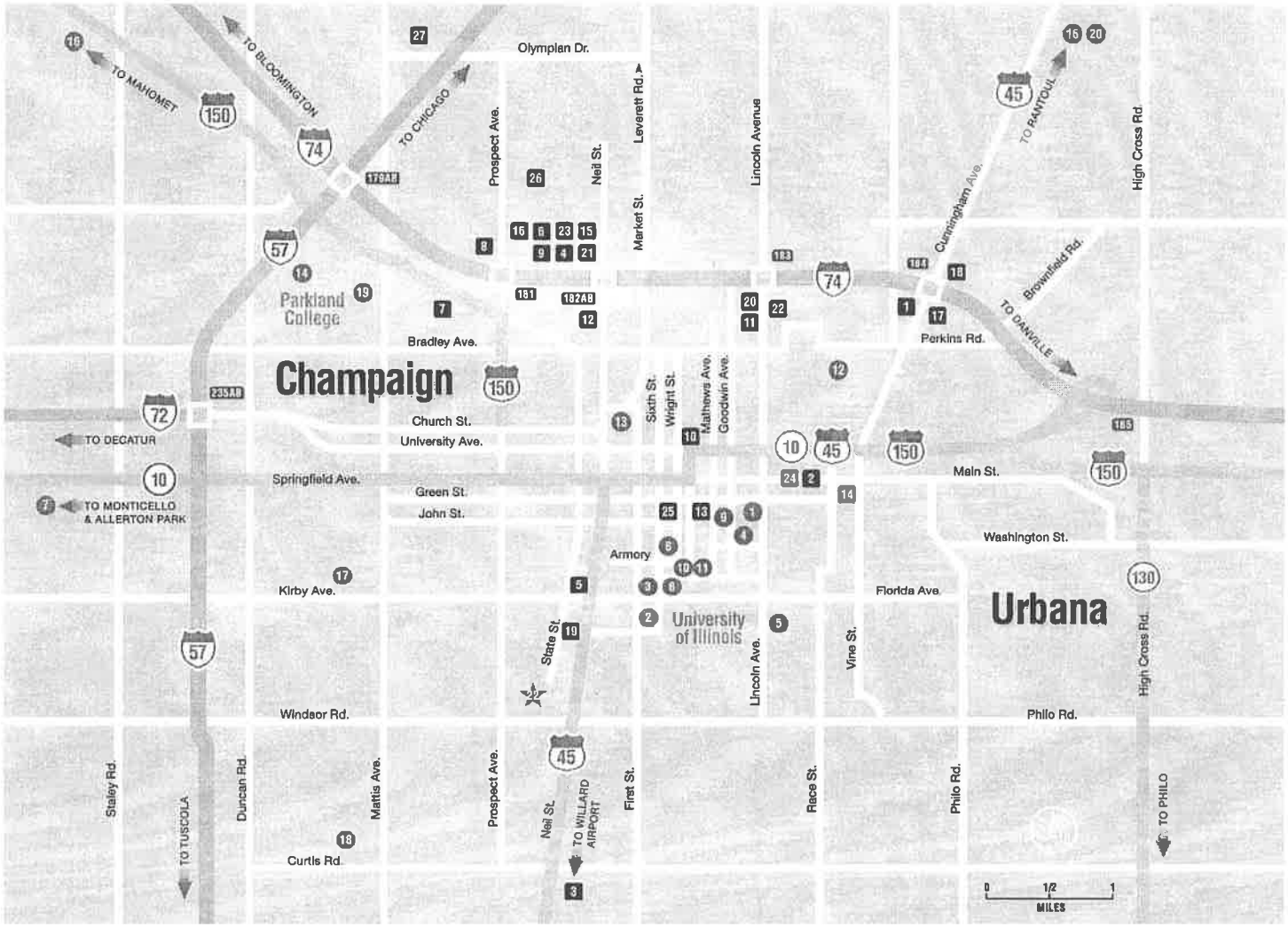
Illinois Soybean Program Operating Board
United Soybean Board
Illinois Department of Agriculture
Blackwell Scientific

Registration fees for graduate students and post-docs sponsored in part by the University of Kentucky – Soy2000

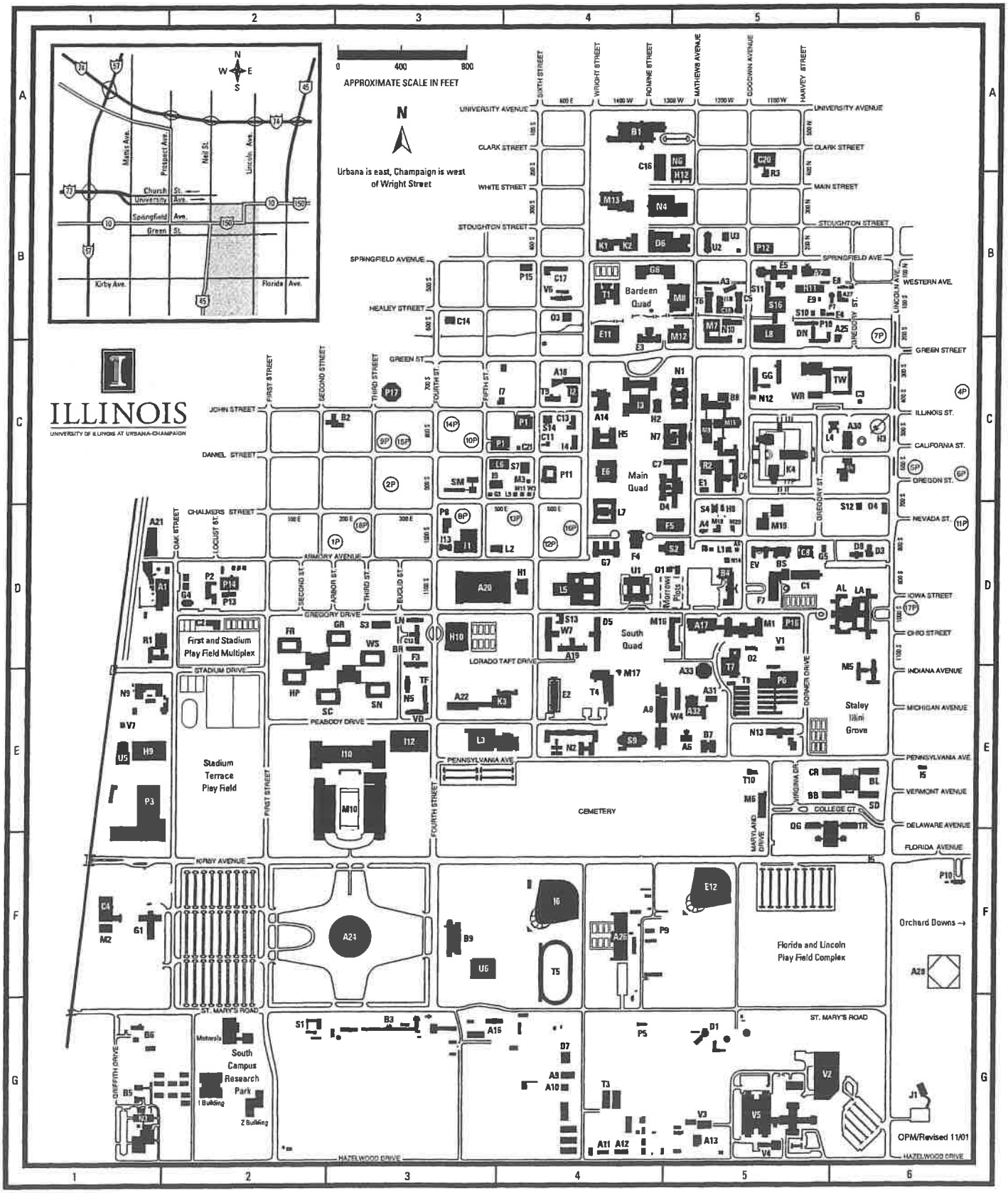


A Attractions

1. Campus Visitors Center
2. Assembly Hall
3. Memorial Stadium
4. Krannert Center forthe Performing Arts
5. The Arboretum
6. The Krannert Art Museum
7. Robert Allerton Park
8. John Philip Sousa Library and Museum
9. The Spurlock Museum
10. Rare Book and Special Collections Library
11. University of Illinois Library
12. Anita Purves Nature Center
13. The Orpheum Children's Science Museum
14. The William M. Staerkel Planetarium
15. Octave Chanute Aerospace Museum
16. The Early American Museum/Mabery Gelvin Botanical Garden
17. Prairie Farm
18. Curtis Orchard
19. Olympic Tribute
20. Hardy's Evergreen Acres & Reindeer Ranch
21. Champaign County Historical Museum
22. Champaign-Urbana Convention & Visitors Bureau

H Accomodations

1. Eastland Suites & Convention Center
2. Best Western-The Lincoln Lodge
3. Best Western-Paradise Inn
4. Baymont Inn & Suites
5. Clarion Hotel and Convention Center
6. Comfort Inn
7. Days Inn
8. Drury Inn
9. Fairfield Inn
10. Hampton Inn
11. Premier Inn
12. Howard Johnson
13. Illini Union
14. Jumer's Castle Lodge
15. La Quinta Motor Inn
16. Marriott Courtyard
17. Motel 6
18. Park Inn & Illini Conference Center
19. Hawthorn Suites Ltd & Conference Center
20. Ramada Limited
21. Red Roof Inn
22. Sleep Inn
23. Super 8
24. Travelodge
25. Quality Hotel University Centre
26. Extended StayAmerica
27. Microtel


ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN
APPROXIMATE SCALE IN FEET
0
400
800
N
Urbana is east, Champaign is west of Wright Street
SIXTH STREET
WRIGHT STREET
ROMINE STREET
MATHEWS AVENUE
GOODWIN AVENUE
HARVEY STREET
UNIVERSITY AVENUE
CLARK STREET
WHITE STREET
STOUGHTON STREET
SPRINGFIELD AVENUE
HEALEY STREET
GREEN ST
JOHN STREET
DANIEL STREET
CHALMERS STREET
ARMORY AVENUE
GREGORY DRIVE
STADIUM DRIVE
PEABODY DRIVE
LORADO TAFT DRIVE
PENNSYLVANIA AVE
KIRBY AVENUE
ST. MARY'S ROAD
HAZELWOOD DRIVE
GRIFFITH DRIVE
FIRST STREET
SECOND STREET
THIRD STREET
FOURTH STREET
FIFTH STREET
OAK STREET
LOCUST ST
WESTERN AVE.
ILLINOIS ST.
CALIFORNIA ST.
OREGON ST.
NEVADA ST.
IOWA STREET
OHIO STREET
INDIANA AVENUE
MICHIGAN AVENUE
VERMONT AVENUE
DELAWARE AVENUE
FLORIDA AVENUE
COLLEGE CT
VIRGINIA DR.
MARYLAND DRIVE
DOHERTY DRIVE
Bardeen Quad
Main Quad
South Quad
Morrow Plots
First and Stadium Play Field Multiplex
Stadium Terrace Play Field
CEMETERY
Staley Illini Grove
Florida and Lincoln Play Field Complex
Orchard Downs →
South Campus Research Park
Motorola
I Building
Z Building
OPM/Revised 11/01