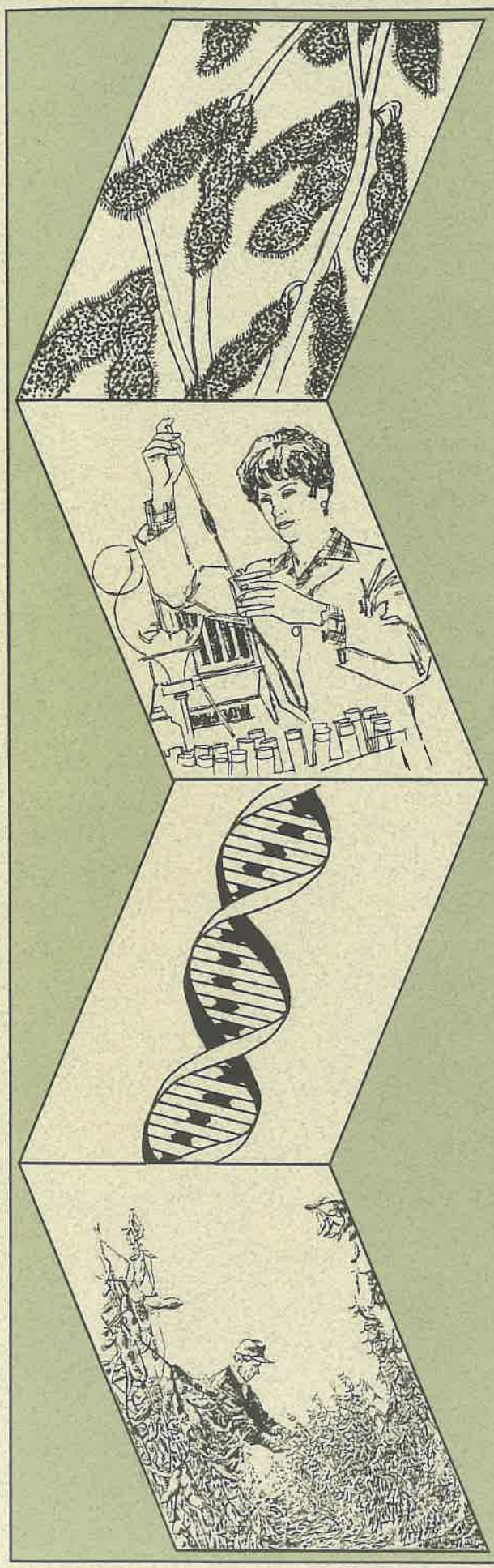

6th Biennial Conference

Molecular and Cellular Biology of the Soybean

Abstracts

August 12-14, 1996

Holiday Inn Executive Center
Columbia, Missouri

University of Missouri
Columbia

GENERAL INFORMATION

MEETING FACILITIES

All conference activities will take place at the Holiday Inn Executive Center. All contributed papers will be presented in Windsor III. The Social Hour and Dinner will be held in Windsor Ballroom of the Executive Center. Tuesday's lunch and invited address will be in the Parliament I, II, and III.

SPEAKER INFORMATION

A slide preview/practice room (Windsor IV) will be available for speakers to review their slides. Speakers should give their slides to the projectionist 30 minutes before the start of the session in which they are to speak. Speakers should also introduce themselves to the presider of the session. This will allow the presider to know the speaker and be certain of the correct pronunciation and affiliation. Slides should be picked up after the session.

POSTER INFORMATION

Posters will be in the Windsor IV Room for the duration of the meeting. Posters have been assigned numbers. Presenters should find the board with their number, and set up their poster any time Monday morning before the start of the first session. Posters may be taken down on Wednesday afternoon. Presenters should plan to be by their posters as indicated in the schedule.

ACKNOWLEDGMENTS

Conference coordinators:	A. P. Rao Arelli David A. Sleper
Logo and design:	Sharon G. Rodes
Clerical support:	Carol A. Smith
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WELCOME TO THE

6th Biennial Conference
Molecular and Cellular Biology of the Soybean

HOLIDAY INN EXECUTIVE CENTER, COLUMBIA, MISSOURI

Monday, August 12, 1996

10:00 a.m. - 1:00 p.m.	Registration	Windsor III
12:45 p.m.	Orientation David A. Sleper	Windsor III
12:50 p.m.	Welcome Michael Chippendale Interim Associate Dean CAFNR,UMC	Windsor III

2:00 p.m. **SIMPLE SEQUENCE REPEAT (SSR) MARKER DEVELOPMENT AND PROGRESS TOWARDS THE INTEGRATION OF SOYBEAN MOLECULAR GENETIC MAPS.** P. B. Cregan, USDA/ARS, Soybean and Alfalfa Research Lab., Beltsville, MD 20705; K. G. Lark, Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112; R. C. Shoemaker, USDA/ARS, Field Crops Research Unit, Iowa State Univ., Ames, IA 50011; J. E. Specht, Univ. of Nebraska, Lincoln, NE 68583; and A. L. Kahler, BioGenetic Services, Brookings, SD 57006.

2:15 p.m. **PROGRESS TOWARDS MAP-BASED CLONING OF THE *Rps1-k* GENE FROM SOYBEAN.** M. K. Bhattacharyya[1], T. Kasuga[1], S. Salimath[1], J. Shi[2], M. Kraft[3], M. Gijzen[4], and R. I. Buzzell[5]. [1]The Plant Biology Division, the Samuel Roberts Noble Foundation, 2510 Noble Park Way, P. O. Box 2180, Ardmore, OK 73401. [2] The Dep. of Plant Biology, 111 Koshland Hall, Univ. of California, Berkley, CA 94720. [3] 17 Fisher Dr., Franklin Park, New Jersey. [4] Agriculture Canada, 1400 Western Road, London, Ontario, Canada N6G 2V4. [5]Research Station, Agriculture Canada, Harrow, Ontario, Canada N0R 1G0.

2:30 p.m. **RAPD AND RFLP MARKERS LINKED WITH THE GENE RESISTANT TO A SMV STRAIN IN CHINA.** Zhiyong Zhang and Junyi Gai, Soybean Research Institute, Nanjing Agricultural Univ., Nanjing, 210095, China. Dongwei Hui and Shouyi Chen, The Institute of Genetics, Academia Sinica, Beijing, 100101, China.

2:45 - 3:15 p.m. Break Late Registration

Presiding: Lila Vodkin, University of Illinois, Urbana.

3:15 p.m. **QTL CONDITIONING CHLORIMURON ETHYL SENSITIVITY IN SOYBEAN AND THEIR EFFECT ON SEED YIELD.** M. A. R. Mian, J. Alvernaz, D. A. Ashley, H. R. Boerma, Dept. of Crop and Soil Sciences, Univ. of Georgia, Athens, GA 30602-7272; E. R. Shipe, Dep. of Agronomy and Soils, Clemson Univ., Clemson, SC 29634-0359; and J. D. Mueller, Dep. of Plant Pathology and Physiology, Clemson Univ., Blackville, SC 29817.

ORAL PRESENTATIONS CONTINUE

MOLECULAR ASPECTS RELATED TO PESTS

Presiding: Nevin Young, University of Minnesota

8:00 a.m. **TWO SIMPLE SEQUENCE REPEAT (SSR) MARKERS THAT FLANK THE MAJOR SOYBEAN CYST NEMATODE RESISTANCE GENE.** J. Mudge, N. D. Young, Dep. of Plant Pathology, Univ. of Minnesota; W. J. Kenworthy, Dep. of Agronomy, Univ. of Maryland; J. P. Kenworthy, P. B. Cregan, Soybean and Alfalfa Laboratory, USDA-ARS, Beltsville, MD and J. H. Orf, Dep. of Agronomy and Plant Genetics, Univ. of Minnesota.

8:15 a.m. **CYTOLOGICAL AND MOLECULAR ANALYSIS OF EARLY RESPONSE TO *HETERODERA GLYCINES* ICHINOHE INFECTION IN PI437654.** R. Mahalingam and H. T. Skorupska. Agronomy Dep., 272 P&AS Bldg., Clemson Univ., PO Box 340359, Clemson, SC 29634-0359.

8:30 a.m. **DIFFERENTIAL AND SYNCYTIUM-SPECIFIC EXPRESSION OF SOYBEAN GENES DURING INFECTION BY SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES* ICHINOHE, SCN)** C.-N. Liu and S. Mackenzie, Dep. of Agronomy, Purdue Univ.,West Lafayette, IN 47907.

8:45 a.m. **ANALYSIS OF DEFENSE RELATED GENES FOR ASSOCIATION WITH SUDDEN DEATH SYNDROME RESISTANCE.** G. A. Torto and D. A. Lightfoot. the Dept. of Plant and Soil Science, Molecular Science Program, Southern Illinois Univ., Carbondale, IL 62091.

9:00 a.m. **ISOLATION OF CANDIDATE DISEASE RESISTANCE GENE FAMILIES IN SOYBEAN.** V. Kanazin[1], L. F. Marek[1], and R. C. Shoemaker[1,2], Dep. of Agronomy[1], and USDA/ARS Field Crops Research Unit[2], Iowa State Univ., Ames, IA 50011.

Dave Sommers – Balloon Tit

TRANSFORMATION

Presiding: Wayne Parrott, The University of Georgia.

2:00 p.m. **IMPROVING PHOSPHORUS UTILIZATION IN SOYBEAN MEAL THROUGH PHYTASE GENE ENGINEERING.** E. A. Grabau, R. Hanlon, C. Hegeman, and J. Li, Dep. of Plant Pathology, Physiology and Weed Science, Fralin Center for Biotechnology, Virginia Tech, Blacksburg, VA 24061-0346.

2:15 p.m. **TRANSFORMATION AND REGENERATION OF SOYBEAN FROM THE SHOOT APICAL MERISTEM OF MATURE SEED.** C. D. Carter and C. M. Baker. Dep. of Plant Science, NPB 247, Box 2140C, South Dakota State Univ., Brookings, SD 57007.

2:30 p.m. **CHROMOSOMAL ABERRATIONS IN THE TRANSGENIC SOYBEAN.** R. J. Singh[1], T. M. Klein[2], T. Hymowitz[1], and C. M. Kostow[2]. [1] Dep. of Crop Sciences, Univ. of Illinois, Urbana, IL 61801; [2]DuPont Agricultural Products, Wilmington, DE 19880.

2:45 p.m. **EVALUATION AND USE OF INSECT RESISTANCE IN TRANSGENIC SOYBEAN.** C. N. Stewart[1*], D. R. Walker[1], M. J. Adang[2], J. N. All[2], H. R. Boerma[1], and W. A. Parrott[1], Deps. of Crop and Soil Sciences[1] and Entomology[2], The Univ. of Georgia, Athens, GA 30602. *Current address: Dep. of Biology, Univ. of North Carolina, Greensboro, NC 27412.

3:00 p.m. ***AGROBACTERIUM* MEDIATED TRANSFORMATION OF SOYBEAN EMBRYONIC AXES.** R. Di and G. B. Collins, Dep. of Agronomy, Univ. of Kentucky, Lexington, KY 40546.

3:15 p.m. **VARIABILITY OF GENOTYPIC RESPONSE OF SOYBEAN TO AGROBACTERIUM.** M. S. Fitter, A. M. Mazurkiewicz, and M. Hinchee, Ceregen, 700 Chesterfield Parkway N., Chesterfield, MO 63198.

3:30 p.m. Break

3:30 p.m. - 5:30 p.m. **Poster Session - Numbers 20-40**

9:15 a.m. **EARLY INDUCTION OF SPECIFIC CHITINASE AND PEROXIDASE ISOZYMES IS ASSOCIATED WITH ROOT-KNOT NEMATODE RESISTANCE IN SOYBEAN.** J. Qiu, J. Hallmann, D. B. Weaver, R. Rodríguez-Kábana, and S. Tuzun, Dep. of Plant Pathology, Dep. of Agronomy & Soils, and Biological Control Institute, Auburn Univ., AL 36849.

9:30 - 10:00 a.m. Break

MOLECULAR ASPECTS OF GENETIC DIVERSITY

Presiding: Peter Gresshoff, The University of Tennessee

10:00 a.m. **CLUSTERED QTL UNDERLYING RESISTANCE TO MANGANESE TOXICITY AND SDS ARE IN REPULSION.** V. Y. Kilo and D. A. Lightfoot, Southern Illinois Univ. at Carbondale, IL 62901-4415.

10:15 a.m. **MOLECULAR EVOLUTIONARY RELATIONSHIPS AMONG THE WILD PERENNIAL RELATIVES OF SOYBEAN.** K. P. Kollipara, R. J. Singh, and T. Hymowitz, Dep. of Crop Sciences, Univ. of Illinois, Urbana, IL 61801.

10:30 a.m. **GENETIC RELATIONSHIP AMONG PLANT INTRODUCTIONS WITH RESISTANCE TO SOYBEAN CYST NEMATODES.** B. W. Diers[1], H. T. Skorupska[2], A. P. Rao Arelli[3], and S. R. Cianzio[4].Dep. of Crop and Soil Sciences, Michigan State Univ., East Lansing, MI 48824[1]; Dept. of Agronomy and Dept. of Biological Sciences,Clemson Univ., Clemson, SC 29631[2];Dep. of Agronomy, Univ. of Missouri, Columbia, MO 65211[3]; Dep. of Agronomy, Iowa State Univ., Ames, IA 50011[4].

10:45 a.m. **AUTOMATED SIZING OF FLUORESCENT LABELED SIMPLE SEQUENCE REPEAT (SSR) MARKERS TO ASSAY GENETIC VARIATION IN SOYBEAN.** Noa Diwan and P. B. Cregan, USDA/ARS, Soybean and Alfalfa Research Laboratory, Beltsville, MD 20705.

MICROSATELLITE BASED INTEGRATION OF RFLP AND RAPD MARKER MAPS OF SDS, SCN RACE 3 AND SCN RACE 14 RESISTANCE QTL.

J. Abu-Thredeih, V.Y. Kilo, T.W. Doubler D.A.Lightfoot, Southern Illinois Univ. at Carbondale.

Soybean sudden death syndrome (SDS) can reduce soybean production by 60%. Since SDS resistance is polygenic breeding and selection of soybean lines containing several SDS resistance genes is difficult. An alternative and more precise method is the use of gene markers known to be linked to SDS resistance genes. RAPD, RFLP and DAF markers have been used to map five important SDS^R QTL in Essex x Forrest. However, these markers are not effective for selection except within the mapping population or related germplasm. Our objective was to integrate the map in ExF with other maps of the soybean genome and to provide improved tools for selection of SDS^R QTL. Microsatellite markers or simple sequence repeat (SSR) markers have been mapped in several RIL populations for which SDS scores exist including ExF, Pyramid x Douglas and Minsoy x Noir. Microsatellite markers closely linked to two SDS^R QTL have been identified and can be used to unequivocally identify and select alleles conferring SDS^R in soybean germplasm. SSR markers SATT79 and SCT28 allowed an SDS QTL from Essex to be anchored and oriented relative to a region of linkage group C2 close to the telomere. However, this region of the genome is not involved in greenhouse assay SDS resistance in MxN. Unfortunately the SSRs are not close enough to allow detection of the equivalent field resistance QTL in PxD. SSR markers SATT9, SATT80 and SOYABAB allowed an SDS QTL from Forrest to be assigned to linkage group N. The QTL has also been mapped with the microsatellite markers in PxD and MxN. However, this region of the genome is not involved in greenhouse assay SDS resistance in MxN. Unfortunately the SSRs are not close enough to allow detection of the equivalent field resistance QTL in PxD. No SSR markers are currently commercially available that map to linkage group G where major loci for SCN race 1, 3, 6, 14 and SDS resistance appear to cluster. Integration on this linkage group relies on RFLP markers and common QTL. The SDS QTL on this linkage group is environmentally sensitive so that the Douglas allele is beneficial in three of six environments infested with SDS. The allelic Pyramid locus is most likely derived from PI88.788 since it is closely linked to SCN race 14 resistance. Breaking this linkage in a cross with a Peking source of resistance is of importance to improving SDS resistance since PI88.788 is a common source of SCN resistance in modern soybean germplasm. Microsattelite markers for this region of the genome are needed.

FINE MAPPING OF CLUSTERED LOCI UNDERLYING THE COINHERITANCE OF FIELD RESISTANCE TO SUDDEN DEATH SYNDROME (SDS) AND CYST NEMATODE (SCN) RACE 3.

T.W. Doubler, S.J.C. Chang, V. Kilo, R. Suttner, P.T. Gibson and D.A. Lightfoot*. The Dept. of Plant and Soil Science, Molecular Science Program, Southern Illinois Univ., Carbondale, IL 62091.

Coinheritance of field resistance of soybean (*Glycine max* (L.) Merr.) to sudden death syndrome (SDS) (caused by the fungus *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & Hans.) and soybean cyst nematode (SCN) (caused by *Heterodera glycines* Ichinohe) sometimes occurs in crosses among adapted cultivars. Our objective was to characterize the loci underlying this coinheritance. One hundred and thirty DNA markers were compared with SDS disease response and SCN score among 100 recombinant inbred lines (RILs) derived from a cross between SDS and SCN resistant 'Forrest' and SDS and SCN susceptible 'Essex'. SDS disease incidence (DI), was determined in replicated sites during four years encompassing five locations. The SCN score was determined in the greenhouse using naturally infested field soil samples. Three separate genomic regions identified by RAPD markers $OI03_{450}$, $OR10_{390}$ and $OW15_{400}$ were associated with mean SCN score ($P < 0.005$) and jointly accounted for about 30% of variability in cyst score. $OI03_{450}$ and the linked (about 1-3 cM) RFLP marker Bng122D identified a QTL for resistance to SCN ($R^2 = 14\%$) within a genomic region that was strongly associated with SDS DI ($R^2 = 20\%$), partly explaining the coinheritance of the two traits. The two markers were polymorphic and associated with both traits ($P < 0.005$) within a near isogeneic line population (n=80) derived from residual heterogeneity in a RIL. Two recombinants were detected and SCN resistance and SDS resistance were still coinherited in these two NILs suggesting close clustering of QTL. The cluster will be analyzed in a larger NIL population (n = 4,000). Additional DNA markers polymorphic within this region are being sought by AFLP and SAMPL as a prelude to gene cloning.

SIMPLE SEQUENCE REPEAT (SSR) MARKER DEVELOPMENT AND PROGRESS TOWARDS THE INTEGRATION OF SOYBEAN MOLECULAR GENETIC MAPS

P.B. Cregan, USDA/ARS, Soybean and Alfalfa Research Lab., Beltsville, MD 20705; K.G. Lark, Dept. of Biology, University of Utah, Salt Lake City, UT 84112; R.C. Shoemaker, USDA/ARS, Field Crops Research Unit, Iowa State Univ., Ames, IA 50011; J.E. Specht, University of Nebraska, Lincoln, NE 68583; and A.L. Kahler, BioGenetic Services, Brookings, SD 57006.

A collaborative effort is proceeding with the goal of developing 500 or more Simple Sequence Repeat (SSR) DNA markers in soybean. The level of informativeness of each new marker locus assessed on a set of 10 soybean cultivars to assure a maximum level of genetic polymorphism of each marker. The average gene diversity of newly developed markers during the past 3 month period was 0.62 when assayed on these 10 soybean cultivars. As markers are developed each is mapped in three different populations including the USDA/Iowa State Univ. F_2 mapping population derived from a cross of *Glycine max* (A81-356022) x *G. soja* (PI 468.916), a second F_2 population derived from a cross of the cultivars Clark x Harosoy, and a set of recombinant inbred lines from Minsoy x Noir 1. As reported previously, there seems to be little or no clustering of markers. For example, in the Minsoy x Noir I population only four of 34 linkage groups do not contain an SSR marker and these groups represent less than 2% of the total linkage map length. A set of selected SSR markers containing mainly $(ATT)_n$ /$(TAA)_n$ and $(AT)_n$ /$(TA)_n$ core motifs were used to characterize each of the mapping populations. At least two markers have been placed in each of 17 of the 24 linkage groups previously defined in the *G. max* x *G. soja* population and commonly referenced in soybean molecular genetic mapping research. These include linkage groups a2, b1, b2, c2, d1, d2, e, f, g, h, i, j, k, l, n, p, and q. By the end of 1996 we hope to detect homologies between all linkage groups of the three mapping populations and identify a set of SSR loci that define each group. This will allow the complete joining of the three maps and provide a standard set of reference loci for scientists engaged in the development or use of molecular genetic maps of the soybean genome.

RAPD AND RFLP MARKERS LINKED WITH THE GENE RESISTANT TO A SMV STRAIN IN CHINA

Zhiyong ZHANG and Junyi GAI, Soybean Research Institute, Nanjing Agricultural University, Nanjing, 210095, China
Dongwei HUI and Shouyi CHEN, The Institute of Genetics, Academia Sinica, Beijing, 100101, China

The strain Sa of Soybean Mosaic Virus (SMV) was identified as a dominant strain in lower Changjiang vally and the resistance of soybeans to Sa were controlled by a single dominant gene Rsa. The objective of the present study was to find RAPD and RFLP markers linked with Rsa.

A cross between Kefeng No. 1 (resistant to Sa and other domestic and foreign strains) and Nannong 1138—2 (susceptible to Sa and other strains) was made. Total genomic DNA was extracted by using CTAB method. Fifteen resistant and 15 susceptible F2 individuals were selected and the extracted DNAs (2μg /plant) were pooled to obtain two bulks. Four hundred and twenty decamer primers (Operon Technologies, inc. Alameda, CA) were used to test the two bulks. RAPD analysis was conducted according to Operen 10—mer kits' product information. A specimen of approximately 5μg DNA was individually digested with one of 5 restriction enzymes (Taq I, Dra I, Hind III, EcoR V and BamH I). The DNA was transferred to nylon membrane via Southern blotting. Blots were hybridized with randomly primed P^{32}—labelled dCTP insert DNA and membranes were exposed to X—ray film for 5—10 days.

After the polymorphic bands of the two bulks, resistant and susceptible, were compared with the two patterns of the parental cultivars, five polymorphic bands were found to fit the Kefeng No. 1—resistant bulk pattern vs. Nannong 1138—2—susceptible bulk. It was also found that the polymorphic DNA fragment OPAS—06_{1800} generatd by the primer OPAS—06 is 23± 6.29cM from Rsa according to the results from 61 F2 plants. OPAS—06_{1800} amplified from Kefeng No. 1 was retrieved and was re—amplified by using the same primer. The re—amplified DNA product was labelled with P^{32} and used as a hybridization probe. Southern blotting results showed that it hybridized to single copy DNA.

A polymorphic RFLP marker was also identified when pK644 was used as a probe with the restriction enzyme Hind III. The marker co—segregated with Rsa in the F2 population of 50 plants. The genetic linkage distance between them was 31 ± 8.15cM. Yu et al (1993) reported that PK644a was tightly linked to Rsv in PI96983, thus Rsa and Rsv were possibly on a same chromosome in Kefeng No. 1 . Accordingly, several resistant genes might be located on a same linkage group since Rsa has been reported to be linked with Rsc, Rsg and Rsh.

MOLECULAR ANALYSIS OF ANTHOCYANIN PATHWAY GENES IN SOYBEAN

Dionysia A. Fasoula, Joselyn J. Todd, Paul A. Stephens, Cecil D. Nickell, and Lila O. Vodkin, 384 Plant and Animal Biotechnology Laboratory, Department of Agronomy, University of Illinois, Urbana, IL 61801

In soybean, pigmentation is present in the flowers, pubescence, pods, hypocotyls, and seed coats and is affected by a number of genes that may encode enzymes in the anthocyanin pathway. Many of these genes are known to act pleiotropically. We are interested in the linkage relationships and molecular interactions between the novel *Wp* locus, which controls the pink flower color, and the loci *I* and *T*, which affect the seed coat color and the pubescence pigmentation.

The mapping populations used in this study segregated for purple/pink flower color, yellow/pigmented seed coat and tawny/gray pubescence. Phenotypic scoring indicated that the pink flower color segregates independently of the pubescence and seed coat coloration. However, the recessive *wp* genotype modifies seed coloration pleiotropically in the presence of the recessive *i* allele.

RFLP mapping was performed using cloned genes encoding enzymes of the soybean anthocyanin pathway as probes. These experiments revealed a *Dra*I/*CHS* (chalcone synthase) polymorphism, which segregated independently of the seed coat color. This is intriguing, in view of the recent finding that a *Hind*III/*CHS* polymorphism represents a *CHS* three-gene cluster and corresponds to the *I* locus. Soybean chalcone synthase is a small multigene family with seven members and the *Dra*I/*CHS* polymorphism is independent of the *Hind*III/*CHS* polymorphism. We are currently developing gene specific probes for the four *CHS* genes that do not belong to the cluster with the aim of assigning a specific *CHS* gene to the *Dra*I polymorphism.

MOLECULAR AND CELLULAR MECHANISMS OF FLOODING TOLERANCE IN SOYBEAN

Tara T. VanToai
USDA-ARS, Soil Drainage Research, Columbus, OH 43210

Genetic variability for flooding tolerance exists in soybean. We have acquired a collection of flood-tolerant soybean lines to be used in the determination of the genetic, molecular, and cellular mechanism of flooding tolerance. Our collection includes: a) soybean from southeastern China that survived the 1991 Yangtze River basin "flood of the century"; b) Midwest soybean that produced 55 bu/A in northwestern Ohio where fields were flooded for the entire month of July 1992; and c) soybean in Iowa that survived and produced 30 bu/A after 2 weeks of submersion during the 1993 Midwest flood. Field, greenhouse and laboratory tests indicate that flooding tolerance of soybean is mediated by at least two mechanisms: a) *innate ability of seedlings to survive complete submergence.* This occurs at the biochemical level and allows the seedlings to survive short durations of submergence. Tolerant seedlings are capable of recovering after 18 h of complete submergence to continue primary root growth and produce vigorous secondary roots. Susceptible soybean either shows only minimum secondary root growth or is dead under the same stress. b) *adaptation ability of established plants to grow under continuous soil waterlogging.* Tolerant plants not only survive, but also grow normally during extended periods of waterlogging. Tolerant lines must develop extensive cellular mechanisms for transportation of oxygen to the roots to support vigorous shoot and root growth and to produce a large number of functional nodules during prolonged waterlogging (10 to 12 weeks). Susceptible soybean lines are chlorotic, stunted and have many dead roots without nodules. The kinetics of aerenchyma (air channels) and adventitious root development in response to flooding stress is correlated with the tolerance to waterlogging in soybean.

CURRENT STATUS OF PRIMARY TRISOMICS AND TETRASOMICS IN SOYBEAN

S. J. Xu, R. J. Singh and T. Hymowitz, Department of Crop Sciences, University of Illinois, Urbana, IL 61801

Primary trisomics are useful cytogenetic tools for chromosome manipulations and chromosomal locations of genes in diploid plant species. The objectives of this study were to report the isolation and identification of a complete set of primary trisomics ($2n=41$) and tetrasomics ($2n=42$) in soybean [*Glycine max* (L.) Merr.] and to develop all primary trisomic lines in a uniform genetic background. The precise identification of primary trisomics was based on the total length, arm ratio, and distribution of heterochromatin and euchromatin, and trivalent configuration of chromosomes at the pachynema stage. The tetrasomics were identified from the selfed progenies of the primary trisomics by counting somatic chromosomes. They were verified by meiotic chromosome pairing and morphological features. Currently, we have isolated and identified all possible 20 primary trisomics and related tetrasomics. Unlike primary trisomics, the tetrasomics expressed various alterations in morphological traits: some (e.g., Tetra 15) were normal, others (e.g., Tetra 13) were dwarf and had slow growth, small leaf, and low seed fertility. Since the primary trisomics were of diverse genetic backgrounds, they are being backcrossed into a uniform genetic background by using the soybean cultivar 'Clark 63'. Thus far, four primary trisomics are in the F_1 generation and sixteen in the BC_2. All primary trisomics are being used in genetic linkage analysis.

TWO SIMPLE SEQUENCE REPEAT (SSR) MARKERS THAT FLANK THE MAJOR SOYBEAN CYST NEMATODE RESISTANCE GENE

J. Mudge and N.D. Young, Dept. of Plant Pathology, Univ. of Minnesota
W.J. Kenworthy, Dept. of Agronomy, Univ. of Maryland
J.P. Kenworthy and P.B. Cregan, Soybean and Alfalfa Laboratory, USDA-ARS, Beltsville, MD
J.H. Orf, Dept. of Agronomy and Plant Genetics, Univ. of Minnesota

A major gene for soybean cyst nematode (SCN) resistance is located near the top of linkage group 'G' of soybean. This gene is significant against multiple nematode races in PI 209332, PI 88788, PI 90763, and 'Peking', and has also been uncovered in PI 437654. In previous experiments, marker-based selection with restriction fragment length polymorphism (RFLP) markers at the F_2 generation was more than 90% accurate in identifying resistant lines at the $F_{5:6}$ generation. Nonetheless, RFLP markers are difficult to use and their usefulness is hampered in soybean by low levels of DNA sequence diversity. Because of these difficulties, we have searched for simple sequence repeat (SSR or microsatellite) markers that would be easy to use, informative, and tightly linked to the major SCN locus. One SSR marker, BARC-Satt038 (subsequently referred to as Satt038), is located on the distal side of the resistance gene approximately 5.4 centimorgans (cM) away. In preliminary studies, Satt038 exhibited a gene diversity value of 0.76 and was polymorphic between several resistant genotypes (PI 88788, PI 90763, and PI 209332) and two susceptible varieties ('Evans' and 'Parker'). A second SSR marker, BARC-Satt130, with a gene diversity value of 0.64, was located on the opposite side of the resistance gene approximately 15 cM away. Surprisingly, Peking exhibited the same sized products as did the two susceptible varieties for both SSR markers. In ongoing studies, we are evaluating these SSRs on a wider range of soybean genotypes, determining their map locations with greater precision, and searching for a additional SSR markers located nearer the SCN resistance gene and exhibiting higher levels of polymorphism.

DIFFERENTIAL AND SYNCYTIUM-SPECIFIC EXPRESSION OF SOYBEAN GENES DURING INFECTION BY SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES* ICHINOHE, SCN)

C.-N. Liu and S. Mackenzie, Department of Agronomy, Purdue University, West Lafayette, IN 47907

Using genetically homogenous (inbred) strain *Hg1*, a gene-for-gene interaction of soybean-SCN was demonstrated in soybean lines PI88287 (resistant to *Hg1*) and PI89008 (susceptible to *Hg1*) (Luedders V. 1987, Crop Sci. 27:604-606; Luedders V. 1990, Ann. Appl. Biol. 116: 321-325). Based on these observation and materials (PI88287, PI89008 and *Hg1*), we initiated this study to clone and characterize soybean genes responsive to SCN infection, aiming at understanding the mechanisms underlying the interaction of soybean-SCN and devising strategies for genetically engineering effective and long-lasting resistance to SCN. Our in-depth time-course observation of *Hg1* developmental stages on soybean roots shows that maximum number of nematode/per unit mass residing in the root around 10 days after inoculation. There were virtually no differences in terms of nematode number per unit of root tissue and in the development of nematodes between resistant and susceptible lines during the first ten days post inoculation. Ten days after inoculation, the number of nematodes per unit of root and the number of nematodes of later stages ($\geq$ L4) in the resistant line was dramatically reduced as opposed to that in the susceptible line. Meanwhile, the ratios of males to females in the resistant line increased. Based on these observations, we selected 100 cDNA clones from *Hg1*-infected PI89008 and PI88287 by subtractive hybridization and mRNA differential display. Further characterization of an initial subset of these clones by soybean genomic blotting, partial sequencing, RT-PCR, and *in situ* hybridization identified two soybean genes which appear to be expressed in a root- and syncytium-specific manner.

ISOLATION OF CANDIDATE DISEASE RESISTANCE GENE FAMILIES IN SOYBEAN

V. Kanazin[1], L.F. Marek[1], and R.C. Shoemaker[1,2], Dept. of Agronomy[1], and USDA/ARS Field Crops Research Unit[2], Iowa State University, Ames, IA 50011

In this study, degenerate oligonucleotide primers designed from conserved sequences in the coding regions of the *N* (tobacco), *RPS2* (Arabidopsis) and *L6* (flax) disease resistance genes were used to amplify related sequences from soybean (*Glycine max* (L.) Merr.). Sequencing of amplification products indicated that at least nine families of resistance gene analogs (RGAs) were detected. Genetic mapping of members of these families located them to eight different linkage groups. Several RGA loci mapped near known resistance genes including a cluster of family 1 RGAs which mapped around the *Rj2* (ineffective nodulation), *Rmd* (powdery mildew resistance) and *Rps2* (*Phytophthora sojae* resistance) disease loci on linkage group J. Primers and probes specific for the nine RGA families were used to screen a bacterial artificial (BAC) library and clones representing 5 of the families were identified. Analysis of the BACs based on restriction enzyme digests indicated that individual BACs contained two to six members of a single RGA family. Clustering and sequence similarity of members of RGA families suggests a common process in their evolution. Our data indicate that it may be possible to use sequence homologies from conserved motifs of cloned resistance genes to identify candidate resistance loci in diverse plant taxa.

FOLIAR APPLICATION OF METHANOL AND UREA INCREASES SOYBEAN GROWTH AND YIELD BY STIMULATING PHYLLOPLANE BACTERIA

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Interest in the growth-enhancing potential of methanol on a variety of crop plants was sparked by a 1992 report from Nonomura and Benson (PNAS 89:9794-9798), which claimed yield increases ranging from 36-100%, but methanol use has not become routine in commercial application. We see two reasons for this: 1) replication of their experiments has produced equivocal results, and 2) no satisfactory mechanism for the effect has been forwarded. These reasons are, of course, related since understanding the mechanism by which methanol affects plants should make it possible to apply the treatment with more success. Two of us (E.M. and J.J., unpublished) have developed an optimized protocol for application of methanol with urea to soybeans and have characterized the conditions under which the treatment is effective. Field trials of the method in the Summer of 1995 resulted in yield increases of 45% over untreated controls. The mechanism by which methanol treatments work appears to be by stimulating the growth of a symbiotic bacterium which is ubiquitously distributed on plant leaves. These bacteria, the PPFMs (pink-pigmented facultative methylotrophs - genus *Methylobacterium*), are able to utilize methanol as a carbon source and secrete cytokinins. Previous work (Holland and Polacco.1992. Plant Physiol. 98:942-948.) has demonstrated that these bacteria are present in sufficient numbers and are metabolically active enough to alter the physiological profile of soybean plants. We believe that foliar applications of methanol initiate a cycle of events that begins with bacterial growth and cytokinin production and proceeds as the plant responds with increased growth. Plant growth in turn generates more methanol, and this provides more stimulation to the PPFMs. The model is supported by field and laboratory studies which correlate changes in PPFM populations with methanol treatments, plant growth, cytokinin production and crop yield. The proposed mechanism also provides answers to some puzzling results raised by earlier work on methanol and plant growth.

IMPROVING PHOSPHORUS UTILIZATION IN SOYBEAN MEAL THROUGH PHYTASE GENE ENGINEERING

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Phosphorus is an essential dietary requirement for animal growth. Abundant stores of phosphorus are present in plant seeds in the form of phytate (*myo*-inositol hexakisphosphate), but this form is poorly utilized by monogastric animals. To meet dietary requirements, inorganic phosphorus is routinely added to swine and poultry rations. Non-utilized phytate is excreted by the animals and applied to soil in manure, which can contribute to environmental pollution in areas of intensive animal production. Increasing regulatory scrutiny of animal waste disposal has fostered interest in finding solutions for decreasing phosphorus output. Phytase enzyme supplements obtained from *Aspergillus niger* or from seeds of genetically-engineered canola or tobacco (1) are now available but they are expensive alternatives. Phytases are enzymes that sequentially remove phosphates from the phytate molecule. Use of phytase supplementation reduces the need for inorganic phosphorus and lowers the percentage of dietary phosphorus that is excreted (2,3). Our goal is to introduce phytase genes directly into transgenic soybean for expression in the developing seed. We are utilizing two different phytase gene sources for our studies, a fungal phytase gene (*phy*A) from *A. niger* and a phytase gene from soybean that is normally expressed after germination. To date we have successfully constructed soybean transformation vectors containing the *phy*A gene under control of two different promoters, the constitutive CaMV 35S promoter and the seed specific soybean β-conglycinin promoter. Vectors were constructed both with and without an ER signal sequence. Particle bombardment was used to recover stably transformed tissue culture lines containing the different constructs. These studies confirmed that the constitutive but not seed specific promoter functioned in tissue culture to express phytase transcripts. In addition, only constructs containing the ER signal sequence to direct the phytase to the endomembrane system for glycosylation expressed active recombinant phytase. The phytase was secreted from the cells and accumulated to high levels in the culture medium. Enzyme activity as measured by pH and temperature optima as well as stability was similar to a commercial fungal phytase preparation. Prior to consumption in animal feed, soybeans are roasted to inactivate antinutrient enzymes such as trypsin inhibitors and then processed to soybean meal. In addition, the feed pelleting process involves high temperatures which makes it unlikely that that the fungal phytase enzyme will remain active in processed feed. Our alternative approach is to utilize a plant phytase gene that should provide an ideal route for the breakdown of seed phytate. It should contain proper targeting information for localization of the phytase to the site of phytate accumulation, the protein bodies. To date no plant phytase genes have been isolated and characterized. To obtain a soybean phytase clone we have partially purified a protein with phytase activity from 10 day old soybeans by ion exchange and affinity chromatography. We have obtained N-terminal amino acid sequence data from the major protein band in order to synthesize oligonucleotides to screen a cDNA library. Once the soybean phytase gene has been isolated, it will be reintroduced into soybean under the control of a seed specific promoter to lower seed phytate content.

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CHROMOSOMAL ABERRATIONS IN THE TRANSGENIC SOYBEAN

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Several means exist for the genetic transformation of soybean [*Glycine max* (L.) Merr.]. One method that has the potential for high efficiency is particle bombardment of embryogenic suspensions cultures. However, plants regenerated from these cultures can exhibit morphological aberrations including leathery dark green leaves and partial seed fertility. The aberrations may be genetic, epigenetic, or chromosomal in nature. We examined cytologically (mitotic prometaphase and metaphase chromosomes) suspension cultures, developing somatic embryos, R_0 and R_1 plants from two soybean lines. Normal soybean contains $2n=40$ chromosomes. Morphological alterations and partial and total seed sterility in R_0 and R_1 were attributed to chromosomal aberrations such as deletions, duplications, trisomics and tetraploidy. Chromosome doubling appears to be genotypically controlled. Meiotic chromosome pairing particularly at pachynema stage may reveal cryptic chromosomal structural changes in morphological looking tetraploids, although some of these plants carried diploid chromosomes. Sterility in these plants may also be due to asynaptic or desynaptic genes. An early chromosome count of cells in culture may be very cost effective for the scientists engaged in transformation research.

AGROBACTERIUM MEDIATED TRANSFORMATION OF SOYBEAN EMBRYONIC AXES

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Although *Agrobacterium*-mediated DNA transfer is commonly used to introduce foreign genes into plants, soybean is not easily transformed utilizing *Agrobacterium*. Soybean has been transformed and regenerated with low frequencies by several different DNA transfer techniques. McCabe et al. (1988) and Christou et al. (1989) reported that fertile transgenic soybean plants were produced from the meristems of immature seeds using electric discharge particle acceleration with DNA-coated gold particles. Using mature soybean cotyledonary nodes, *de novo* multiple shoots have been regenerated by Wright et al. (1986). Transgenic soybean plants have been produced using cotyledonary nodes and *Agrobacterium*-mediated DNA transfer (Hinchee et al., 1988; Di et al., in press). Our most recent work involves the transformation and regeneration of soybean embryonic axes with a construct containing the β-glucuronidase (GUS)-intron gene using *Agrobacterium*. The transformation rate as measured by the number of blue GUS spots or sectors among several cultivars were compared. It was found that the differences among cultivars were minimal with slightly better response with the cultivar Jack in comparison to Calhoun, Flambeau and J103. Co-culture of embryonic axes with *Agrobacterium* for more than one day was found to be more favorable for transformation. The survival rate of embryonic axes and their abilities to produce multiple shoots after transformation were also compared against the co-culture time with *Agrobacterium*. With the combination of embryonic axes and our system for *Agrobacterium* DNA transfer, we have developed a procedure for soybean that assures a higher transformation rate than most presently available protocols. In addition, the multiple shoots generated from embryonic axes can be efficiently rooted and transferred to soil.

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GENE DISCOVERY IN PLANT NODULATION RESPONSES

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The genetic analysis of nodulation-related genes has resulted in considerable insights into the symbiotic process. Several approaches were used: (i) chemical mutagenesis induced non-nodulation and supernodulation mutants, which demonstrated new regulatory mechanisms of nodule development, such as autoregulation and systemic suppression of nodulation; (ii) isolation of nodule-specific or enhanced gene products (nodulins) and their responsible genes, which allowed the spatial and time-dependent verification of their expression, analysis of promoter regions for common motifs and regulatory elements, and gave hints at novel mechanisms; and (iii) biochemical analysis followed by "text-book" associations of function and already known genes.

While these approaches have not been fully exhausted, new strategies are being put into place. These are: (i) positional cloning or map-based cloning; (ii) insertional mutagenesis using either T-DNA or transposable elements in high transformation model legumes such as *Lotus japonicus*, and (iii) PCR methods based on mRNA population profiling after RT-PCR and arbitrary primer amplification (e.g., differential display).

These strategies have the potential to find new genes involved in signal reception, signal transmission as well as plant and cellular responses. For example, differential display analysis of mRNA isolated from the leaves of *Bradyrhizobium*-inoculated and uninoculated soybean plants allowed the detection of stress-related proteins and proteins related to cell walls and plant resistance genes. This suggests a mechanism of systemic perception of the root inoculation and infection response, which may be related to autoregulation of nodulation.

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A COMPARISON OF PINITOLS IN SOMATIC AND ZYGOTIC SOYBEAN EMBRYOS

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Soybean somatic embryos cannot be efficiently germinated and converted to plants. They may have some biochemical deficiency relative to zygotic embryos, a possibility of which are certain soluble carbohydrates. Axis and cotyledon tissues of dry soybean seeds have at least 15 different soluble carbohydrates including sucrose and free *myo*-inositol, D-pinitol, and D-*chiro*-inositol plus five series of their galactosyl oligomers: raffinose series, galactinol series, galactopinitol A series, galactopinitol B series, and fagopyritol B series. Sucrose (10% of dry weight of axis tissues) and stachyose (12%) were the primary components, with lesser amounts of raffinose (2%) and galactosyl cyclitols (2%). Only trace amounts of reducing sugars were detected. Sucrose accumulated during tissue growth. Soluble oligosaccharides and galactosyl cyclitols accumulated during yellowing of tissues and continued to accumulate after mass maturity during drying. A low ratio of sucrose to oligosaccharides correlated with prolonged storability of seeds. Mature, dry somatic embryos also had sucrose (10-15% of dry weight), stachyose (3-4%), and raffinose (1-2%) as the major soluble carbohydrates, plus small amounts of reducing sugars, *myo*-inositol, and galactinol. In contrast to zygotic embryos, somatic embryos did not accumulate D-pinitol, D-*chiro*-inositol, ciceritol, or digalactosyl *myo*-inositol. Some somatic embryos had trace amounts of galactopinitol A, galactopinitol B, and fagopyritol B1. Free D-pinitol and D-*chiro*-inositol were not detected. During the slow drying treatments after "maturation", somatic embryos lost their green color, stachyose increased six-fold, maltose declined to very low levels, and sucrose levels remained unchanged. Only some somatic embryos had galactosyl cyclitols, and the level of galactosyl cyclitols appeared to decrease rather than increase during slow drying treatments. Trace amounts of galactosyl cyclitols may have come from residual explant tissues. Like soybean somatic embryos, alfalfa somatic embryos also did not accumulate pinitol or galactosyl pinitols. Soybean zygotic embryos matured *in vitro* accumulated less D-pinitol and galactosyl pinitols during maturation and drying those matured *in planta*. Our results are consistent with the interpretation that somatic embryos are deficient in the expression of D-pinitol biosynthesis.

EVIDENCE FOR CYTOKININ PRODUCTION BY PLANT-ASSOCIATED METHYLOTROPHS

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Pink-pigmented facultative methylotrophic bacteria (PPFMs) of the genus *Methylobacterium* have been known to have an association with many, if not all, plant species and appear to be seed transmitted. Evidence exists for a complex interaction between the PPFM and its plant host (Basile et al., *Bull Torrey Bot. Club* **96**: 711-714, 1969; Corpe and Basile, *Dev. Ind. Microbiol.* **23:** 483-493, 1982). Experiments from our laboratory have shown that PPFMs, or their spent medium, can increase the germination rate of heat-treated soybean seed by 9-16% over controls, and that this effect can be mimicked by the addition of the plant hormone, zeatin, to the control medium (Holland and Polacco, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45**: 197-209, 1994).

Using immunoaffinity chromatography and HPLC separation , we have obtained significant amounts of zeatin and zeatin riboside from the spent medium of PPFM leaf isolates from soybean and barley. Radioimmunoassay with zeatin riboside standards indicates that approximately 20 ng/L zeatin and 9 ng/L zeatin riboside (34 ng/g cell d.w. and 16 ng/g cell d.w. respectively) were recovered from the barley isolate, and 44 ng/L zeatin and 9 ng/L zeatin riboside (314 ng/g cell d.w. and 64 ng/g cell d.w. respectively) were recovered from the soybean isolate. These recoveries were not corrected for losses during processing, and may be low estimates.

Cytokinin production by plant-associate microbes is currently best understood in the context of pathogenesis and symbiosis; the production of cytokinins by a commensal plant-associated bacteria is novel and the implications are not as clear.

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CLUSTERED QTL UNDERLYING RESISTANCE TO MANGANESE TOXICITY AND SDS ARE IN REPULSION.

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Manganese nutrition toxicity in soybean (*Glycine max* L. Merr.) and Sudden Death Syndrome (SDS) both cause leaf chlorosis in soybeans and are most severe in acid soils. Our objective was to examine the genetic basis of this correspondence by detecting Quantitative Trait Loci (QTL) conditioning resistance to manganese toxicity (Mn^R) and comparing them to SDS^R QTL mapped previously. Mn^R was analyzed in Essex, Forrest and their F_2 derived $F_{5:9}$ progeny. Traits were determined with plants grown in hydroponics containing 125 μM Mn, the toxic level and included chlorosis, wilt, height, shoot weight and root weight. Six QTL conditioning Mn^R were detected. Jointly the QTL can explain about 75% of each trait. Three unlinked markers found associated with Mn toxicity resistance are not associated with SDS resistance; $OU15_{800}$ (LG siu3) was associated with the chlorosis and height Mn toxicity traits. $OC10_{500}$ (LG unknown) was associated with the wilt Mn toxicity trait. $OC11_{400}$ (LG unknown) was associated with the wilt, chlorosis, root and shoot dry weight Mn toxicity traits. The three other Mn^R QTL corresponded with the map positions of SDS^R QTL SDS associated markers $OCO1_{650}$ and $OO04_{1075}$, that map to a 20 cM interval on linkage group N, were associated significantly with most Mn toxicity traits including; chlorosis, wilt, height, root and shoot weight. In contrast two SDS QTL on linkage group G was only associated with chlorosis ($OE02_{1000}$, MnQTL2) or chlorosis, wilt and shoot weight ($OG13_{490}$, MnQTL1). For each of these QTL the beneficial allele for Mn resistance was derived from Essex whereas the SDS resistance allele was derived from Forrest. Therefore, QTL for Mn toxicity and SDS resistances are in repulsion. It is possible that soybean breeders selecting in the acidic soils in the southern US have increased SDS susceptibility in soybean germplasm by unintentional selection for resistance to manganese toxicity. This would explain the earlier appearance of SDS in the south. Since SDS resistance and Mn toxicity resistance QTL were in repulsion, either linkage exists or the physiological bases of the Mn and SDS responses share some common elements. Leaf Mn deficiency has been reported to be associated with increased SDS severity in the field. Marker saturation and subline population analyses may allow the QTL to be separated. Markers capable of distinguishing the QTL could be used to break linkage between SDS resistance and Mn toxicity susceptibility. The greenhouse Mn resistance reaction may predict partial SDS resistance better than greenhouse assays with *Fusarium solani*, the incitant of SDS.

GENETIC RELATIONSHIP AMONG PLANT INTRODUCTIONS WITH RESISTANCE TO SOYBEAN CYST NEMATODES

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Soybean cultivars available in the U.S. have resistance to soybean cyst nematodes (SCN) (*Heterodera glycines* Ichinohe) derived from only a few plant introductions (PIs). Many other resistant PIs have been identified and these could be used as resistance sources for broadening the diversity of resistance genes in cultivars. The objective of the research was to study the relationship among cyst nematode resistant PIs using genetic markers and to describe the resistance response of the PIs to races 1, 2, 3, 5, and 14 of *H. glycines*. This can provide information on PIs which are genetically unique and may have new resistance genes. A group of 38 resistant PIs and 12 cultivars were tested with 167 RFLP markers. The PIs were polymorphic for 73% of the clones which revealed 250 polymorphic DNA fragments that were scored. A cluster analysis of the RFLP data uniquely grouped the northern and southern cultivars with two exceptions. The groupings of the PIs for both cluster analysis and principal component analysis (PCA) were not associated with either their maturity group or place of origin. There was an association between these groupings and resistance responses to races of *H. glycines*. One major group of PIs was distant from all previously used sources of resistance. This group was mostly resistant to race 3 and could potentially provide new genes for resistance to this race. The other PIs grouped with sources of resistance previously used by breeders.

MOLECULAR ANALYSIS OF ANTHOCYANIN PATHWAY GENES IN SOYBEAN

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In soybean, pigmentation is present in the flowers, pubescence, pods, hypocotyls, and seed coats and is affected by a number of genes that may encode enzymes in the anthocyanin pathway. Many of these genes are known to act pleiotropically. We are interested in the linkage relationships and molecular interactions between the novel *Wp* locus, which controls the pink flower color, and the loci *I* and *T*, which affect the seed coat color and the pubescence pigmentation.

The mapping populations used in this study segregated for purple/pink flower color, yellow/pigmented seed coat and tawny/gray pubescence. Phenotypic scoring indicated that the pink flower color segregates independently of the pubescence and seed coat coloration. However, the recessive *wp* genotype modifies seed coloration pleiotropically in the presence of the recessive *i* allele.

RFLP mapping was performed using cloned genes encoding enzymes of the soybean anthocyanin pathway as probes. These experiments revealed a *Dra*I/*CHS* (chalcone synthase) polymorphism, which segregated independently of the seed coat color. This is intriguing, in view of the recent finding that a *Hind*III/*CHS* polymorphism represents a *CHS* three-gene cluster and corresponds to the *I* locus. Soybean chalcone synthase is a small multigene family with seven members and the *Dra*I/*CHS* polymorphism is independent of the *Hind*III/*CHS* polymorphism. We are currently developing gene specific probes for the four *CHS* genes that do not belong to the cluster with the aim of assigning a specific *CHS* gene to the *Dra*I polymorphism.

POSTER SESSION

1. **SCREENING FOR FLOODING TOLERANCE OF SOYBEAN.** Jose D. Alves, N. Nurjani and Tara T. VanToai, Universidade Federal de Lavras, Conselho Nacional de Desenvolvimento Cientifico e Technologico, Brazil, The Ohio State Univ., and USDA-ARS, Soil Drainage Research Unit, Columbus, OH 43210-1086.

2. **TRANSFORMATION OF SOMATIC EMBRYOS OF SOYBEAN WITH CHITINASE AND β-1,3-GLUCANASE GENES VIA PARTICLE BOMBARDMENT.** S. Chanprame and J. M. Widholm, Dep. of Crop Sciences, Univ. of Illinois at Urbana-Champaign, Edward R. Madigan Lab., Urbana, IL 61801.

3. **COMPARISON OF OIL, PROTEIN AND SUGARS CONTENT OF SOYBEAN (*Glycine* MAX {L.] MERR.) SOMATIC AND ZYGOTIC EMBRYOS.** Sermsiri Chanprame and Jack M. Widholm, Dep. of Crop Sciences, Univ. of Illinois at Urbana-Champaign, Edward R. Madigan Lab., Urbana, IL 61801.

4. **LINKAGE ESTIMATION AND GENETIC INSTABILITY AT THE *k2 Mdhl-n y20* CHROMOSOMAL REGION IN SOYBEAN.** X. F. Chen and R. G. Palmer, Dep. of Agronomy, Iowa State Univ., Ames, IA 50011 and USDA/ARS Field Crops Research Unit.

5. **BIOCHEMICAL AND CYTOLOGICAL ANALYSES OF STORAGE PROTEINS IN SOYBEAN SHRIVELED SEEDS.** Z. J. Chen, H. Ilarslan, R. G. Palmer and R. C. Shoemaker, Dep. of Agronomy, USDA-ARS-FCR, Iowa State Univ., Ames, IA 50011.

6. **MAPPING QTL FOR SOYBEAN SEED SIZE USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS.** J. Chung, J. E. Specht, G. L. Graef, M. J. Livingston and D. E. Delaney, Dep. of Agronomy, Univ. of Nebraska, Lincoln, NE 68583-0915.

7. **A DENATURING POLYACRYLAMIDE GEL SYSTEM FOR HIGH THROUGHPUT ANALYSIS OF SIMPLE SEQUENCE REPEAT (SSR) DNA MARKERS.** P. B. Cregan and C. V. Quigley, USDA/ARS, Soybean and Alfalfa Research Laboratory, Beltsville, MD 20705.

16. **MORPHOLOGICAL AND CYTOLOGICAL CHARACTERISTICS OF SOYBEAN SUSPENSION CULTURES THAT INFLUENCE TRANSFORMATION BY PARTICLE BOMBARDMENT.** C. Hazel, C. Kostow, C. Lee, C. Sanders, B. Schweiger, L. Szymanski and T. Klein, DuPont Agricultural Products, Experimental Station, Wilmington, DE 19880.

17. **MONITORING MULTIPLE RESISTANCE TO SOYBEAN CYST NEMATODE (SCN) USING MOLECULAR MARKERS IN A BREEDING POPULATION.** J. A. Heer, E. R. Shipe, A. P. Rao Arelli, H. T. Skorupska, Agronomy Dep. Clemson Univ., P. O. Box 340359, Clemson, SC 29634-0359 and Dep. of Agronomy, Univ. of Missouri, Columbia, MO 65211.

18. **PURIFICATION AND CHARACTERIZATION OF A PHYTATE-DEGRADING ENZYME FROM GERMINATION SOYBEAN COTYLEDONS.** C. E. Hegeman and E. A. Grabau, Dep. of Plant Pathology, Physiology, and Weed Science, Fralin Biotechnology Center, Virginia Tech, Blacksburg, VA 24061-0346.

19. **SOYBASE, A SOYBEAN GENOME DATABASE: STORAGE PROTEINS OF SOYBEAN.** M. R. Imsande, D. Grant, and R. C. Shoemaker, Dep. of Agronomy and USDA/ARS Field Crops Research Unit, Agronomy Hall, Iowa State Univ., Ames, IA 50011.

20. **MICROSPORE SEGMENTATION AND EMBRYO FORMATION IN CULTURED ANTHERS OF SOYBEAN.** E. Kaltchuk-Santos*, M. H. B. Zanettini* and J. E. Mariath**, *Depto. de Genética, **Depto. de Botânica, IB-UFRGS, Caixa Postal 15053, Porto Alegre, RS, Brasil.

21. **MAPPING OF QTL AND IDENTIFICATION OF cDNA ASSOCIATED WITH *PHYTOPHTHORA SOJAE* TOLERANCE OF SOYBEAN.** Namik Kaya, Tara VanToai, James Specht and A. F. Schmitthenner, Dep. of Horticulture and Crop Science and Dep. of Plant Pathology, The Ohio State Univ.; USDA-ARS, Soil Drainage Research, Columbus, OH 43210 and Dep. of Agronomy, Univ. of Nebraska, Lincoln, NE 68583.

22. **MULTIPLE-LOCUS BULK SEGREGANT ANALYSIS OF SCN LOCI IN PI437.654 USING RAPD AND AFLP MARKERS.** P. Keim, A. R. Ferreira, J. M. Schupp, K. Clayton, D. M. Webb, Northern Arizona University and Pioneer Hi-Bred, Int.

29. **MOLECULAR MARKERS ASSOCIATED WITH SOYBEAN SEED PROTEIN AND OIL ACROSS POPULATIONS AND LOCATIONS.** M. A. R. Mian, H. R. Boerma, S. H. Lee, M. A. Bailey, D. A. Ashley, W. A. Parrott, Dep. of Crop and Soil Sciences, Univ. of Georgia, Athens, Ga 30602-7272; T. E. Carter, Jr., USDA-ARS, Dep. of Crop Science, North Carolina State Univ., Raleigh, NC 27695-7631; Emerson Shipe, Dep. of Agronomy, Clemson Univ., Clemson, SC 29634-0359; and Richard Hussey, Dep. of Plant Pathology, Univ. of Georgia, Athens, GA 30602-7274.

30. **GENETIC MAPPING OF A YIELD DEPRESSION LOCUS NEAR A MAJOR SOYBEAN CYST NEMATODE RESISTANCE GENE.** J. Mudge and J. H. Orf, Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, St. Paul, MN 55108; V. C. Concibido, R. L. Denny, and Nevin Young, Dep. of Plant Pathology, Univ. of Minnesota, St. Paul, MN 55108.

31. **RATE-REDUCING RESISTANT TO *Fusarium solani* f. sp. *phaseoli* UNDERLIES FIELD RESISTANCE TO SUDDEN DEATH SYNDROME.** V. N. Njiti, R. J. Suttner, P. T. Gibson and D. A. Lightfoot. Dep. of Plant and Soil Science, Southern Illinois Univ., Carbondale, IL 62091. L. E. Gray, USDA/ARS, Dep. of Crop Science, Univ. of Illinois, Urbana, IL.

32. **UTILIZATION OF SEQUENCE CHARACTERIZED AMPLIFIED REGIONS FOR MARKER-ASSISTED SELECTION OF RESISTANCE TO SUDDEN DEATH SYNDROME AND CYST NEMATODE IN SOYBEAN.** R. R. Prabhu, T. W. Doubler, and D. A. Lightfoot, Dep. of Plant and Soil Science, Southern Illinois Univ., Carbondale, IL 62901.

33. **RFLP MARKER ANALYSIS OF RESISTANCE TO SOYBEAN CYST NEMATODE RACE 1 IN PEKING.** B. X. Qiu, A. P. Rao Arelli, and D. A. Sleper. Dep. of Agronomy, Univ. of Missouri, Columbia, MO 65211.

34. **SOYBEAN EMBRYOGENIC CULTURE: MEDIUM OPTIMIZATION.** V. M. Samoylov, D. M. Tucker and W. A. Parrott, Dep. of Crop and Soil Sciences, Univ. of Georgia, Athens, GA 30602-7272.

SCREENING FOR FLOODING TOLERANCE OF SOYBEAN

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Flooding is common in heavy soils with poor drainage. The decrease in seed yield is linearly related to the length of the flooding period and varies from 0.8 to 2.3 bushels per acre per day, depending on cultivar, soil type and the growth stage when the flooding occurs. Soybean germplasm has been identified that continues to fix nitrogen and produce dry matter during prolonged flooding. An efficient and reproducible screening technique for flooding tolerance is essential for the mapping of QTL and identification of DNA markers closely linked to the tolerant trait. This study determined the responses of 21 soybean lines in our collection to complete submergence and to continual soil waterlogging using laboratory, greenhouse, and field screening tests. Tolerance to submergence was determined by the viability of 3-d-old seedlings after two days of submersion in water. Tolerance to continual soil waterlogging was determined by leaf color, leaf photosynthetic rate, biomass and seed yield. The experiment was repeated three times with three replicates each time. The correlation coefficient of tolerance to submergence and tolerance to waterlogging was 0.7 and 0.5 for greenhouse and field conditions, respectively. Under field conditions, tolerance to diseases that are prevalent in flooded soil may play an important role in the growth and productivity of soybean.

TRANSFORMATION OF SOMATIC EMBRYOS OF SOYBEAN WITH CHITINASE AND β-1,3-GLUCANASE GENES VIA PARTICLE BOMBARDMENT

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Somatic embryos of soybean (*Glycine max* L.) "Jack" were bombarded with 1.0 μm diameter tungsten particles coated with various plasmids. Each plasmid contained the *hph* (hygromycin phosphotransferase) gene as selectable marker and one of the following genes; maize chitinase 2 (Chi2), bean chitinase 18 (Chi18) and maize ß-1,3-glucanase (ß-gluII). All of the genes were under the control of the 35s CaMV promoter. Following selection, genomic DNA of the hygromycin resistant lines were isolated, and the PCR for the presence of those genes were performed. Some of the PCR positive-lines were able to regenerate into plants and these plants showed the presence of the transgene by PCR of the leaf DNA. Leaves of some of the transformed lines had 3-4 time higher chitinase enzyme activity than the wild type. Northern blots indicated that the transgenic lines produced the 1.2 kb messager RNA for chitinase. The transgene can be detected in the first generation progeny (T_1) of the transformed line and gene expression in these progeny is being studied. Characterization of the ß-gluII transformed lines is in progress as well.

COMPARISON OF OIL, PROTEIN AND SUGARS CONTENT OF SOYBEAN (GLYCINE MAX [L.] MERR.) SOMATIC AND ZYGOTIC EMBRYOS

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Five different developmental stages of soybean c. Jack somatic embryos and seven different times after pollination (25 to 65 DAF) of field grown zygotic embryos were used. Zygotic embryos accumulated oil at a low level at 25 to 50 DAF and the significant accumulation commenced at 55 DAF(16.0%) that reached a mean of 21.3% at 65 DAF. Somatic embryos had elevated levels of oil with the maximum of only 4.7 % throughout development. Soybean zygotic embryos accumulated high levels of protein after 25 DAF and had elevated levels throughout development with the high of 40.8 %. Protein accumulation in somatic embryos was decreased from 43.5% to 24.2% as maturation approached. Somatic embryos accumulated much higher galactose and fructose levels than zygotic embryos. For sucrose, somatic embryos mimic the accumulation pattern of zygotic embryos with low levels. Raffinose was detected initially at late stages in both somatic and zygotic embryos and the accumulation rapidly increased as the embryos matured.

LINKAGE ESTIMATION AND GENETIC INSTABILITY AT THE *k2 Mdh1-n y20* CHROMOSOMAL REGION IN SOYBEAN

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Ten mutants have been reported at the *k2 Mdh1-n y20* chromosomal region in soybean. The genetic distances among the *k2* (tan saddle seed coat), *Mdh1-n* (mitochondrial malate dehydrogenase 1 null), and *y20* (yellow foliage) loci were not resolved previously. The objectives of this study were to determine the genetic distances among these three loci, and to investigate whether genetic instability exists at this chromosomal region.

The results indicated that the genetic distance between the *k2* and the *Mdh1-n* loci was about 1.00 centiMorgans estimated in about 5400 F2 plants in coupling phase constructed by crossing Genetic Type T261 (*k2 Mdh1-n*) with wild type, and that the genetic distance between the *k2* and the *Mdh1-n y20* loci was about 3.30 centiMorgans estimated in 661 F2:3 families with tan saddle seed coat segregating yellow plants derived from crossing T317 (*Mdh1-n y20*) with T239 (*k2*) and L67-3483 (*k2*).

Genetic instability was evident by introducing the *w4-m* and *Y18-m* mutable systems into the *k2* genetic background. Eleven out of 977 total F2 families derived from crossing T239 (*k2*), T261 (*k2*) and L67-3483 (*k2*) with lines that were suspected to contain active transposable elements contained new mutants with tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage phenotypes. Among the eleven F2 mutant families, ten out of 381 were associated with T239 (*k2*) genetic background and one out of 273 was associated with T261 (*k2*) genetic background; however, no mutation events out of 323 were identified to be associated with L67-3483 (*k2*) genetic background. Allelism and inheritance studies indicated that these eleven mutants were new mutants at the *k2 Mdh1-n y20* chromosomal region. The genetic mechanism responsible for this high frequency of instability at the *k2 Mdh1-n y20* region was hypothesized to be a receptor element activities at this chromosomal region.

BIOCHEMICAL AND CYTOLOGICAL ANALYSES OF STORAGE PROTEINS IN SOYBEAN SHRIVELED SEEDS

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A shriveled seed mutant T311 was found among progeny of a cross between P2180 and AP2. Shriveled seeds are generally small and have puckered/dimpled seed surface. To demonstrate the association between storage protein and the shriveled seeds, plants of T311 and P2180 were grown at specific temperatures allowing expression of the shriveled seed phenotype. Comparative analyses between T311 and P2180 showed that total protein content was decreased in the shriveled seeds. Appearance of α and α' subunits of conglycinin was delayed in shriveled seeds of T311. SEM analyses showed that mature shriveled seeds had larger protein bodies than wild type seeds. Protein bodies in shriveled seeds of T311 had more scattered globoid crystals and fewer globoid regions than P2180 seeds. Energy disperse X-ray analyses detected the presence of elements P, Ca, Mg in the globoid crystals of T311 shriveled seeds while in P2180 round seeds, elements K and S were observed in addition to elements P, Ca, Mg.

MAPPING QTL FOR SOYBEAN SEED SIZE USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

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Seed size is an important yield component in the soybean. Seed size in this plant species is inherited quantitatively, has a high heritability, and is governed almost entirely by additive gene action. The identification of Quantitative Trait Loci (QTL) for seed size has the potential to improve the efficiency of selection for developing extremely small or large-seed soybean cultivars. A 68-plant F_2 segregation population derived from a mating between 'Mercury' (small seed) and PI 467.468 (large seed) was evaluated with random amplified polymorphic DNA (RAPD) markers to identify QTL related to seed size. The genetic map consisted of 113 RAPD loci that converged into 29 linkage groups and spanned a genetic distance of 1043 cM. Significant ($P < 0.01$) associations were found between several RAPD markers and QTLs. Based on single-factor ANOVAs, three markers, OPL9a on linkage group 16, and OPM7a and OPAC12 on linkage group 19 were significantly ($P < 0.01$) associated with QTL for seed size. Using the QTL log-likelihood scans of MAPMAKER-QTL, three QTLs for seed size were detected. The QTL in the interval between marker OPM2 and OPY10c on linkage group 6 accounted for 40.8% of seed size variation, the QTL in the interval between marker OPL9a and OPV14 on linkage group 16 accounted for 40.1% of seed size variation, and the QTL in the interval between marker OPL9b and OPV7b on linkage group 17 accounted for 39.8% of seed size variation. All of the MAPMAKER-detected QTLs were also detected by single-factor ANOVAs at $P < 0.05$ (i.e., OPL9b, OPAS18, OPP10, and OPL9a). This study demonstrated that the RAPD marker technique is a convenient and rapid means of identifying QTLs controlling important quantitative traits in soybean F_2 populations. We believe it has substantial potential for manipulating those traits in soybean genetics and breeding.

A DENATURING POLYACRYLAMIDE GEL SYSTEM FOR HIGH THROUGHPUT ANALYSIS OF SIMPLE SEQUENCE REPEAT (SSR) DNA MARKERS

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The analysis of SSR markers requires the ability to distinguish small differences in the length of DNA fragments. This requirement often necessitates the use of DNA sequencing gels with ^{32}P or ^{33}P labeling as the best method of obtaining sufficient resolution. In addition, double stranded fragments amplified from certain SSR alleles tend to associate and form higher molecular weight products when separation is performed under non-denaturing conditions. In these instances a clear determination of allele size and/or genetic status (homo- vs. heterozygote) is difficult. Furthermore, in the context of a plant breeding program one is faced with the necessity of high sample throughput. To meet these requirements and constraints, a denaturing polyacrylamide gel electrophoresis system was designed that can readily distinguish SSR alleles that vary by as little as 4 basepairs, does not require the use of radio labeled compounds, and which is run on a microtiter plate format with a multi-channel pipette used for both set-up of polymerase chain reactions (PCR) as well as gel loading. Using this system a total of 192 individual samples can be genotyped on one gel. As currently configured a 35 (width) x 30 (length) cm, 1.5 mm thick gel (6% acrylamide, 5.6M urea, 30% formamide, 1X TAE). A 32 tooth comb (0.9 cm center to center) or a 64 tooth comb (0.45 cm center to center) is used. After a pre-running, four sets of 32 or 64 samples are loaded every 40 to 60 min followed by electrophoresis at 60 watts constant power. Following electrophoresis gels are stained using the single strand DNA-specific stain SYBR® Green I (Molecular Probes, Eugene, OR) and photographed with a SYBR® Green gel stain photographic filter on a standard UV transilluminator.

MAPPING QTL ASSOCIATED WITH SCLEROTINIA STEM ROT RESISTANCE IN SOYBEAN USING RAPDs

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Sclerotinia sclerotiorum is the causal organism of sclerotinia stem rot in soybean. The wide host range of 400 dicotyledenous species and long-term survival of this pathogen in the soil assures outbreaks of disease under favorable environments. Since disease management options are limited, resistance, thus far unidentified in soybeans, is the most cost-effective and long-term management strategy. The objective of this study was to differentiate susceptible and resistant soybean genotypes, and identify putative QTL for resistance using polymorphic RAPD markers. A leaf test, refined at the University of Nebraska for soybeans, is consistently effective in differentiating the most susceptible and the most resistant soybean lines. The laboratory leaf assay is highly correlated with the ranking of disease severity index (DSI) in replicated field trials, is non-destructive and can be used to screen segregating populations. Soybean genotypes that differ in their reaction to the pathogen were identified based on the leaf assay and field results. Williams 82, the susceptible genotype, had a DSI rating of 64 and a mean lesion size of 5.5 cm^2. Five resistant genotypes were used, and ranged from a 2-year mean DSI of 13 for Dairyland DSR-173 to 22 for Vinton 81. The lesion size ranged from 1.7 cm^2 for Dassel to 2.7 cm^2 for Vinton 81. The six cultivars were screened with 1,000 RAPD primers to identify polymorphisms between Williams 82 and the five more resistant genotypes. The polymorphism ranges from 22% for DSR-173 to 28% for Corsoy 79. In the Williams 82 X Corsoy 79 cross 278 polymorphic primers were identified, marking potentially 334 loci. A mapping population of 60 F_2 plants from the Williams 82 X Corsoy 79 cross was analyzed for both disease severity and RAPD markers to identify putative QTL for resistance to sclerotinia stem rot.

GENOTYPIC MAPPING OF POST-STRESS ROOT GROWTH IN SOYBEAN.

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Under severe moisture stress in the field, the soybean plant introduction PI416937 exhibits delayed leaf wilting. This plant introduction also expresses a relatively high post-stress root growth after drought stress in the greenhouse. Post-stress root growth is a quantitatively inherited trait which is difficult to measure. Genetic markers offer plant breeders the potential to select for this trait. An F_4-derived soybean, *Glycine max* (L.) Merr., population developed from a cross of 'Young' x PI416937 was evaluated with restriction length polymorphism markers to identify QTL related to post-stress root growth. The F_4-derived lines were evaluated for segregation and a genetic map consisting of 155 RFLP markers was constructed. The lines were grown in a greenhouse in 10-cm peat pots to the V3 stage of development and exposed to three cycles of drought stress. Each cycle consisted of drought stress until all leaves on the plant exhibited wilting symptoms followed by the addition of water and the recovery of turgidity. No roots were present outside the pots at the end of the third cycle. Peat pots containing the plants were placed in 20-cm plastic pots filled with soil and allowed to recover under well-watered conditions for two additional weeks. Roots, which grew after moisture stress, were present predominantly in the soil surrounding the smaller peat pot. This permitted an accurate assessment of post-stress root growth. At harvest, the shoot was removed at the root-shoot junction and the soil external to the peat pot gently washed from the roots and root dry weight was determined. The Young x PI416937 population exhibited a heritability of 40% and transgressive segregation. The association of molecular markers with the rooting trait was determined by single factor analysis of variance. Eight putative independent marker loci were associated with post-stress root growth in this population. These marker loci together explained most of the variation in the trait. PI416937 contributed the favorable alleles at five of the marker loci, whereas Young did so at the remaining three loci.

A SOLID MEDIA BASED PLANT REGENERATION METHOD FOR SOYBEAN VIA SOMATIC EMBRYOGENSIS

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Genetic engineering of soybeans (Glycine max L.) has been difficult, partially due to inefficient plant regeneration protocols. Plants are often stunted, with poor root systems and low seed yields. We have developed a solid media-based system using zygotic cotyledons explants to produce somatic embryos that will regenerate into plants. Experiments have been conducted to compare subculture intervals, auxin levels, pH levels and plant cultivars. The solid media-based protocol has resulted in more normal looking plants, with strong root systems and initial yields of up to 130 seeds per plant. All subsequent sexual seed generation plants have appeared normal in the greenhouse. Currently the soybean cultivars that have been successfully regenerated are Jack, Flambeau and J-103. The basic protocol consists of a series of embryo initiation, proliferation and maturation stages with minor cultural and media adjustments for each cultivar. Histological studies have been completed for each developmental phase. The solid media protocol has resulted in sufficient regeneration efficiency that we are evaluating the technique in conjunction with various genetic transformation protocols. A study has been initiated to compare field performance.

MOLECULAR MARKERS ASSOCIATED WITH TOBACCO RINGSPOT VIRUS IN SOYBEAN

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A soybean, *Glycine max* (L.) Merr., population derived from the cross of 'Young' x PI 416937 was used to identify molecular markers associated with tobacco ringspot virus resistance. Young is resistant and PI 416937 is susceptible to tobacco ringspot virus. One hundred and twenty F_4- derived lines were scored for segregation at 155 restriction fragment length polymorphism markers. Plants were naturally infected with tobacco ringspot virus and were scored for viral infection in Athens, GA in 1994. Data were analyzed using single-factor analysis of variance. Three independent quantitative trait loci (QTL) were found to be related to tobacco ringspot virus resistance. A major QTL was found on Linkage Group F and accounted for 82% of the variation in viral infection. The other two QTL were found on Linkage Groups R and G and accounted for 12% and 8% of the variation, respectively. For the major QTL, the allele for resistance was contributed by Young, whereas for the minor QTL the alleles for resistance were contributed by PI 416937. The major QTL for tobacco ringspot virus is in the same linkage group with at least seven other disease resistance loci in soybean. Resistance to tobacco ringspot virus is therefore controlled by one major and few minor genes.

EFFICIENT PLANT REGENERATION THROUGH SOMATIC EMBRYOGENESIS FROM GERMINATED COTYLEDON OF THE SOYBEAN

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The objective of the present study was to find a rapid and efficient plant regeneration procedure through somatic embryogenesis by using germinated cotyledon as the source of explants. In the experiment of genotypes and explant types, 11 cultivars and breeding lines were used and explants were taken from the cotyledon and hypocotyl of the 5—6 days old seedlings which were cut into 2—3mm pieces and inoculated on Medium C (see the following). In the experiment of induction medium and regeneration medium, the germinated cotyledon of Hongyin No. 1 was used as explants.

Induction media were as follows:

Medium A, MS with 3% sucrose, 2 mg/L 2,4—D
Medium B, MS nutrients with B_5 vitamins, 3%sucrose, 0.2% casein hydrolysate, 2 mg/L 2,4—D, 1 mg/L BA, 1 mg/L IAA
Medium C, MS with 3% sucrose, 2 mg/L 2,4—D, 1mg/L KT, 1mg/L NAA
Medium D, MS with 20% potato extract solution, 2mg/L 2,4—D
Medium E, MS with 3% sucrose, 0.25 mg/L TDZ (Thidiazuron)

Regeneration media were as follows:

Medium a, N_6 with 2% sucrose
Medium b, MS with B_5 vitamins, 6% sucrose, 0.2% casein hydrolysate
Medium c, Medium C without auxins
Medium d, MS with 4% sucrose, 0.5mg/L IAA, 0.3mg/L BA
Medium e, MS with 3% sucrose, 0.5mg/L BA, 0.5mg/L KT

The callus induction frequencies of cotyledon and hypocotyl from a same genotype was about the same and up to 100% for Hongyin No. 1, Nannong 73—935, Heinong 35. For Nannong 86—4, the frequency of hypocotyl was 87.6%, twice as that of cotyledon, and vise versa for Huaidou No. 2. The somatic embryogenesis frequencies from cotyledon explants of Hongyin No. 1, Nannong 73— 935, NJ89—1, Huaidou No. 2 and Heinong 26 were higher than those from hypocotyl explants, especially of the first two cultivars, the former was about 30—33%, more than 4 —5% of the latter. There existed genotype x explant type interaction for callus induction and somatic embryogenesis. But on the average, both explant types could produce high frequency of callus and the cotyledon explants appeared to be better than the other in somatic embryogenesis. On induction media, the calli could be grouped as embryogenic callus, non —embryogenic callus, and rhizogenic callus, according to their morphological performance and somatic —embryogenic ability. The highest embryogenic callus frequency was 34.3% on Medium E; the next was 23.6% on Medium C. The others were much less than these two. It was observed that the number of somatic embryoids increased well, after changing subculture onto Medium E. Accordingly, TDZ was more favorable to embryogenic callus induction than 2,4—D, BA, KT and their combinations.

The embryogenic calli were transferred from induction media onto regeneration media in the following way: A —a, B—b, C—c, D—d, and E—e. The highest germination frequency was 83% on C—c, and the next being 63% on E—e; but the highest number of plantlets per callus was 2.4 on the latter set and the next being 2.0 on the former set. The other three sets were inferior. The frequencies of plant regeneration calculated from frequency of induction by frequeucy of regeneration from embryogenic calli for C—c and E—e were 19.5%—21.6%, and up to 30% might be expected if using E—c set. It seemed that additional auxins were not necessary based on MS with 3% sucrose, for germination of the embryogenic calli.

SOYBASE, A DATABASE FOR SOYBEAN GENOME AND GENETIC DATA

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SoyBase was designed to give geneticists and breeders easy access to data from many laboratories, the literature and other databases (Genbank, GRIN, PVP, BIOSIS, AGRICOLA). It is an ACE-type database with hypertext links between classes and an easy to use graphical interface. This means, for example, that following a path through the database from a locus' map position to detailed information about that gene or marker as simple as clicking on the links. Other related information is clearly indicated and is also available through the hypertext links.

SoyBase currently contains data on a number of topics.

Class	Number	Description
Map Collection	6	Classical genetic map and 5 molecular marker maps
Locus	563	Morphological, biochemical and molecular markers
Probe	358	DNA markers with images of the autoradiograms for most of the core set of markers developed at ISU
Gene	396	Data include gene class, alleles and 2 point data
QTL Study	33	Data on 21 agronomic traits including yield, seed quality and disease resistance
Germplasm	>30,000	Soybean germplasm with pointers to GRIN and PVP databases and hypertext links to the enzyme, storage protein, nodulin and allele classes
Reaction or Pathway	48	Clickable diagrams of 376 metabolic pathways covering 524 enzymes and 539 metabolites
Enzyme	685	Data include EC number, purification, clones, physical properties and the species and cultivars studied
Traits	234	Traits associated with entries in GRIN and PVP databases
Sequence	580	All available Glycine DNA sequences with pointers to the Genbank records
Pathology	15	Information on soybean diseases including causative organism, differentials, phenotypic scores and distribution
Storage Protein	14	Data on 6 vegetative and 11 seed storage proteins
Nodulin	37	Data on the nodulins of soybean including gene and protein information, and probe and antibody availability
Author	>22,000	Names of all of the authors of the >18,000 AGRICOLA, BIOSIS and direct entry papers related to soybean
Colleague	>250	Names and address of soybean-associated people

SoyBase can be accessed via the WWW at the National Agriculture Library (http://probe.nalusda.gov:8300/plant/index.html) and is also available in a Macintosh version. Additional soybean genome data not yet in the database is available on the SoyBase WWW server (http://129.186.26.94).

If you have research information, colleague information or corrections that should be included in SoyBase, please contact David Grant at this meeting, by phone at (515) 294-1205 or by email at dgrant@iastate.edu. We can accept data in almost any form and will reformat it to fit the database as needed.

INCREASING LYSINE IN TRANSGENIC SOYBEAN SEEDS

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Animal feeds derived from various grains are often deficient in some of the ten essential amino acids. Lysine, in particular, is a limiting essential amino acid in some highly consumed grains, such as corn. One way to supplement animal feed is to add fermentation-produced crystalline lysine. However, we have undertaken an approach to fulfill animal dietary needs for lysine by raising the amount of lysine in certain crop plants, such as soybean.

In order to raise the lysine level in soybeans we circumvented the normal feedback regulation of two enzymes in the lysine biosynthetic pathway, aspartokinase (AK) and dihydrodipicolinic acid synthase (DHDPS). Lysine-feedback-insensitive bacterial DHDPS and AK enzymes encoded by the *Corynebacterium dap A* gene and a mutant *E. coli lys C* gene, respectively, were linked to a chloroplast transit peptide and expressed from a seed-specific promoter in transgenic soybean seeds. Expression of *Corynebacterium* DHDPS plus lysine-insensitive *E. coli* AK in soybean transformants caused several hundred-fold increases in free lysine and increased total seed lysine content by as much as 5 -fold. Accumulation of two lysine catabolites, saccharopine and α-amino adipic acid, have also been observed. The non-protein bound lysine in the transgenic soybean seeds has been shown to be biologically available to animals under typical processed soybean conditions.

FUNGAL PHYTASE GENE CONSTRUCTS FOR SOYBEAN TRANSFORMATION

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Soybean meal is a major component of livestock feed. The phosphorus content of soybean seeds is primarily in the form of phytate (61%). Phytate has a *myo*-inositol backbone with six bound phosphates. Nonruminant animals such as swine and chickens lack the ability to break down phytate in order to utilize the phosphorus. Phytate is an antinutrient that chelates cations such as magnesium, iron, calcium, and zinc so they are no longer available to the animal. Phytate also binds to proteins and causes a decrease in digestibility. The high concentration of phosphorus excreted from livestock can become an environmental pollutant. Current solutions to the nutritional requirement for phosphorus include supplementing the feed with inorganic phosphorus, and adding a microbial phytase enzyme (1) that sequentially hydrolyzes phytate to *myo*-inositol and inorganic phosphorus. Supplementation with inorganic phosphorus solves the nutritional problem but does not help the environmental problem of excreted phytate. The addition of phytase aids both the nutritional and the environmental problem but is an expensive alternative for farmers. Our goal is to transform soybean with a phytase (*phy*A) gene from *Aspergillus niger*. Four vectors were constructed containing the *phy*A gene and hygromycin resistance gene as a selectable marker. The fungal *phy*A ER signal sequence and single intron were replaced by either a plant ER signal sequence from the patatin gene (2), or an in-frame ATG for control constructs lacking the signal sequence. Two different promoters were utilized. Two vectors were constructed with the constitutive dual enhanced cauliflower mosaic virus 35S promoter, and the other two with a seed specific, soybean β-conglycinin promoter (3). The 35S promoter constructs were tested in nonregenerable tissue culture cells for phytase expression, activity, and stability. Prior to regeneration of transgenic soybean, the existing vectors are being further modified to contain a vacuolar sorting sequence from barley lectin at the carboxy terminus of *phy*A (4). This sorting sequence is desirable to correctly compartmentalize the *phy*A gene product to the vacuole. We anticipate that the construct containing the seed specific promoter, the ER signal sequence and the vacuolar sorting sequence will give optimal expression in transgenic soybean for lowering phytate levels.

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MORPHOLOGICAL AND CYTOLOGICAL CHARACTERISTICS OF SOYBEAN SUSPENSION CULTURES THAT INFLUENCE TRANSFORMATION BY PARTICLE BOMBARDMENT

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A reproducible and efficient means for producing fertile transgenic soybean remains an important goal of agricultural biotechnology. One promising method involves the bombardment of embryogenic suspension cultures (Finer and Nagasawa, Plant Cell Tissue and Organ Cult. 15:125; Parrott, et al., Plant Cell Rep. 7:615). However, this method suffers from several limitations. One limitation is the high rates of tissue culture induced mutations observed in plants regenerated from embryogenic suspensions (see for example Singh et al., this conference). This limitation has been addressed by us and other groups by introducing genes into relatively young cell cultures (preferably between 3 and 8 months after induction). Another limitation is the variable number of transformants that are recovered between experiments. We have performed bombardment experiments on a number of embryogenic cultures over the last several years. Particular morphological and histological characteristics of the cell cultures appear to be correlated with rates of stable transformation. Cultures that produce large numbers of stably transformed clones (from about 20 to 100 per bombardment) are characterized by relatively tightly packed clusters of globular stage embryos while cultures yielding few transformants harbor more elongated structures. Histological examination indicates that the outer layers of transformable cultures consist of relatively small, cytoplasmically rich cells. In contrast, the peripheral layers of non-transformable cultures appear to be composed of larger cells with pronounced vacuoles which contain numerous starch granules. We have also examined the number of mitotically active cells in the outer cell layers using DAPI, a DNA-specific dye. The results show differences in the numbers of dividing cells in different cultures and even for the same culture sampled at different times. The differences in the position and number of dividing cells may help explain the variation in transformation rates found between different cultures and different experiments.

MONITORING MULTIPLE RESISTANCE TO SOYBEAN CYST NEMATODE (SCN) USING MOLECULAR MARKERS IN A BREEDING POPULATION

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Soybean germplasm J87-233, developed by L.D. Young at Jackson, TN, is resistant to soybean cyst nematode (SCN) races 1, 2, 3, 5 and moderately resistant to race 14. Three resistant sources, Peking, PI 88788 and PI 90763, provided genes for resistance within the pedigree of J87-233. Peking contributed resistance to races 1, 3 and 5. The PI 88788 provided genes for resistance to races 3 and 14 while PI 90763 provided resistance for races 1, 2, 3 and 5. Peking, PI 88788, PI 90763 and PI 437654 (resistant to all known races of SCN) were characterized using approximately 400 RAPD and RFLP molecular markers. A cross between J87-233 and cultivar 'Hutcheson' was made in an attempt to incorporate SCN resistance genes into the superior genetic background of 'Hutcheson'. 'Hutcheson', maturity group V, is highly susceptible to SCN infection.

Molecular markers that detect chromosomal regions associated with SCN resistance were used to trace loci for resistance within the $F_{2:3}$ population of the cross J87-233 x 'Hutcheson'. The progeny population was assayed for individual genotype reaction to each of five SCN races: 1, 2, 3, 5 and 14.

We identified molecular polymorphisms between the three resistant sources in J87-233, and described differences between J87-233 and 'Hutcheson' for the regions where putative SCN resistance loci reside. Significant SCN resistance loci have been detected for SCN race 1 with the clone BLT65, and probe K069. Four of the 125 progeny genotypes have a female index of < 10% for race 1 resistance, and all four carry the resistance phenotype for these two markers. Ten other genotypes also carried the resistant molecular pattern for these two markers and expressed moderate resistance to SCN infection. Further discrimination between molecular marker pattern and SCN resistance is required to subsequently trace resistance genes as selective breeding pressure is applied.

PURIFICATION AND CHARACTERIZATION OF A PHYTATE-DEGRADING ENZYME FROM GERMINATION SOYBEAN COTYLEDONS

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Much of the phosphorus in soybean meal is sequestered in an organic form known as phytate (*myo*-inositol hexaphosphate). Because non-ruminant animals lack enzymes necessary for phytate degradation, phytate-bound phosphorus passes through the gastrointestinal tract undigested. This necessitates phosphorus supplementation, which increases feed costs and contributes to phosphorus pollution. Phytase (E.C. 3.1.3.8) is an enzyme that breaks down phytate, liberating inorganic phosphate. Fungal phytase has successfully been used as a dietary supplement in place of inorganic phosphorus. Nutritional availability of phosphorus also has been increased by the overexpression of a fungal phytase gene in plants (1). Phytase occurs naturally in soybean, but is not synthesized until seed germination. An endogenous soybean enzyme would likely be more suited to hydrolyzing soybean phytate, which accumulates in protein bodies of developing seeds. Our goal is to isolate a soybean phytase gene for characterization and reintroduction under control of specific signals to insure correct expression and accumulation in the seed. Using amino acid sequence data from purified soybean phytase, we will generate probes for screening a cDNA library from 10-day old germinating soybean cotyledons. We have purified a major form of soybean phytase from cotyledons of 10-day old germinating seedlings to apparent homogeneity by sequential ammonium sulfate precipitation, lectin affinity, and anion exchange chromatography. A partial amino acid sequence of this protein was obtained by automated Edman degradation. A BLAST search of the 20 amino acid sequence was performed against the Swiss & PIR & Translated Release 92 protein database. Our sequence showed similarity to soybean vegetative storage proteins (VSPs) alpha and beta (64.6% and 80.5%, respectively). The VSPs are glycoproteins that possess acid phosphatase activity, but do not hydrolyze phytate (2). It has been suggested that VSPs are encoded by a family of two to four genes, and that related sequences are present in the soybean genome (3). It is possible that the phytate-degrading protein we isolated is a member of the VSP family.

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SOYBASE, A SOYBEAN GENOME DATABASE: STORAGE PROTEINS OF SOYBEAN

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Storage proteins of soybean are of great interest and importance to geneticists and molecular biologists, but until now there have been no comprehensive overviews of the topic available by computer. SoyBase recently was expanded to include broad, up-to-date information of seed storage proteins as well as vegetative storage proteins.

The major seed storage proteins in soybean are glycinin (11S globulin) and β-conglycinin (7S globulin). These constitute up to 50% of dry weight of seeds, and therefore are of nutritional and economic value as well as targets for improvement of seed quality. In general, soybean seed proteins are low in cysteine and methionine. Mature glycinin is hexameric, with each subunit pair containing an acidic and a basic subunit. Five genes encode the glycinin subunits; each has been cloned and sequenced. β-conglycinin is encoded by at least 15 cloned genes, several of which are closely linked. It typically is trimeric, with the β-subunit in some cultivars totally lacking methionine. A total of 9 seed storage proteins are detailed in SoyBase.

Storage proteins also occur in vegetative tissues of soybean. These vegetative storage proteins (VSP) are of importance because they can be hydrolyzed, translocated, and reassimilated throughout plant development. Major VSP's have been purified, and their genes have been cloned. Six individual vegetative storage proteins are described in SoyBase.

Data in SoyBase cover purification, molecular weights and subunitation, gene symbols, isozymes, regulation, enzyme data, plant source, existence of mutants and sequences, availability of molecular probes, clones, and transgenic plants, as well as linkage and mapping details for all vegetative and seed storage proteins of soybean. Complete reference citations are accessible for all data.

These and all SoyBase data are available to the public through the National Agricultural Library at http://probe.nalusda.gov:8300. Updates are made on a regular basis. For more information contact SoyBase curator David Grant (dgrant@iastate.edu) or assistant curator Marcia Imsande (mimsande@iastate.edu).

MICROSPORE SEGMENTATION AND EMBRYO FORMATION IN CULTURED ANTHERS OF SOYBEAN

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The succesful development of haploid plantlets by anther culture is not yet reported in soybean, and only fragments of basic information are available. The current study had two aims: (i) to investigate the effects of different cold pretreatments on cytological events of pollen embryogenesis; (ii) to verify the androgenetic response of three Brazilian soybean cultivars for different floral bud sizes. For the first aim, young inflorescences (3.0-3.5 mm of lenght), of the cultivars IAS 5 and RS 7, were subject to 4°C for 0, 5 and 10 days, and then placed on Induction Medium (B5 long [Carolina Biological Supply Co., Burlington, NC, USA] + 2.0mg/l 2,4D + 0.5mg/l BAP + 9% sucrose) . Cytological examination of the *in vitro* anthers were done during the first four weeks of culture through squashing 60 anthers per treatment in propionic carmine. Microspores were staged and classified under a Zeiss Axioplan Universal microscope.Symmetrical-binucleate grains has been considered one of the routes in soybean callus formation. This study showed that, in cold pretreated anthers of cv. RS7, the percentage of symmetrical-binucleate microspores increased with the length of *in vitro* incubation. However, for cv. IAS 5, the percentage of such microspores was reduced drastically with the cold treatments. Both cultivars presented multinucleate pollen formation. An overall frequency of 0.31% was obtained by the end of one month incubation. These multinucleate grains might be originated by asymmetrical or symmetrical mitosis, and both vegetative and generative nuclei can divide. The data sugested an unusual vigorous mitotic activity from the generative nuclei during multinucleate pollen formation. The low temperature pretreatment did not affect the percentage of multinucleate grains in culture. For the second aim, floral buds of four different intervals of size (1.5-1.9mm, 2.0-2.4mm,2.5-2.9mm and 3.0-3.5mm) were colected.They were cold treated (4°C) dung 10 days and plated in the Induction Medium. There were excised 600 anthers per cultivar for each bud size. After three months, the cultivar IAS 5, DECADA and RS 7 presented the following overall percentages of calli: 45.48%, 64.61% and 70.09% respectively. The averages of calli with androgenetic structures was 0.67% in cv IAS 5 and 0.36% in DECADA. Such structures didn't occure in cv RS 7 . In both cultivars, IAS 5 and DECADA, the androgenetic structures were formed just on anthers from the two first intervals of floral bud sizes (1.5-1.9 and 2.0-2.4).The androgenetic structures were similar to the somatic embryos obtained from imature zygotic cotyledons. Then to confirm this observation,the structures were fixed in glutaraldeide 2.5%, embebed in hydroxyethylmethacrylate and sectioned at 4µm for histological analysis. The sections were stained with toluidine blue. Anatomical data showed clearly an embryo at late torpedo stage . The procambium has bifurcated the shoot apical meristem and enters into the cotyledon. The shoot meristem is located at the apical notch. A suspensor was not found and the apical root meristem was not well defined as in zygotic embryos. The observations suggested that the tissues of the anther embryo were not fully determined.

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MAPPING OF QTL AND IDENTIFICATION OF cDNA ASSOCIATED WITH *PHYTOPHTHORA SOJAE* TOLERANCE OF SOYBEAN

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One of the most destructive diseases of soybean is Phytophthora root rot (prr) caused by the fungus *Phytophthora sojae*. Contrary to the Phytophthora resistant trait which is controlled by a single gene, tolerance to prr is a quantitative trait controlled by a small number of genes. The QTL associated with prr tolerance is identified using the Harosoy Isoline x Clark Isoline mapping population that segregates for prr tolerance. Phytophthora tolerance is assayed by the inoculum layer tests. The Amplified Fragment Length Polymorphism (AFLP) is used to add additional DNA markers to the Harosoy x Clark linkage map. QTL for prr tolerance is mapped using the MAPMAKER-QTL program. In addition, the cDNAs expressed in the tolerant responses are isolated by the differential display technique using bulk segregant analysis.

MULTIPLE-LOCUS BULK SEGREGANT ANALYSIS OF SCN LOCI IN PI437.654 USING RAPD AND AFLP MARKERS.

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We have been analyzing a ~300 RIL population from PI437.654 X BSR101 in order to map resistance genes to soybean cyst nematode (SCN) race 3. Initial studies using > 400 RFLP markers detected significant affects of three QTL on linkage groups A, G, and M. While the M locus later proved to be due to unrelated epistasis (*Theor. Appl. Genet.* - 91:574-581), this was determined after this project was initiated. In order to saturate these regions with genetic markers, we constructed three-loci bulks using the RILs and RFLP markers flanking the SCN resistance regions. We identified ~20 RILs for each bulk that were fixed for RFLP marker alleles closest to the QTL and contained even distributions of nonlinked RFLP marker alleles. Nearly 1,000 RAPD primers were used against the parents and the three-loci bulks in combination with *Taq*I restricted and nonrestricted templates to identify QTL-linked markers. RAPD markers differing between the bulks were placed on the genetic map by using a random set of 42 RILs from the same population. While many of the markers were located in the QTL regions on A, G, and M, others mapped to dispersed locations of the genome. In order to understand how markers identified by the three-loci bulks could map to distant genomic regions, we analyzed the bulks with >600 AFLP markers during the construction of an AFLP map. AFLP markers also were identified by the bulks as residing in the QTL regions. However, like the RAPD markers about 50% of the AFLP markers mapped to distant regions of the genome. These markers were not clustered indicating that their identification was not due to inadvertent inclusion of additional genetic contrasts in the bulks. Rather, their identification from the bulks seems to be locus not region specific. In addition, we have identified additional random genetic markers in the QTL containing regions that were not identified by the three-loci bulks. Our data indicate that bulk segregant analysis is useful for identifying markers in specific genetic regions, but that some markers may be missed and that other markers will be falsely identified.

EFFECT OF *HETERODERA GLYCINES* ON ISOFLAVONOID PRODUCTION OF *GLYCINE MAX* AND NODULATION BY *BRADYRHIZOBIUM JAPONICUM* USDA110

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Studies were conducted to determine the effect of *H. glycines* isolate TN1 on isoflavonoid production in soybean cultivars Essex and Hartwig. Isoflavonoid production was indirectly measured by incubating ethanolic root extracts with *Rhizobium fredii* USDA191 containing the reporter gene *lacZ* fused to the common nodulation genes *nodABC*. β–galactosidase activity was measured in Miller units. Activity in extracts from roots of both *H. glycines*-resistant (Hartwig) and -susceptible (Essex) cultivars infected with TN1 was significantly higher than non-infected roots at two and three days post inoculation. The identity of the compounds which activate the transcription of *nodABC* genes was verified by HPLC and GC/MS. Daidzein and genistein were the two main compounds that promoted *nodABC* expression. Daidzein levels increased four or three fold in infected roots over non-infected roots at two and three days post inoculation, respectively. Genistein levels increased two fold in infected roots over non-infected roots at both two and three days post inoculation. Previous studies have indicated that suppression of *B. japonicum* nodulation of soybean by *H. glycines* is population specific. Therefore, the interaction of *H. glycines* isolate TN1 and *B. japonicum* USDA110 on soybean cultivars Essex and Hartwig were investigated. Two day radicals were inoculated with eggs of *H. glycines* isolate TN1 or *B. japonicum* USDA110, either together or separately, and were grown in soil in the greenhouse for 33 days. Root and shoot weight, number of nodules and nitrogenase activity were measured. The interaction between *H. glycines* TN1 and *B. japonicum* USDA110 led to intermediate roots and shoot weights compared to the control treatments. Infection by *H. glycines* isolate TN1 led to an increase in the number of nodules, but a decrease in the weight of nodules. Furthermore, *H. glycines* isolate TN1 infection decreased nitrogenase activity as measured by acetylene reduction.

Maintenance of Genetic Diversity in an Outcrossing Population of Soybeans

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Soybean can be treated as an outcrossing crop by harvesting seed from male-sterile plants. Insect-mediated outcrossing in populations segregating for male sterility generates large numbers of hybrid seeds but reduces the control of the breeder in parental selection. Long term changes in the genetic makeup of outcrossing soybean populations will impact the effectiveness of this breeding system. Objective: Determine if allelic variation at RFLP, morphological and flowering trait loci is maintained during outcrossing cycles in a male-sterile soybean population. Approach: A population of soybeans was derived by making all possible two way crosses between 39 female and 4 male sterile maintainer lines. This population was grown in isolation and subjected to forced outcrossings. Seed born on male sterile plants was harvested and a random selection of this seed gave rise to the next generation for nine consecutive cycles. Pure lines which represent cycles 3,8, and 9 were derived by single seed decent. The parents and cycles 3 and 8/9 were scored for four mtDNA types, nine nuclear RFLP loci, and seven loci controlling five morphological traits. Drift contributed to the loss of cytoplasmic types before cycle 3 while selection against plants with the 'Soja' cytoplasmic type caused it's loss between cycles 7 and 9. No cytonuclear interactions were observed suggesting this selection was directly against the 'Soja' mtDNA. No significant change in nuclear allele frequencies occurred during outcrossing but cluster analysis showed an increase in genetic similarity among lines with continued cycles of outcrossing. Field data was taken on flower load, length of flowering, height, flower color, and plant architecture on individual lines to evaluate traits that might influence the success of plants in an outcrossing system by their attractiveness to pollen vectors. Analysis of these traits showed that overall variation was maintained throughout the cycles. No selection occurred for larger flower load, longer flowering period, flower color or flowering on branches. The data suggests that taller plants flowering above the leaf canopy competed more successfully in this outcrossing population. The number of lines with overlapping flowering periods increased from 75% in the parents to 100% in cycle 8/9. The increase maximized the chance for each parent to contribute to the pollen pool. However, the overall genetic structure of the populations suggests that assortive mating rather than random mating predominates in this outcrossing population.

IDENTIFICATION OF MOLECULAR MARKERS IN SOYBEAN USING RFLP, RAPD AND AFLP DNA MAPPING TECHNIQUES

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Three different DNA mapping techniques, RFLP, RAPD and AFLP were used on identical soybean germplasm to compare their ability to identify markers to develop a genetic linkage map. Polymorphisms present in fourteen different soybean cultivars were demonstrated using all three techniques. AFLP, a novel PCR based technique, was able to identify multiple polymorphic bands in a denaturing gel using 60 of 64 primer pairs tested. AFLP relies on primers designed in part on endonuclease restriction site sequences and on three selective nucleotides. The 60 diagnostic primer pairs tested for AFLP analysis each distinguished on average six polymorphic bands. Using specific primers designed for soybean from EcoRI and MseI restriction site sequences and three selective nucleotides, as many as 12 polymorphic bands per primer could be obtained with AFLP techniques. Only 35 percent of the RAPD reactions identified a polymorphic band using the same soybean cultivars and in those positive reactions, typically only one or two polymorphic bands per gel were found. Identification of polymorphic bands using RFLP techniques was the most cumbersome, because Southern blotting and probe hybridization were required. Over 50 percent of the soybean RFLP probes examined did not distinguish even a single polymorphic band. The RFLP probes that distinguished polymorphic bands seldom identified more than one polymorphic band.

LOCALIZATION OF CHOLINEPHOSPHOTRANSFERASE EXPRESSION IN DEVELOPING SEEDS

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Previous studies have shown that CDP-choline:diacylglycerol cholinephosphotransferase (CPT) is a key component of membrane lipid synthesis in vegetative tissues and triglyceride synthesis in developing soybean seeds. Enzyme levels are known to be regulated developmently, environmentally and hormonally. The enzyme is present at a low level in extracts from seeds during late-R4. The activity increases until R5 then decreases to undetectable levels before the end of seed fill. Tissue specific regulation of these events was investigated by analyzing the expression of CPT mRNA in seeds from late-R4 through mid-R5 stages using fluorescent *in situ* hybridization (FISH).

Tissue hybridizations were conducted using a 400 bp fragment from the 5′ coding region of the CPT mRNA. Sense and antisense riboprobes were made by *in vitro* transcription using fluorescein-UTP. Sections were counter stained with propidium iodide.

The antisense probe revealed expression of CPT in all samples, while the sense probe showed no detectable signal. In late-R4 seeds, detectable CPT expression was confined to the outer integument. Early-R5 seeds showed detectable expression in all seed tissues except the aleurone layer. By mid-R5 high level expression was confined to the cotyledons, while lower level expression could be detected in the inner integument. Image analysis of early-R5 seeds showed that the hybridized mRNAs were localized in the cytoplasm.

CONSTRUCTION AND CHARACTERIZATION OF A BACTERIAL ARTIFICIAL CHROMOSOME LIBRARY FROM SOYBEAN

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A bacterial artificial chromosome (BAC) library suitable for map-based cloning and physical mapping has been constructed in soybean. The BAC vector, capable of maintaining large insert DNA, was developed by Shizuya et al. (P.N.A.S. 89:8794 1992) for use in human genome mapping. The first plant BAC work was in Rod Wing's laboratory at Texas A&M (Woo et al., NAR 22:4922 1994). Our soybean BAC library uses the pBeloBAC 11 vector. Large insert DNA was isolated from HindIII partial digests of megabase DNA prepared from nuclei embedded in agarose microbeads. The nuclei were prepared from young leaves of the Williams 82 soybean cultivar. A random selection of 160 BACs generated from two ligations prepared from DNA size-selected from partial digests of two different microbead preparations ranged in size from 40 to 350 kb with an average insert size of 150 kb. The BAC library is stored as individually picked clones representing 4-5 genome equivalents (approximately 40,000) in 384 well microtiter dishes. We have prepared pooled DNA samples suitable for screening the library with PCR based technologies as well as membranes for screening by hybridization methods.

EVALUATION OF CYTOKININS INCLUDING THIDIAZURON FOR INITIATION OF MULTIPLE SHOOTS FROM SOYBEAN COTYLEDONARY NODES.

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Treatments of thidiazuron (TDZ), benzylaminopurine (BAP), and TDZ plus BAP in a B5 basal medium (Gamborg et al. 1968) were evaluated as shoot inducing cytokinins for soybean cotyledonary nodes. The high level of TDZ (10μM) tested nearly suppressed all shoot formation while the low level (1μM) did not decrease shoot number. All treatments containing TDZ inhibited adventitious root formation. The BAP plus low TDZ (1μM) treatment did not decrease the number of shoots formed but did produce an interesting morphology in the cotyledonary node region suggestive of a flush of arrested shoots that was not seen in the treatment containing BAP alone. Using the existing cotyledonary node protocol of Di et al. (1996), these possibly arrested shoots would not develop further. An additional experiment has been initiated to alter the duration of exposure to TDZ and also to evaluate 2-isopentenyl phosphate (2iP) as a replacement auxin for indole butyric acid (IBA) in the existing protocol. This is being done in an attempt to break the possibly arrested development of these shoots.

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MOLECULAR MARKERS ASSOCIATED WITH SOYBEAN SEED PROTEIN AND OIL ACROSS POPULATIONS AND LOCATIONS

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Two soybean [*Glycine max* (L.) Merr.] populations, 'Young' X PI 416937 and PI 97100 X 'Coker 237', were used to identify quantitative trait loci (QTL) related to seed protein and oil content. For the Young X PI 416937 population, 120 F_4-derived lines were scored for segregation at 155 restriction fragment length polymorphism (RFLP) markers. The F_4-derived lines and two parents were grown at Plains, GA, and Windblow and Plymouth, NC in 1994, and evaluated for seed protein and oil. For the PI 97100 X Coker 237 population, 111 F2-derived lines were evaluated for segregation at 153 RFLP markers. Phenotypic data were obtained in two different locations (Athens, GA, and Blackville, SC) in 1994. Based on single-factor ANOVA for the Young X PI 416937 population, five of seven independent markers associated with seed protein and all four independent markers associated with seed oil in the combined analysis over locations were detected at all three locations. For the PI 97100 X Coker 237, single-factor ANOVA revealed that three of four independent markers for seed protein and two of three independent markers for seed oil were detected at both locations. In both populations, single-factor ANOVA revealed consistent QTL across locations. However, interval mapping of the PI 97100 X Coker 237 population indicated that QTL identified at Athens for seed protein and oil were different from those at Blackville. Comparison of these results with previous studies supports the population specificity of important QTL, and evidence for additional QTL for seed protein and oil.

GENETIC MAPPING OF A YIELD DEPRESSION LOCUS NEAR A MAJOR SOYBEAN CYST NEMATODE RESISTANCE GENE

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The use of cultivars with resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN), is currently the most effective method for controlling this destructive pathogen. However, cultivars selected for resistance to SCN typically have lower yields than susceptible cultivars when grown in the absence of SCN pressure. Previously, a major locus conferring SCN resistance had been found on linkage group 'G'. One possible reason for the decreased yield may be linkage drag between this resistance locus and a yield depression locus. Preliminary yield data from two PI 209332-derived populations were used to determine the relationship of any putative yield depression loci and the resistance gene on 'G'. The first population consisted of 98 $F_{5:7}$ lines from the cross 'Evans' x PI 209332. The second population was derived from an $F_{2:3}$ line from the cross M83-15 x M85-1430 (with PI 209332-derived resistance) which was heterozygous for molecular markers in the region of the 'G' resistance locus. Individual plants from this line were crossed to 'Evans'. In 1994, $F_{5:6}$ plants from the latter population were yield-tested in St. Paul, MN. In 1995, both populations were grown in Waseca (non-SCN-infected) and New Richland, MN (SCN-infected) with two replications at each site. Maturity, height, lodging, and yield data were also used to identify QTLs linked to molecular markers both on 'G' and in the rest of the soybean genome. The placement of this putative yield depression locus in relation to the resistance gene on 'G' should allow the use of molecular markers to identify recombinants which show resistance without any accompanying yield depression. Further yield trials and the use of larger populations currently being developed, will be used to confirm and further define the location of the putative yield depression locus and its relationship to the resistance gene.

RATE-REDUCING RESISTANCE TO *Fusarium solani* f. sp. *phaseoli* UNDERLIES FIELD RESISTANCE TO SUDDEN DEATH SYNDROME.

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Yield losses occur in soybean (*Glycine max* (L.) Merr.) due to sudden death syndrome, caused by *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & Hans. Our objective was to determine whether tolerance or partial resistance to infection underlies field resistance to SDS. Seven field resistant cultivars and five susceptible cultivars were planted at two locations. Twenty taproots per cultivar were sampled every 1 to 3 weeks from the V0 to R8 growth stages, 8 to 121 days after planting (DAP). Six taproot sections per plant were tested for *F. solani* on a selective medium. Infection frequency (IF, the percentage of infected plants), and infection severity (IS, the percentage of infected root segments) were scored. Leaf symptoms were recorded and standardized to the R6 stage and the disease index was calculated as (DI*DS)/9. Infection was detected at the V1 stage (within 15 DAP) and reached a maximum by about R1 (55-68 DAP). All cultivars were infected by *F. solani*. However, seasonal mean IF and mean IS were significantly ($P< 0.05$) lower among the resistant cultivar class. After R1, infection was also significantly lower among the resistant cultivar class within individual sampling dates. Also, disease index and IF were correlated ($P<0.05$, $r=0.38$ and 0.61). Therefore, in the resistant cv.s Forrest, Ripley, Jack, PI520,733, ExF44, ExF59 and ExF78 late season rate-reducing (partial) resistance decreased the disease index, IF and IS, probably by extending the latent period of *F.solani*. Among susceptible cv.s, Essex and Asgrow 5403, had reduced leaf symptoms but high IF and IS suggesting tolerance to *F.solani* that alleviated SDS.

UTILIZATION OF SEQUENCE CHARACTERIZED AMPLIFIED REGIONS FOR MARKER-ASSISTED SELECTION OF RESISTANCE TO SUDDEN DEATH SYNDROME AND CYST NEMATODE IN SOYBEAN

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The disease sudden death syndrome in soybean is characterized by extensive root lesions, leaf necrosis and results in dramatic yield losses. Resistance to the disease caused by the fungus, *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & hans., type A, is controlled by several genes, is incomplete and quantitative. RAPD and RFLP markers identified four QTL that accounted for approximately 65% of total phenotypic variability in mean disease index (DI), 50% in mean disease severity (DS) and 35% in mean yield. Due to the proximity of the markers to the QTL, efforts were focused on RAPD markers OIO3, OEO2 and RFLP markers K455D, CHS4 located on linkage groups, G, G C2, and A respectively. Marker OIO3 is close (1 - 3 cM) to a QTL cluster containing the major SCN resistance gene that partly explains the co-inheritance of resistance to SDS and SCN. *CHS4* is a candidate for the I gene that maps to MLG A close to *Rhg4*. SCAR primers designed from the ends of K455D amplified a 1.4 kb in the parents, Essex and Forrest that was not polymorphic. The 1.4 kb band was sensitive to Mg^{2+} concentration but was insensitive to a range of annealing temperatures. SCAR primers designed from the sequenced ends of the cloned RAPD marker, OIO3, produced a non-polymorphic 450 bp allele in both Essex and Forrest. SCAR-derived parental fragments from both OIO3 and K455 were sequenced a second time to determine appropriate primer sequences that would yield polymorphic fragments for marker assisted selection of SDS and SCN resistant lines. Results of the screening will be presented.

RFLP Marker Analysis of Resistance to Soybean Cyst Nematode Race 1 in Peking

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Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is one of the most serious pests of soybean [*Glycine max*(*L.*) Merrill]. SCN can cause up to 90% reduction in seed yield. Based on classical genetic studies, there are three genes controlling resistance to SCN Race 1 in 'Peking', but little is known about molecular markers associated with resistance to SCN Race 1. We conducted molecular marker analysis on 200 $F_{2:3}$ populations derived from a cross between Peking, resistant to Race 1, and 'Essex', susceptible to SCN. The index of parasitism (IP) was calculated based on the ratio of the number of white females of a given individual to the mean number of cysts on Essex. The IP values were used as the phenotypic reaction of each individual plant to SCN Race 1 in the SAS regression analysis. A total of 215 probes have been screened and 55 (25.5% polymorphism) were found to be polymorphic between Peking and Essex. To date, five RFLP markers have been shown to be associated with resistance to SCN Race 1 in Peking. Both RFLP markers A597 and A18, which are located in linkage group E, explained 18.2% ($p<0.0001$) and 15.7% ($p=0.0001$) of the phenotypic variation, respectively. Marker pT05 in linkage group B explained 13.3% ($p=0.0001$) of the phenotypic variation, and markers k104 in linkage group A and A102 in linkage group I explained 10.2% ($p=0.0001$) and 9.9% ($p=0.001$) of the phenotypic variation, respectively. These molecular markers together explained 67.5% of the total phenotypic variance in the resistance response to SCN Race 1. Based on our earlier classical genetic studies considering a three-gene hypothesis conferring resistance to SCN Race 1 in Peking, associated RFLP markers were found in four linkage groups. This implies that Peking may have some minor genes together with major genes conditioning complete resistance to SCN Race 1.

SOYBEAN EMBRYOGENIC CULTURE: MEDIUM OPTIMIZATION

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Development of stable, reproducible tissue culture systems is one of the most essential steps in legume biotechnology. Maintenance of repetitively embryogenic cultures of soybean in liquid medium is a very convenient system for genetic engineering and in vitro propagation. The objective of this study was to optimize the growth of soybean embryogenic cultures in liquid medium. Such cultures were first made possible by the development of Finer and Nagasawa (FN) medium, but not all soybean cultures respond well to this medium. Consequently, embryogenic cultures of cv. Jack were maintained on FN medium and used as a control. A number of variables, such as sucrose content, total nitrogen amount, and source of nitrogen were tested.
Sucrose concentration and total nitrogen content were found to be the major factors controlling growth rate of soybean embryogenic cultures. Total biomass of soybean embryos cultured for three weeks on standard FN medium with 6% sucrose or FN with 3% sucrose increased 8.6 and 10.9-fold, respectively. Although lower sucrose content (3%) in a standard FN medium substantially enhanced overall growth of embryogenic clusters, it also promoted development of necrotic areas which hampered further proliferation. This was overcome by lowering the amount of total nitrogen from the 50 mM in FN to 35 mM. This was accomplished by substitution of $(NH_4)_2SO_4$ for NH_4NO_3, which also increased SO_4^{-2} from 1.7 mM in FN to 5.2 mM in the new medium. The ammonium/nitrate ratio of 0.25 in FN medium was retained in the new medium. These changes make it possible to obtain about 11.7 and 14.5-fold increases in total biomass of soybean embryogenic tissue in media with 6 and 3% sucrose content, respectively, over a three-week period. Embryogenic clusters proliferated on the new medium can develop into normal, mature embryos capable of conversion. Due to its lowered sucrose and nitrogen content, we are referring to the new medium as FN Lite.

GENETIC DIVERSITIES OF CHLOROPLAST AND MITOCHONDRIA GENOMES OF THE WILD SOYBEAN, *G. SOJA*, IN CHINA

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Polymorphisms of genomes of chloroplast(cp) and mitochondria(mt) have been shown in the wild soybean, *G. soja,* with RFLPs. However, no organelle genome about the Chinese wild population has been investigated. 754 plants of wild soybean collected from overall districts of China were evaluated for RFLP profiles of cp- and mt-DNA. The cp genome revealed the two profiles, cpII and cpIII equivalent to Close *et al.*(1989), of RFLP with *EcoRI-H2 clone*. The cpIII was predominant and distributed all over the China and the cpII was limited to the southern China. The cpII was considered to evolve from the cpIII and to be the progenitor of cpI which was predominant in soybean cultivars. The cp genome indicated that soybean would be domesticated in the southern China. The mt genome revealed the fifteen types in combination of RFLP profiles with *HindIII-coxII, BamHI-coxII* and *BamHI-atp6*. Of these the mtIVa and mtIVb were predominantly observed in northern and western and in southern districts, respectively, and occupied 77.5% of the plants tested. The six mt types unique to the Chinese wild soybean were observed with less than 2% and could be found only in the southern district. Therefore, the southern China has conserved the abundant genetic diversity of mt genome in wild soybean and was the habitat of mtIVb, which was also the cultivar type of mt genome. As the combined types of cp and mt genomes were expected from the frequencies of cp and mt types, cpIII-mtIVa was predominant in northern and western districts and cpII-mtIVb in southern district, respectively. However, no the two opposite types, cpII-mtIVa and cpIII-IVb, were observed in the Chinese population. This implicated that the specific cp and mt genomes might be evolutionally associated with each other. Modal types of cultivar organelle genome are the cpI-mtIVb and cpII-mtIVc, and different from the wild soybeans. The cpII-mtIVb may be an ancestor close mostly to above the two cultivar types and evolve into the domesticates with the mutation on cp or mt genome.

USING AFLP MARKERS TO DETERMINE THE CONTRIBUTION OF PARENTAL GENOMES TO POPULATIONS

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Estimates of proportions of parental contribution to germplasm populations are useful to breeders. The objective of this research was to assess the ability of amplified fragment-length polymorphism (AFLP) markers to characterize the contributions of the original parents to later cycles of a soybean [*Glycine max* (L.) Merr.] population that had undergone recurrent selection. The AFLP technique was invented in 1993 (Zabeau, 1993). Its advantage over other DNA marker techniques includes the detection of a large number of polymorphism from a single PCR reaction. Use of four primers yielded a total of 47 polymorphic bands. Estimates of parental contribution were consistent between primers and between cycles and conformed to those expected based on pedigrees. Standard errors of estimates were 3 to 5%, indicating satisfactory precision. The AFLP markers should provide relatively inexpensive, precise estimates of parental contribution.

POLLEN AND ANTHER CULTURE OF SOYBEAN

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The development of doubled haploid plants from pollen grains has been recognized as a method for rapidly producing homozygous plants from heterozygous breeding lines. The techniques for applying this approach have been established in a number of crops, including rice, barley, and rapeseed. Doubled haploid plants can be used to reduce the time needed for the production of new cultivars. Doubled haploid plants are also useful in mapping and genetic engineering research.

Doubled haploid plants from uninucleate soybean microspores have recently been developed at the Rice Research Station. Both immature individual pollen and immature anthers have been utilized as explant material. Callus can be obtained from isolated microspores, but no plant regeneration has occurred to date. High rates of callus can be obtained from individual pollen when cultured with the intact anther. Microscopic sections have confirmed that callus derived from the cultured anthers originated from microspores and not maternal tissue. Calli are transferred to differentiation medium. Within 4 weeks embryos are removed and transferred through a series of media for maturation, desiccation and germination. Plants have been obtained from cultivars and F_1 genotypes. Research is continuing on optimizing these techniques for efficient production of doubled haploids from a range of soybean genotypes.

RFLP ANALYSIS of SCN RESISTANT PI LINES FOR GENETIC DIVERSITY

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Soybean cyst nematode (SCN, *Heterodera glycines*) resistant cultivars have proven highly successful in reducing yield losses of soybean. In the U.S.A., improved soybean cultivars have derived SCN resistance from only a few plant introductions (PI). Additional germplasm is available which could be used as new sources of resistance for broadening the genetic diversity of resistance. For this purpose, 54 soybean PI lines representing resistance to most populations (or races) of SCN and 4 susceptible controls were assayed by RFLPs with five restriction enzymes, EcoRI, EcoRV, HindIII, DraI, and Taq I.

These RFLP profiles could be used as indicators to characterize genetic diversity, relationships of the SCN resistant PI lines, and to evaluate the usefulness of RFLP markers and loci for SCN resistance. To this date, 244 of the 729 bands detected with 45 probes provided polymorphism for the PI lines. Cluster analysis based on these RFLP bands was conducted using Ward's minimum variance method. The data suggested two main clusters based on the PIs' geographic origin. One included most PI lines from China, the other contained all the PI lines from Korea (except one), plus several from China; while the PIs from Japan and Russia were separated into the two clusters.

The cluster representing China region included the four important SCN resistant PI lines, PI 88788, Peking, PI 90763, and PI 437654. These four lines not only have contributed genes for SCN resistance for breeding all resistant soybean cultivars in the U. S. A., but have been used as standard host differentials in the SCN race classification system. More over, these four were grouped into three sub-clusters: PI 88788 was placed in one cluster; Peking and PI 90763 belonged to another; and PI 437654 was found in the third cluster. Additionally, PI 437654 was clustered along with PI 438489B, and both of them are resistant to all SCN races.

These tentative results suggest that SCN resistant PI lines from Korea may represent unrelated germplasm. They may be genetically different from the four differentials, and may be potential sources for finding unique genes for SCN resistance.

RFLP analysis is in progress using fifty additional probes to determine if they will contribute and add precision for the survey of SCN resistant germplasm.

THE SOYBEAN RETROELEMENT *SIRE-1* IS AN ENDOGENOUS RETROVIRUS

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SIRE-1 was initially reported to be a multicopy, interspersed collection of large genomic elements from *G. max* with weak sequence similarity to *copia/Ty1*-like retrotransposons (Laten and Morris, 1993, Gene 134:153). We have since strengthened this assessment with sequence analysis of a truncated 2.4 kb cDNA clone containing part of the 5' long terminal repeat (LTR), a tRNA primer binding site, and a 617-codon ORF encompassing the *gag* and protease regions (Bi and Laten, 1996, Plant Mol. Biol., in press). The protease sequence most closely resembles those of *copia/Ty1*-like retrotransposons. A full-length copy of the *SIRE-1* element was recovered from a genomic library. We have completed the preliminary DNA sequence (one strand) of a subclone containing a 4.2 kb *Xba*I fragment from this genomic insert. This fragment contains the distal 3.7 kb of *SIRE-1* and 538 bp of presumably single-copy, flanking DNA. Analysis and translation of the *SIRE-1* portion of the sequence produced 2 ORFs. ORF1 is truncated at its 5' end, but the 62-amino acid theoretical peptide is strikingly similar to the peptide sequence of the carboxyl portion of the Rnase H from *copia* (56% identity, 74% similarity). ORF2 is 578 codons in length. The translated amino acid sequence bares a striking resemblance to viral envelope glycoproteins and other eukaryotic membrane glycoproteins. The amino third of the sequence is rich in proline, serine and threonine, with the latter two possibly serving as O-glycosylation sites. A small number of asparagines in this region might serve as N-glycosylation sites. There is a typical hydrophobic signal peptide near the amino terminal and a characteristic hydrophobic "anchor" near the C-terminus. Computer prediction of secondary structural features revealed a pattern of helical domains similar to that found in mammalian retrovirus surface proteins. Other characteristic nucleotide sequence features found in both retroviruses and LTR retrotransposons, including promoter consensus sequences, a polyadenylation signal, and a polypurine tract for the initiation of retroelement plus-strand synthesis, are present at the appropriate locations. The 538 nucleotides adjacent to the 3' end of this *SIRE-1* copy can be translated into an uninterrupted ORF, strongly suggesting that the integration event disrupted a functional gene. Our data support the conclusion that *SIRE-1* is an endogenous family of proretroviruses whose genomic structure is based on a *copia/Ty1*-like organization. The genomic organization of all animal retroviruses (from vertebrates and *drosophila*) is patterned after *gypsy/Ty3*-like retrotransposons. Neither retroviral genomes nor virions have been reported in plants. We have no data that would indicate whether or not *SIRE-1* is infectious. We have detected transcripts in RNA slot blots from leaf tissue using both *gag*-specific and ORF2-specific probes, but are deferring any interpretation of these findings until more definitive experiments are run.