Proceedings of the
4th Biennial Conference on

# Molecular and Cellular Biology of the Soybean

July 27-29, 1992
Scheman Building
Iowa State University
Ames, Iowa

## General Information

### Meeting Facilities

All conference activities will take place at the Iowa State Center and Scheman Building. All contributed papers will be presented in Benton Auditorium, Scheman Building. The barbecue on Monday evening will be located in the courtyard south of the Scheman Building.

### Speaker Information

Persons making presentations should bring their slides to the projectionist in Benton Auditorium 30 minutes before the start of the session. Speakers are encouraged to introduce themselves to the person presiding over the session in which they will speak. This will allow the presiding officer to know who will be presenting the paper and to be certain of correct pronunciation and affiliations. Speakers are requested to pick up their slides within 30 minutes after the session is adjourned.

### Poster Information

Posters will be displayed in the 1st floor lobby of Benton Auditorium. Posters may be set up anytime after 1000 a.m. on Monday, 27 July, and should be taken down by 1300 p.m., Wednesday, 29 July.

### Acknowledgements

Mary Ann Simpson, Personnel Services (Housing)
Tom McCormick, Office of Continuing Education
Janet Gardner, Office of Continuing Education
Mary Adams, Office of Continuing Education
Roberta Kerkove, Department of Agronomy
Kayla Polzin, USDA-ARS-FCR, Department of Agronomy
Stephanie Lonning, USDA-ARS-FCR, Department of Agronomy

**Schedule of Papers for the**

**4th Biennial Conference on**

**Molecular and Cellular Biology of the Soybean**

**Monday Afternoon (July 27, 1992)  1300-1630**

**Building:** Benton Auditorium, Scheman Building, Iowa State Center

**1300-1305 -** Introductory remarks

**1305-1430 -** **Presiding:** Jack Widholm, University of Illinois

**1305-1320** (1)*
GENOTYPE EFFECTS ON REPETITIVE EMBRYOGENESIS AND PLANT REGENERATION OF SOYBEAN
M.A. Bailey and W.A. Parrott, Department of Agronomy, University of Georgia, Athens, GA 30602-7272

**1320-1335** (2)
INTRODUCTION OF A *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* (*BTK*) TOXIN GENE INTO SOYBEAN
W.A. Parrot[1], M.A. Bailey[1], M.J. Adang[2], H.R. Boerma[1], and J.N. All[2]. Department of Agronomy[1] and Department of Entomology[2], University of Georgia, Athens, GA 30602-7272

**1335-1350** (3)
EFFECT OF PETUNIA TRANSFORMATION BOOSTER SEQUENCE
Buising, C.M.[1], Benbow, R.M.[1,2], [1]Department of Zoology and Genetics, [2]Nucleic Acid Research Facility, Iowa State University, Ames, IA 50011-3223

**1350-1405** (4)
IMPROVING THE QUALITY OF SEED PROTEINS IN SOYBEAN
J.A. Townsend, L.A. Thomas, E.S. Kulisek, M.J. Daywalt, K.R.K. Winter and S.B. Altenbach, Pioneer Hi-Bred, Inc., Johnston, IA.

**1405-1430**
UPDATE ON TRANSGENIC PLANT REGULATIONS: CURRENT ACTIVITIES
Quentin Kubicek, USDA-APHIS-BBEP, Hyattsville, MD 20782

**1430-1500  Break**

**Numbers in parenthesis refer to the abstract presented in the back of this booklet.*

**1500-1630 - Presiding:** Halina Skorupska, Clemson University

**1500-1515** (5)
NEW PRIMARY TRISOMICS OF SOYBEAN IDENTIFIED BY PACHYTENE CHROMOSOME ANALYSIS

F. Ahmad, R.J. Singh and T. Hymowitz, Department of Agronomy, University of Illinois, Urbana, IL 61801, U.S.A.

**1515-1530** (6)
GENOMES OF THE GENUS *GLYCINE* WILLD

T. Hymowitz and R.J. Singh, Department of Agronomy, University of Illinois, Urbana, IL 61801, U.S.A.

**1530-1545** (7)
TRYPSIN AND CHYMOTRYPSIN INHIBITORS IN THE WILD PERENNIAL *GLYCINE* SPECIES

K.P. Kollipara and T. Hymowitz, Department of Agronomy, University of Illinois, Urbana,IL 61801, U.S.A.

**1545-1600** (8)
RAPD MARKER ANALYSIS OF GLYCINE TAXA

K.G. Lark and J. Evans, Department of Biology, University of Utah, Salt Lake City, UT 84112

**1600-1615** (9)
CYTOPLASMIC BASIS OF SELECTION IN AN OUTCROSSING SOYBEAN (*G. MAX*) POPULATION

D.J. Lee, C.A. Caha, G.L. Graef and J.E. Specht, University of Nebraska, Lincoln, NE

**1615-1630** (10)
CYTOPLASMIC DIVERSITY IN THE GENUS *GLYCINE* USING MITOCHONDRIAL RFLPS

E.A. Grabau, A. Pesce, J. Li, M. Peters, and W.H. Davis, Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0331, USA.

**1830-2100 Barbecue Picnic and Reception**
South Courtyard of the Scheman Building

**Tuesday Morning (July 28, 1992) 0830-1200**

**0830-1000 Presiding:** David Webb, Pioneer Hi-Bred International

**0830-0845** (11)
SELECTION OF *HETERODERA GLYCINES* POPULATIONS ON RESISTANT SOYBEAN GENOTYPES

R.L. Ruff and G.L. Tylka, Department of Plant Pathology, Iowa State University, Ames, Iowa

**0845-0900** (12)
RFLP MAPPING OF SOYBEAN CYST NEMATODE RESISTANCE IN SOYBEAN

K. Rorick and S. Mackenzie, Purdue University, West Lafayette, IN 47907

**0900-0915** (13)
MAPPING RESISTANCE TO *HETERODERA GLYCINES* I

H.T. Skorupska, Department of Agronomy and Soils and Department of Biological Sciences, Clemson University, and A.P. Rao-Arelli, Department of Agronomy, University of Missouri-Columbia

**0915-0930** (14)
RFLP MAPPING OF CYST NEMATODE RESISTANCE GENES IN SOYBEAN (*GLYCINE MAX*)

V. Concibido*, S. Boutin*, R. Denny*, H. Ansari*, J. Orf**, and N. D. Young*, *Department of Plant Pathology, **Department of Plant Breeding and Genetics, University of Minnesota, St. Paul, MN 55108

**0930-0945** (15)
A SIMPLE DNA MINIPREP PROCEDURE FOR PCR AMPLIFICATION ANALYSIS

G. Luo, A.G. Hepburn, and J.M. Widholm, University of Illinois, 1201 West Gregory, Urbana, Illinois 61801

**0945-1030 Break**

**1030-1145 Presiding:** Alan Atherly, Iowa State University

**1030-1045** (16)
GENETIC, BIOCHEMICAL, AND PHYSIOLOGICAL CHARACTERIZATION OF SOYBEAN NECROTIC ROOT MUTANTS

R.M. Kosslak, J. Dieter, R.G. Palmer, Iowa State University, Ames IA 50011, and B.A. Bowen, Pioneer Hi-Bred Intl., Johnston, IA 50131

**1045-1100** (17)
PLANT GENETIC STUDY OF RESTRICTED NODULATION OF SOYBEAN

D. Qian, F. Allen, G. Stacey, and P.M. Gresshoff, Center for Legume Research, University of Tennessee, Knoxville, TN 37996

**1100-1115** (18)
DNA AMPLIFICATION ANALYSIS OF NODULATION REGIONS IN SOYBEAN (GLYCINE MAX (L) MERRILL)

P.M. Gresshoff, D. Landau-Ellis, A. Kolchinsky, A. Jones, R. Prabhu, B. Bassam, J. Deckert, and G. Caetano-Anollés. Plant Molecular Genetics, Center for Legume Research, The University of Tennessee, Knoxville, TN 37901-1071, USA

**1115-1130** (19)
EVIDENCE OF INTERACTIONS IN NITROGEN METABOLISM BETWEEN SOYBEAN AND A PHYLLOPLANE BACTERIUM

M.A. Holland, N.E. Stebbins, and J.C. Polacco, Biochemistry Department, University of Missouri, Columbia, MO 65211

**1130-1145** (20)
CLONING OF SOYBEAN SEED COAT DIHYDROFLAVONOL REDUCTASE (DFR) GENE BY POLYMERASE CHAIN REACTION

Chang-sheng Wang and Lila Vodkin, Department of Agronomy, University of Illinois, 1201 W. Gregory Dr. Urbana, IL 61801, USA

**1145-1330 Lunch**

**Tuesday Afternoon (July 28, 1992) 1330-1630**

**1330-1445 Presiding:** Eve Wurtele, Iowa State University

**1330-1345** (21)
ISOLATION OF A LATE EMBRYOGENIC ABUNDANT GENE (Lea) IN SOYBEAN (*GLYCINE MAX* L.)

Calvo, E.S., Wurtele, E.S., Shoemaker, R.S., Iowa State University and USDA-ARS-FCR, Ames, Iowa, 50011

**1345-1400** (22)
DEVELOPMENTAL AND TISSUE SPECIFIC REGULATION OF SOYBEAN CHOLINEPHOSPHOTRANSFERASE

N. Cook, T. Cheesbrough, Biology/Microbiology Department, South Dakota State University, Brookings, SD

**1400-1415** (23)
BIOCHEMICAL CHARACTERIZATION OF A SOYBEAN WITH HIGH STEARIC ACID SEED OIL

Terence Hui and Basil J. Nikolau, Dept. Biochemistry & Biophysics, Iowa State University, Ames, IA 50011

**1415-1430** (24)
MOLECULAR CLONING AND CHARACTERIZATION OF 3-METHYLCROTONYL-CoA CARBOXYLASE FROM SOYBEAN
Jianping Song and Basil J. Nikolau, Dept. Biochemistry & Biophysics, Iowa State University, Ames, IA 50011

**1430-1445** (25)
METABOLIC ORIGINS OF UREA IN SOYBEAN
M.A. Holland and J.C. Polacco, Biochemistry Department, University of Missouri, Columbia, MO 65211

**1445-1515 Break**

**1515-1630 Presiding:** Sally Mackenzie, Purdue University

**1515-1530** (26)
*Eu4* IS THE ONLY FUNCTIONAL GENE FOR THE UBIQUITOUS UREASE IN SOYBEAN
R.S. Torisky and J.C. Polacco, Department of Biochemistry, University of Missouri, Columbia, MO 65211

**1530-1545** (27)
PRESENCE OF PROANTHOCYANIDIN IN SOYBEAN SEED COAT
Joselyn Todd and Lila Vodkin, Department of Agronomy, University of Illinois, Urbana, IL 61801

**1545-1600** (28)
QUANTITATIVE AND QUALITATIVE VARIATION OF CELL WALL PROTEINS IN SOYBEAN LINES WITH ANTHOCYANIN MUTATIONS
Christopher D. Nicholas, Jon T. Lindstrom, J. Scott Schmidt, and Lila O. Vodkin, Department of Agronomy, University of Illinois, Urbana, Illinois 61801

**1600-1615** (29)
HERITABILITY OF GENETIC MARKERS IN SOYBEAN GENERATED BY SHORT TANDEM REPEAT LENGTH POLYMORPHISM
P.B. Cregan, M.S. Akkaya, and A.A. Bhagwat, United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, Maryland 20705-2350 USA

**1615-1630** (30)
USE OF A REPEATED SEQUENCE IN MAPPING THE SOYBEAN GENOME
K.G. Lark, K. Chase and T. Malcalma, Department of Biology, University of Utah, Salt Lake City, UT 84112

**Wednesday Morning (July 29, 1992) 0830-1200**

**0830-1200 Presiding:** Randy C. Shoemaker, USDA-ARS-FCR, Iowa State University

**0830-0845** (31)
PARALLEL RFLP MAPPING IN SOYBEAN (*GLYCINE MAX*), MUNGBEAN (*VIGNA RADIATA*), AND COWPEA (*V. UNGUICULATA*)

N. Young*, D. Menancio-Hautea*, C. Fatokun*, L. Kumar*, D. Danesh*, and R. Shoemaker**. *Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota. **USDA/ARS, Department of Agronomy, Iowa State University, Ames, Iowa.

**0845-0900** (32)
RESTRICTION ENZYME COMPARISON OF HOMEOLOGOUS REGIONS WITHIN AND BETWEEN G. MAX AND G. SOJA

K.M. Polzin and R.C. Shoemaker, USDA/ARS Field Crops Research Unit, Agronomy Hall, Iowa State University, Ames, IA 50011

**0900-0915** (33)
SATURATING THE SOYBEAN MOLECULAR GENETIC MAP

A. Nickell, T. Olson, L. Lorenzen, Iowa State University, S. Lonning, K. Polzin, and R. Shoemaker, USDA-ARS-FCR, Iowa State University

**0915-0930** (34)
POSITIONING CONVENTIONAL GENETIC MARKERS ON THE SOYBEAN RFLP MAP - A PROGRESS REPORT

J.E. Specht and R.C. Shoemaker, Univ. of Nebraska - Lincoln, and Iowa State Univ.

**0930-0945** (35)
COMPUTER GENERATED GRAPHICAL GENOTYPES AND DNA MARKER PEDIGREES

S. Boutin*, L. Lorenzen**, R. Shoemaker**, and N. Young*, *Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota; **Department of Agronomy, Iowa State University, Ames, Iowa

**0945-1000**
ROLE OF LAWRENCE BERKELEY LABORATORY IN PLANT GENOME DATABASE EFFORTS

J. McCarthy, Genome Computing Group, Lawrence Berkeley Laboratory, Berkeley, CA 94704

**1000-1030 Break**

**1030-1200**
GENOME DATABASE REPORTS AND DISCUSSION

**ADJOURN**

(36)
ANALYSIS OF A GENOMIC DNA LIBRARY OF SOYBEAN (*G. MAX*)
D.D. Meling and T.C. Olson, Department of Agronomy, Iowa State University, Ames, IA 50011

(37)
ANTISENSE ANALYSIS OF NODULIN GENE FUNCTION
S. Li, R. Kosslak, R. Honeycutt, J. Imsande, and A.G. Atherly, Zoology and Genetics Department, Iowa State University, Ames, IA 50010

(38)
A SIMPLE SYSTEM FOR THE PRODUCTION OF TRANSGENIC SOYBEAN CALLI
G. Luo, A.G. Hepburn, and J.M. Widholm, University of Illinois, 1201 West Gregory, Urbana, Illinois 61801

(39)
MALATE DEHYDROGENASE NULL MUTANTS IN SOYBEAN
Reid G. Palmer and Christine Gietl; USDA ARS FCR, Iowa State University and Technical University of Munich, Germany

(40)
KINETICS OF NODULE INITIATION IN *GLYCINE SOJA*
Q. Jiang, D.L. Eskew, G. Caetano-Anollés and P.M. Gresshoff, Plant Molecular Genetics, Institute of Agriculture and Center for Legume Research, The University of Tennessee, Knoxville, TN 37901-1071, USA

(41)
USING RESTRICTION FRAGMENT LENGTH POLYMORPHISMS TO DESCRIBE ELITE SOYBEAN VARIETIES
D.M. Webb[1], H.J. Jessen[1], J.M. Schupp[2], and P. Keim[2], Pioneer Hi-Bred Int.[1], P.O. Box 1004, Johnston, IA 50131, and Dept. of Biological Sciences[2], Northern Arizona Univ., Flagstaff, AZ 86011

(42)
ISOLATION OF MONOSOMIC ALIEN ADDITION LINES IN SOYBEAN
R.J. Singh, K.P. Kollipara, and T. Hymowitz, Department of Agronomy, University of Illinois, Urbana, IL 6801, U.S.A.

(43)
A TENTATIVE RELATIONSHIP BETWEEN GENETIC & PHYSICAL DISTANCE IN THE SOYBEAN GENOME
X. Duan, R. Shoemaker[1] and A.G. Atherly, Department of Zoology and Genetics. [1]USDA/ARS, Iowa State University, Ames, USA

(44)
LENGTH POLYMORPHISM OF SHORT TANDEM REPEAT DNA SEQUENCES IN SOYBEAN

M.S. Akkaya and P.B. Cregan, United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, Maryland 20705-2350 USA

(45)
CHARACTERIZATION OF A cDNA CLONE FOR SOYBEAN CHOLINEPHOSPHOTRANSFERASE

M. Meyer, T. Wang, T. Cheesbrough, Biology/Microbiology Department, South Dakota State University, Brookings, SD

(46)
PATTERNS OF $^{14}$C-PHOTOSYNTHATE MOVEMENT DURING EARLY OVULE AND EMBRYO DEVELOPMENT IN SOYBEAN

M.A. Chamberlin and H.T. Horner, Bessey Microscopy Facility and Department of Botany, and R.G. Palmer, Departments of Genetics and Agronomy, Iowa State University, Ames, Iowa 50011

(47)
RFLP ANALYSIS OF NEAR ISOGENIC LINES DIFFERING AT THE *Rps1* LOCUS

M.K. Bhattacharyya, M.L. Kraft and R.A. Dixon, Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402 and R.I. Buzzell, Agriculture Canada, Research Station, Harrow, Ontario Canada NOR 1G0

(48)
AGRONOMIC CHARACTERISTICS THAT IDENTIFY HIGH YIELD, HIGH PROTEIN SOYBEAN GENOTYPES

J. Imsande, Department of Agronomy, Iowa State University, Ames, IA 50011

# GENOTYPE EFFECTS ON REPETITIVE EMBRYOGENESIS AND PLANT REGENERATION OF SOYBEAN

M.A. Bailey and W.A. Parrott, Department of Agronomy, University of Georgia, Athens, GA 30602-7272

Repetitive somatic embryogenesis is a regeneration system suitable for mass propagation and genetic transformation of soybean. The objective of this study was to examine genotypic effects on induction and maintenance of embryogenic cultures, and on yield and conversion of resulting somatic embryos. Somatic embryos were induced from nine genotypes (cvs. Bragg, Centennial, Century, Davis, Hutcheson, Lee, Peking, Williams, and PI 417138) by explanting 100 immature cotyledons per genotype on MSD40 medium (MS salts, B5 vitamins, 40 mg/l 2,4-D, and pH 7.0). Six genotypes selected for further study (Century, Davis, Lee, Hutcheson, Peking, and PI 417138) were used to initiate embryogenic liquid cultures in 10A40N medium. These cultures were evaluated for growth rate, yield of cotyledon-stage somatic embryos, and subsequent plant recovery.

Significant differences in frequency of induction were observed among genotypes. However, this step was not limiting for plant regeneration, since induction frequency in the least responding genotype was sufficient to initiate liquid embryogenic cultures. Genotypes also differed in growth rate of liquid cultures, embryo yield and plant recovery. Growth rate was not a reliable indicator of embryo yield. Rigorous selection for a repetitive culture phenotype consisting of nodular, compact, green spheres increased embryo yield relative to that of unselected cultures, but did not affect the relative ranking of genotypes. We conclude that the genotypes used in this study differ at each stage of plant regeneration from repetitively embryogenic cultures, but that genotypic effects can be partially overcome by protocol modifications.

# INTRODUCTION OF A *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* (*Btk*) TOXIN GENE INTO SOYBEAN

W.A. Parrott[1], M.A. Bailey[1], M.J. Adang[2], H.R. Boerma[1], and J.N. All[2].
Department of Agronomy[1] and Department of Entomology[2], University of Georgia, Athens, GA 30602-7272

Lepidopteran insects are one of the major pests of soybeans grown in the Southeastern United States. Soybeans transgenic for a *Btk* gene were obtained from embryogenic cultures of F376, a genotype specifically developed and selected for its ability to grow well in liquid culture and provide a high recovery rate of plants from somatic embryos. Somatic embryos were induced on Murashige & Skoog medium supplemented with B5 vitamins and 40 mg/l of 2,4-D, and used to establish embryogenic cultures in liquid medium with 5 mg/l 2,4-D. These embryogenic cultures were subsequently subjected to microprojectile bombardment with a Biolistic PDS 1000 device, followed by selection on 50 mg/l of hygromycin. Resistant embryogenic cell lines were selected, and subsequently transferred to growth regulator-free medium to permit recovery of mature somatic embryos. Following a desiccation period, the somatic embryos were returned to growth regulator-free medium for their conversion into plants. Standard Southern analysis was used to verify transformation, and gene expression was quantified with ELISA. The gene construct consisted of a 1.8 kb region of a *Btk* gene toxin region driven by the CaMV 35S promoter with an alfalfa mosaic virus leader sequence. Expression of the transgene in different $R_0$ plants ranged from undetectable to 1 ng/mg protein. These levels are comparable to the level of expression of this construct in tobacco plants. Bioassays using neonate larvae of velvetbean caterpillar have been conducted on $R_1$ plants to determine if expression levels were sufficiently high to deter feeding.

EFFECT OF PETUNIA TRANSFORMATION BOOSTER SEQUENCE

Buising, C. M.[1], Benbow, R. M. [1,2], [1] Department of Zoology and Genetics, [2] Nucleic Acid Research Facility, Iowa State University, Ames, Iowa 50011-3223

The effects of petunia *t*ransformation *b*ooster *s*equence (TBS) on the expression and persistence of supercoiled plasmid constructs in dicotyledonous (tobacco and soybean) and monocotyledonous (maize) plant cells were compared. Expression vectors containing bacterial genes coding for ß-glucuronidase and neomycin phosphotransferase, or phosphinothricin acetyltransferase, were introduced by microprojectile bombardment into leaves and cotyledons of *Nicotiana tabacum* cv. Xanthi, embryonic axes and somatic embryogenic cultures of *Glycine max* (L.) Merr., or into suspension cultures of *Zea mays* cv. Black Mexican Sweet (BMS). Petunia TBS increased the transformation frequency 7.8- to 16-fold (Wilcoxon matched-pairs signed-ranks test) in tobacco, and 1.7- to 2.4-fold in maize. Although TBS contains a well defined transcription enhancer element, no significant differences were observed on the enzyme levels of ß-glucuronidase and neomycin phosphotransferase in transgenic tobacco or soybean, or of ß-glucuronidase and phosphinothricin acetyltransferase in transgenic BMS cells. TBS did not appear to alter the integration patterns of exogenous DNA in either tobacco or maize transformants and, in particular, no effect was observed in the complexity of the integration patterns. In tobacco, transformants usually contained at least one copy of the introduced gene in a non-rearranged form, and often contained multiple copies of the gene in rearranged forms as well. In maize, however, most transformants contained only rearranged copies. In tobacco, TBS had no significant effect on the segregation of the exogenous genes in $R_1$ progeny, or on the linkage of two genes introduced in the same construct. Computer analysis of the TBS sequence identified that it contains major and minor DNA unwinding elements, scaffold attachment regions, ARS-like consensus sequences, pyrimidine tracts, and topoisomerase II binding site elements which may suggest a role for common modular elements in transformation.

IMPROVING THE QUALITY OF SEED PROTEINS IN SOYBEAN

J.A. Townsend, L.A. Thomas, E. S. Kulisek, M.J. Daywalt, K.R.K. Winter and S.B. Altenbach, Pioneer Hi-Bred, Inc., Johnston, IA.

Transgenic soybean plants have been produced by cocultivation with Agrobacterium tumefaciens. A set of twenty independent transformants of a commercial variety, 9341, have been characterized by expression analysis of two chimeric genes which were introduced into the soybean chromosome. They were the B-glucuronidase (gus) gene from Escherichia coli and the methionine-rich seed storage protein (bex) gene from Brazil nut, Bertholletia excelsa. The genes were introduced on a common T-DNA in binary plasmid pPHI1816. In histochemical analyses the $T^0$ plants expressed the gus gene in one of three patterns. Three of the twenty (0.15) had the enzyme localized in the epidermal of L1 cell layer. Five of the twenty (0.15) had the enzyme localized in the epidermal or L1 cell layer. In twelve of the twenty plants (0.60) gus was present in both L1 and L2 tissues. All plants transformed in the L2 layer transmitted the new traits to progeny. None of the L1 transformants did. Thus 17 of 20 plants (0.85) were germline transformants. Bex protein has been detected and quantified in seed derived from these transgenics. A direct ELISA assay with purified polyclonal antibody was used. Protein extracted from meal of $T^2$ seed is as much as 20% Bex. The increase in methionine content of soybean meal and its potential as an animal feed ingredient is discussed.

# NEW PRIMARY TRISOMICS OF SOYBEAN IDENTIFIED BY PACHYTENE CHROMOSOME ANALYSIS

F. Ahmad, R.J. Singh and T. Hymowitz, Department of Agronomy, University of Illinois, Urbana, IL 61801, U.S.A.

Primary simple trisomics ($2n = 2x + 1$) are useful for locating genes on the chromosomes and for associating linkage groups with the specific chromosomes. The objective of this study was to identify new primary simple trisomics (Triplos) of soybean [*Glycine max* (L.) Merr.] by pachytene chromosome analysis. The cytological identification was based on association of the extra chromosome in a trivalent configuration, chromosome length, arm ratio and distribution of euchromatin and heterochromatin. Thus, primary simple trisomics representing chromosomes 2, 3, 8, 10, and 14 were identified for the first time in soybean.

The extra chromosome present in Triplo 2 was shown to be different from the one present in Triplo 4 and Triplo 5 by crosses between them and subsequent cytological observation of chromosome pairing in the $2n = 42$ chromosome $F_1$ plants. Likewise, Triplo 5 was proven to be different from Triplo 3, and Triplo 8 to be different from Triplo 1 and Triplo 5.

Together, with the four previously identified primary trisomics, nine of the possible 20 primary simple trisomics of soybean have been successfully identified cytologically. These cover 55.7% of the total nuclear genome and 61.8% of the total nuclear euchromatin of soybean. These trisomics will provide a basis for cytogenetic mapping of the soybean genome by assigning genetic and molecular markers to specific chromosomes.

GENOMES OF THE GENUS *GLYCINE* WILLD.

T. Hymowitz and R.J. Singh, Department of Agronomy, University of Illinois, Urbana, IL 61801, U.S.A.

The genus *Glycine* Willd. is divided into two subgenera *Glycine* and *Soja* (Moench) F.J. Herm. The subgenus *Soja* is composed of *G. max*, the cultivated soybean ($2n=40$) and its wild annual counterpart *G. soja* ($2n=40$). Both species hybridize rather easily, generate viable fertile hybrids and differ only by a reciprocal translocation or by a paracentric inversion. Pachytene analysis revealed that both species carry similar genome designated by the symbol GG.

The subgenus *Glycine* currently contains 15 wild perennial species. Crosses among diploid ($2n=40$) A (*G. argyrea, G. canescens, G. clandestina,* and *G. latrobeana*) genome and diploid ($2n=40$) B (*G. latifolia, G. microphylla*, and *G. tabacina*) genome species show almost complete genome homology and are fertile. Crosses between genomically unlike species show frequent pod abortion, hybrid inviability and total sterility. The diploid ($2n=40$) C genome species are *G. curvata* and *G. cyrtoloba. Glycine falcata* ($2n=40$) carries the F genome designation.

*Glycine tomentella* ($2n=38$) carries the EE genome designation while the cytotype $2n=40$ currently carries the genomic designation DD. *Glycine tomentella* ($2n=78$) is an allopolyploid species complex carrying D and E, A and E, or perhaps other genomic combinations. *Glycine tomentella*, $2n=80$, is an allopolyploid species complex carrying A and D genome species. *Glycine tabacina* ($2n=80$) is a species complex consisting of two groups. One group of allopolyploids carry both A and B genome species. The second group consists of segmental allopolyploids carrying two B genome species. The genomic relationships of *G. albicans, G. arenaria, G. hirticaulis,* and *G. lactovirens* have yet to be determined.

## TRYPSIN AND CHYMOTRYPSIN INHIBITORS IN THE WILD PERENNIAL *GYLCINE* SPECIES

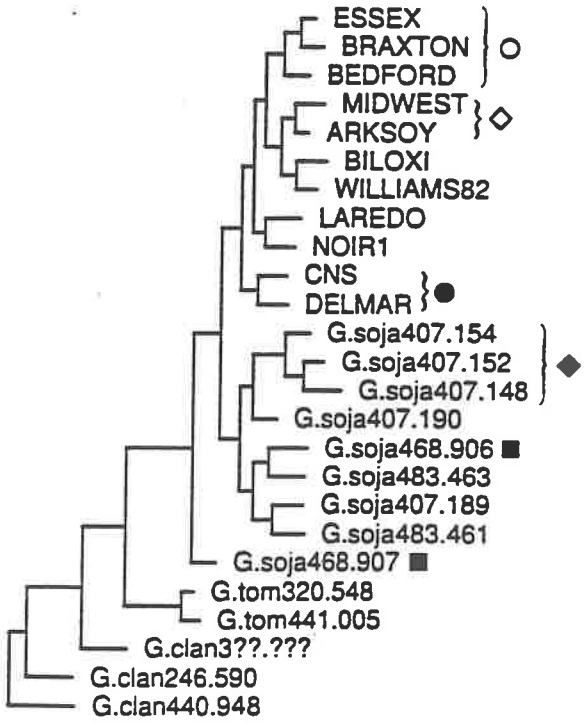
K.P. Kollipara and T. Hymowitz, Department of Agronomy, University of Illinois, Urbana, IL 61801, U.S.A.

A total of 568 accessions from 12 wild perennial species of the genus *Glycine* was screened for the presence or absence of immunocross-reactive proteins to a monoclonal antibody against the major soybean Bowman-Birk inhibitor (BBI) using competitive enzyme linked immunosorbent assay. Wild perennial *Glycine* species with A (*G. arenaria, G. argyrea, G. canescens,* and *G. clandestina*), E (*G. tomentella*, $2n = 38$), and F (*G. falcata*) genomes contained proteins that were crossreactive to the monoclonal antibody. By contrast, the B (*G. microphylla, G. latifolia,* and *G. tabacina*, $2n = 40$) and C (*G. cyrtoloba* and *G. curvata*) genome species did not contain the monoclonal antibody crossreactive proteins (BBI-nulls). The D genome *G. tomentella* ($2n = 40$) had both BBI-positive and BBI-null accessions. Accessions in the polyploid species *G. tabacina* ($2n = 80$) and *G. tomentella* ($2n = 78$ and 80) that have A or E genomes as part of their genome composition were BBI-positive. The presence or absence of mAB 238 crossreactive proteins can be used as a marker in the genome analysis of *Glycine* species. All the wild perennial *Glycine* species contained trypsin and chymotrypsin inhibitors. There were highly significant variations among the wild perennial species in the electrophoretic profiles of trypsin and chymotrypsin inhibitors, migration patterns of anti-soybean Kunitz trypsin inhibitor and anti-BBI immunocrossreactive proteins, and trypsin and chymotrypsin inhibitor activities of seeds. These variations were greater in A genome species than those in B or C genome species. Most of the protease inhibitors found in the wild perennial species had both trypsin and chymotrypsin inhibitor activities. All the wild perennial species also contained DNA sequences that crosshybridized to the soybean BBI cDNA and produced transcripts that were of same size as but less abundant than those of soybean. The pattern of developmental expression of these transcripts in *G. clandestina* was same as that found in the soybean seed.

# RAPD MARKER ANALYSIS OF GLYCINE TAXA

K.G. Lark and J. Evans, Department of Biology, University of Utah, Salt Lake City, UT 84112

An undergraduate laboratory class ( Basha, Bogden, Copeland, Ellison, Horne, Lee, McDonald, Pierson, Schuster, Wilhelm and Yu) analyzed 11 *G.max*, 9 *G. soja*, 2 *G. tomentella* and 2 *G. clandestina* using RAPD markers. In all, almost 600 characters were analyzed of which about 500 were informative. The data were analyzed using a parsimony algorithm developed by Swofford (PAUP). Phylograms were produced for different numbers of characters and different groups of plants. The results were biologically compatible with known genetic origins, physiological data and geographical distribution.




Stability of the phylograms was analyzed in terms of the number of characters used. Pairing of accsessions was determined as a function of other components of the phylogenetic tree. Although phylogeny studies usually avoid instances of genetic introgression in developing an evolutionary tree, our results suggest that this type of analysis may be used with cultivars derived from breeding programs to genetically analyze portions of the soybean genome. Two parameters which measure different aspects of the phylogram have been used to assess results: the **consistancy index** and the **patristic distance**. The information derived from these parameters will be discussed.

# CYTOPLASMIC BASIS OF SELECTION IN AN OUTCROSSING SOYBEAN (G. MAX) POPULATION

D.J. Lee, C.A. Caha, G.L. Graef and J.E. Specht, University of Nebraska, Lincoln, NE

Restriction fragment length polymorphisms (RFLPs) in chloroplast and mitochondrial DNAs were used to assess cytoplasmic variation in a population of soybeans subjected to continuous cycles of forced outcrossing. Six groups based on cytoplasmic RFLPs were identified among the original 39 female parents of the population. Changes is cytotype frequencies were monitored among randomly sampled individuals from the population. This analysis revealed that after seven cycles of outcrossing, only two of the six parental cytoplasmic types remained in the population. In addition to these cytoplasmic RFLPs, changes in the frequencies of nuclear alleles were monitored in the population. Reproductive success in this outcrossing mating system may be related to the plants' cytoplasmic genotype or specific cytonuclear interactions.

## CYTOPLASMIC DIVERSITY IN THE GENUS *GLYCINE* USING MITOCHONDRIAL RFLPS.

E.A. Grabau, A. Pesce, J. Li, M. Peters, and W.H. Davis, Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0331, USA.

Soybean cultivars and plant introductions have been examined for cytoplasmic diversity using mitochondrial restriction fragment length polymorphisms (RFLPs) to classify them into groups. Earlier studies identified four cytoplasmic groups within *Glycine max* (1). This classification was based on Southern hybridization patterns using a 2.3 kb HindIII cloned fragment as a probe. This clone was obtained from 'Williams 82' and is specific to cultivars whose maternal ancestors are derived from 'Lincoln' (2). Other mitochondrial probes showed a very limited degree of diversity in *Glycine max*. We have now extended those studies to include over 300 *Glycine soja* plant introductions as well as old domestic varieties of soybean from the USDA soybean germplasm collection. The results to date suggest that cultivated soybean (*Glycine max*) and its wild annual counterpart (*Glycine soja*) are not easily distinguishable at the cytoplasmic level since most fall into the same groups described earlier. We have examined the origins of the 300+ plant introductions and present a summary of their distribution with respect to cytoplasmic group. We have also initiated the sequencing of the 2.3 kb HindIII fragment in order to examine a possible role and origin of this specific region in the Lincoln cytoplasm.

References:

(1) E.A. Grabau, W.H. Davis, N.D. Phelps and B.G. Gengenbach (1992). Crop Sci. *32*: 271-274.

(2) E.A. Grabau, W.H. Davis and B.G. Gengenbach (1989). Crop Sci. *29*: 1554-1559.

# SELECTION OF HETERODERA GLYCINES POPULATIONS ON RESISTANT SOYBEAN GENOTYPES

R.L. Ruff and G.L. Tylka, Department of Plant Pathology, Iowa State University, Ames, Iowa.

Genes for parasitism in soybean cyst nematode (SCN), Heterodera glycines Ichinohe, have not been identified. Races of SCN are heterogeneous field populations consisting of individuals possessing unknown types and numbers of alleles for the parasitism of soybeans. SCN races are characterized by their ability to parasitize four SCN-resistant soybean genotypes relative to a susceptible soybean genotype. Specific SCN isolates were selected on SCN-resistant soybean genotypes to stabilize alleles for parasitism of the resistant soybean genotypes, and thus reduce the genetic heterogeneity of the nematode population. Initially, natural SCN field populations were collected and found to contain few to moderate numbers of individuals capable of parasitizing the resistant soybean genotypes Peking, Pickett, and PI 88788. Single adult SCN females were collected from the roots of these resistant genotypes, the females were crushed individually, and the eggs from individual females were used to inoculate a plant of the same genotype as the plant from which the cysts and eggs were recovered. The inoculated plants were grown for approximately 60 days at 25-27 C, after which eggs were collected from cysts recovered from the roots. This cycle of selection was repeated several times, maintaining the SCN isolates on the same soybean genotype from which they were recovered. After many selection cycles, the homogeneity of the SCN isolates for parasitism of soybean genotypes was evaluated by inoculating standard susceptible and SCN race differential soybean genotypes. An SCN isolate selected on Pickett was moderately stable and homogeneous for parasitism of Pickett and no other SCN-resistant soybean genotype after eight selection cycles. This isolate remained stable and homogeneous for this trait when tested after fourteen selection cycles. SCN isolates selected for several cycles on Peking and PI 88788 are currently being evaluated for stability of alleles for parasitism of SCN-resistant soybean genotypes and results will be presented. Investigations into the genetics and mechanisms of soybean resistance to SCN should be made more efficient by use of selected SCN populations such as the ones described above.

RFLP MAPPING OF SOYBEAN CYST NEMATODE RESISTANCE IN SOYBEAN.

K. Rorick and S. Mackenzie. Purdue University, West Lafayette, IN 47907

Two plant introduction lines, PI89008 and PI88287, together with an inbred nematode population, Hg1, were used in a crossing program to ascertain the inheritance of soybean cyst nematode (SCN) resistance in soybean (Glycine max.). Restriction fragment polymorphism analysis is being used to locate potential SCN resistance loci within the soybean genome. Because crop production problems associated with SCN infestation involve a host-parasite genetic interaction, the genetic diversity among nematode populations is being analyzed by RAPD analysis in combination with a denaturing gradient gel electrophoresis system.

MAPPING RESISTANCE TO HETERODERA GLYCINES I.

H.T. Skorupska, Department of Agronomy and Soils and Department of Biological Sciences, Clemson University, and A.P. Rao-Arelli, Department of Agronomy, University of Missouri-Columbia

Conventional genetic-breeding approach treats resistance to H. glycines as a complex traits and does not distinguish individual genes for resistance. We will discuss genic sources for resistance and their application in molecular mapping using gene deployment strategy.

# RFLP MAPPING OF CYST NEMATODE RESISTANCE GENES IN SOYBEAN (GLYCINE MAX)

V. Concibido*, S. Boutin*, R. Denny*, H. Ansari*, J. Orf**, and N. D. Young*, Department of Plant Pathology, ** Department of Plant Breeding and Genetics, University of Minnesota, St. Paul, MN 55108

The soybean cyst nematode (SCN), Heterodera glycines (Ichinohe), is one of the most destructive soybean diseases in Minnesota and throughout the midwest. To date, host plant resistance has been the most effective control measure and breeders have successfully incorporated resistance from various wild sources into soybean cultivars. However, resistance to SCN is polygenic and complex, screening germplasm is tedious, and SCN race determination is extremely difficult.

Tagging genes of interest with tightly linked restriction fragment length polymorphism (RFLP) markers offers several advantages as a breeding tool. Using RFLPs, it is possible to identify resistant lines rapidly by eliminating actual nematode tests, remove undesirable genes originating from the donor line, and combine different types of SCN resistance genes in a common soybean background.

Near isogenic lines provide an efficient and targetted approach to mapping genes of interest with RFLPs. We have analyzed a set of backcross inbred lines that are nearly isogenic for SCN resistance derived from P.I. 209332 using fifty RFLP markers. Five genomic regions seem to be related to resistance. Currently, we are analyzing 56 F2 lines from a cross between one of the backcross inbred lines and a susceptible soybean parent. Segregation of the putative resistance loci is being compared to SCN disease response using RFLP markers, potentially confirming our mapping of SCN resistance loci with near isogenic lines.

This research was supported by a grant from the Minnesota Soybean Research and Promotion Council. This abstract is published as a contribution of the series of the Minnesota Agricultural Experiment Station on research conducted under Project 015, supported by G.A.R. funds.

# A SIMPLE DNA MINIPREP PROCEDURE FOR PCR AMPLIFICATION ANALYSIS

G. Luo, A. G. Hepburn, and J. M. Widholm, University of Illinois, 1201 West Gregory, Urbana, Illinois 61801.

A simple and reliable DNA miniprep procedure has been developed to provide DNA for PCR amplification analysis. This procedure has been successfully used to identify transgenic materials and to detect polymorphism using random primer PCR (RAPD). A small amount of plant tissue was macerated in a small amount of extraction buffer for 3 minutes using a Mini-Beadbeater (Biospec Products). The sample was then boiled for 10 minutes followed by 5 minutes centrifugation (all of these steps can take place in a single microfuge tube). The supernatant was then collected and used directly for PCR amplification analysis. 10mg soybean leaf tissue or 50mg soybean callus tissue were sufficient for each miniprep and the crude extract from each miniprep was sufficient for at least 10 PCR amplification reactions. This procedure has also been successfully applied to tobacco leaf and callus tissue as well as corn leaf tissue. Although the optimal amount of tissue used varied between different kinds of tissues, once it is established, the results were very consistent. This procedure is particularly desirable in at least two situations: 1) when only a small amount of tissue is available; 2) when a large number of samples must be analyzed (total time for DNA preparation = time for collecting tissue samples + 3 minutes for grinding per sample + 10 minutes for boiling + 5 minutes for centrifugation). Furthermore, the fact that all the DNA extraction steps are contained in a single tube minimizes the chance of contamination and therefore it is especially ideal for PCR analysis.

GENETICS, BIOCHEMICAL, AND PYSIOLOGICAL CHARACTERIZATION OF SOYBEAN NECROTIC ROOT MUTANTS

R. M. Kosslak, J. Dieter, Iowa State University, Ames, IA 50011, R. G. Palmer, USDA-ARS, Ames, IA 50011, and B. A. Bowen, Pioneer Hi-Bred Intl., Johnston, IA 50131.

Three mutants (nr1 nr1, nr2 nr2, nr3 nr3) which cause necrosis in soybean roots under axenic conditions have been isolated. All three mutations are inherited as single-gene recessives and cause a progressive necrosis of the root system over time. Analysis of the isoflavonoid composition of 5-, 7-, and 14-day old roots from nr1 nr1 and Nr1 Nr1 suggest that enzymes which convert the isoflavones daidzein and genistein into more complex isoflavonoids (e.g. pterocarpans, pterocarpenes, coumestans, and arylcoumarins) are more active in nr1 nr1 roots than in Nr1 Nr1 roots. Changes in the composition of these complex isoflavonoids correlate well with the progression of necrosis in the mutant. Through the use of inhibitors and elicitors of $C_6$-$C_3$ phenylpropanoid and $C_{15}$ flavonoid biosynthetic genes we have found that the synthesis of these compounds is not merely incidental, but actually causes necrosis in the nr1 nr1 mutants. We are now investigating whether Nr1 is a specific repressor of isoflavonoid biosynthetic genes in wild-type roots, or if it regulates other stress-induced genes (e.g. chitinases, peroxidases, etc.) commonly associated with necrosis in soybean.

PLANT GENETIC STUDY OF RESTRICTED NODULATION OF SOYBEAN

D. Qian, F. Allen, G. Stacey, and P. M. Gresshoff Center for Legume Research, University of Tennessee, Knoxville, TN 37996

Two preliminary screenings of twenty three soybean accessions, including *Glycine soja* (PI468.397), indicated that TN4-86, BARC-2 (Rj4) B, andPI468.397 showed restricted nodulation with *Bradyrhizobium japonicum* strain 61A101C. Cultivar Essex gave effective nodulation. Average number of nodules per plant for TN4-86, BARC-2 (Rj4) B, PI468.397, and Essex were 0, 0.08, 0, and 10.5. The F1s of crosses of TN4-86 X Essex, BARC-2 (Rj4) B X Essex, and BARC-2 (Rj4) B X TN4-86 showed an inability to nodulate with strain 61A101C; whereas the F1s of Essex X PI468.397 showed effective nodulation response. Segragation ratio of effective nodulation to non-nodulation in F2 of Essex X PI468.397 showed no significant difference ($P \leq 0.05$) from 3:1 ratio. It appears that the nodulation response in PI468.397 is controlled by a single recessive gene. In contrast, non-nodulation response in TN4-86 appears to be controlled by dominant gene(s). Allelism of the gene(s) in TN4-86 to Rj4 will be tested by using the F2 segregates of BARC-2 (Rj4) B X TN4-86. The study of kinetics of nodule initiation and development on primary root in TN4-86, BARC-2 (Rj4) B, PI468.397, and Essex (positive and negative control) were carried out by modified fast clearing and staining method over a 14 d period after inoculation with strain 61A101C. There were no significant differences among the number of markedly curled root hairs (MC) on the roots of the four accessions 2 days after inoculation. The development of cortical cell division foci (CDF) beyond stage III was rare in TN4-86 and BARC-2 (Rj4) B, but a few stage IV developments were observed in PI468.397. PI469.397 may harbor a new gene governing non-nodulation distinct from BARC-2 (Rj4) B, but still expressed with inoculum of the 61A101C-type. The average number of nodules per plant 34 days after inoculation for TN4-86, BARC-2 (Rj4) B, PI468.397, and Essex were 0.08, 0, 0.06, and 6.8 respectively.

# DNA AMPLIFICATION ANALYSIS OF NODULATION REGIONS IN SOYBEAN (GLYCINE MAX (L) MERRILL)

P. M. Gresshoff, D. Landau-Ellis, A. Kolchinsky, A. Jones, R. Prabhu, B. Bassam, J. Deckert, and G. Caetano-Anollés. Plant Molecular Genetics, Center for Legume Research, The University of Tennessee, Knoxville, TN. 37901-1071, USA

DNA amplification methods based either on 'classical' polymerase chain reaction (PCR) or DNA amplification fingerprinting (DAF) (see Caetano-Anollés et al (1991) BioTechnology **9**: 553-557) were used to provide molecular information on the genetic and genomic arrangement of soybean nodulation control regions. Research focused on the region controlling the supernodulation (Nts) phenotype. We took advantage of the pUTG-132a probe, which is closely linked to the *nts* locus (see Landau-Ellis et al (1991) MGG **228**:221-226). Partial sequencing and restriction mapping allowed the characterization of the polymorphism between *G. max* cv. Bragg and *Glycine soja*. PCR primers within or outside the deletion were used to develop a diagnostic test for the polymorphism. Preliminary results suggest competition between amplification products in the heterozygote leading to apparent 'recessiveness' of the larger PCR product. The same primers were also valuable for the confirmation of genomic lambda clones homologous to probe pUTG-132a. Single primer amplification of genomic DNA from *G. max* and G. soja gave a number of amplification polymorphism. Genetic behavior of these was studied in F2 families. While several polymorphisms segregated in the expected Mendelian manner for a dominant allele, others gave results consistent with recessive segregation. Bands disappeared in F1 hybrids (both genetic and synthetic mixtures were tested), but reappeared in the F2. We propose that the length of an amplification product influences its efficiency for further amplification. If only one molecular species is present, lowered efficiency is concealed. In F1 hybrids and synthetic DNA mixtures efficiency differences are noticeable and appear as apparent 'recessives'. The significance of our finding needs to be tested on a broader scale, but may have utility in understanding segregation in other DAF and RAPD marker systems.

# EVIDENCE OF INTERACTIONS IN NITROGEN METABOLISM BETWEEN SOYBEAN AND A PHYLLOPLANE BACTERIUM

M.A. Holland, N.E. Stebbins, and J.C. Polacco, Biochemistry Department, University of Missouri, Columbia, MO 65211

Pink-pigmented, facultatively methylotrophic bacteria of the genus *Methylobacterium* are normally associated with seeds and leaves of soybean plants. We recently reported (Plant Physiol. 97:1004-1010) that the urease activity of these bacteria is a functional complement to that of the ubiquitous urease of the plant, mitigating the accumulation of urea in developing seeds and germinating seedlings. We further showed (Plant Physiol. 98:942-948) that a class of pleiotropic urease mutants in soybean affect the phenotype of the bacterium. These results show that the relationship between the bacterium and the plant is an intimate one. We are examining nitrogen metabolism as it relates to urease activities in the plant and the bacterium for evidence of interactions between the two. Data are presented which suggest that ureides, urea, and ammonia all are exchanged between the plant and the bacteria. In certain soybean urease mutants, ureides may actually be metabolized through a bacterial intermediate.

Cloning of Soybean Seed Coat Dihydroflavonol Reductase (DFR) Gene by Polymerase Chain Reaction

Chang-sheng Wang and Lila Vodkin, Department of Agronomy,
University of Illinois,1201 W. Gregory Dr. Urbana, IL 61801, USA

Dihydroflavonol-4-reductase(DFR) is necessary for the conversion of dihydroflavonols into anthocyanins. Two degenerate primers were designed based on the published DFR gene sequences of maize, snapdragon, and petunia in order to clone soybean DFR gene by polymerase chain reaction (PCR). A 200 bp genomic DNA fragment amplified at 37°C annealing temperature was cloned and sequenced. Similarites in the amino acid sequences of this 200 bp fragment to the sequences of snapdragon, petunia, and maize are 50%, 62% and 64%, respectively. Interestingly, this 200 bp fragment was very faint, or did not show up, when PCR reactions were performed at higher (45-55-60°C) annealing temperatures. However, a 980 bp DNA fragment, which was amplified at higher temperatures is not homologous to the 200 bp fragment by Southern hybridization assay. These results suggest that it is not necesssary to run the PCR reaction at a higher annealing temperature to obtain a specific fragment especially when degenerate primers are used in the PCR reactions. Some of the correct bands are lost when the annealing temperature is raised. This might be due to the complicated interactions between primers and the template DNA.

Four messages of 200, 210, 280 and 500 bp which are homologous to the 200 bp soybean DFR gene fragment were amplified at 45°C by one step RT-PCR reaction using poly-A RNA from seed coats of T157, an imperfect black cultivar. These data indicate that at least four DFR genes are transcribed in soybean seed coats. The four DFR clones will be used to study whether there are any differences in the expression and regulation of these transcripts among mutable isogenic lines of Clark during seed coat development (Chandlee and Vodkin, 1989. Theoret. Appl. Gen. 77: 587-594).

ISOLATION OF A LATE EMBRYOGENIC ABUNDANT GENE (Lea) IN SOYBEAN (*Glycine max* L.)

Calvo, E.S.; Wurtele, E.S; Shoemaker, R.S. Iowa State University and USDA-ARS-FCR, Ames, Iowa, 50011.

Embryogenesis and seed development are two unique aspects of plant biology that have been explored through molecular biology techniques in the past ten years. It has been shown that gene expression during embryogenesis is temporally modular, and genes representative of the different modules or programs have been isolated. We report here the isolation of a soybean late embryogenic cDNA using the carrot EMB-1 cDNA, a member of the post-abscision program, as a probe in the soybean seed cDNA library. The soybean cDNA, Sle, codes for a 112 amino acid, hydrophilic, glycine rich polypeptide. Sequence comparison with the EMB-1 protein and other homologs in the database reveals a high degree of conservation with an average of 70% amino acid identity and 85% similarity. However, SLE protein contains a 20 aminoacid highly hydrophilic motif that is present only once in its homologs but twice in soybean. Southern blot analysis suggests that the Sle gene is present in more than one copy in the soybean genome. Northern blot analyses show that SLE mRNA is seed-specific, and is found in comparable levels in both embryo axis and the cotyledons. Although present at earlier stages of seed development (10 DAF), SLE mRNA levels increases dramatically at later stages. SLE mRNA accumulation is also developmentally regulated in soybean somatic embryos. However, somatic embryos at the globular stages have levels of SLE mRNA similar to those found in the mature seed (45 DAF). Abscisic acid (ABA) increases SLE mRNA levels both in zygotic and somatic embryos. Neither ABA or desiccation induce Sle expression in 4 weeks old soybean plants. We are currently sequencing and analyzing genomic clones in order to gain insight into the mechanisms of regulation of Sle expression.

# DEVELOPMENTAL AND TISSUE SPECIFIC REGULATION OF SOYBEAN CHOLINEPHOSPHOTRANSFERASE

N. Cook, T. Cheesbrough, Biology/Microbiology Department, South Dakota State University, Brookings, SD

Phosphatidylcholine (PtdCho) is a major component of plant membranes; it is the most abundant phospholipid in subcellular membranes. PtdCho is synthesized by two pathways in most plant tissues (Moore, T., 1977, Plant Physiol. 60:754 ). The nucleotide pathway involves the formation of CDP-choline followed by the transfer to phosphorylcholine to diacylglycerol. The methylation pathway forms PtdCho by sequential methylation of phosphatidylethanolamine. Both of these pathways are present in most soybean tissues, though their relative levels change from tissue to tissue (Mudd, H., Dakto, A. 1990, Inform 1:289). Methylation may be the major pathway in vegetative tissues. In developing seeds, the nucleotide pathway is present at high levels while the methylation pathway is not detectable (Cheesbrough, T., 1989, Plant Physiol. 90: 760). Enzymes in the nucleotide pathway, particularly CDP-choline:1,2-diacylglycerol cholinephosphotransferase (CPT), IUB# 2.7.8.2, are also regulated by environmental (Cho,S., Cheesbrough,T., 1990, Plant Physiol., 93:72 ) and hormonal stimuli (Moore, T. et al. 1983, Phytochemistry 22:2421).

Previous enzymatic and *in vivo* labeling experiments have indicated that the level of CPT activity may vary from tissue to tissue (Mudd, H., Dakto, A. 1990, Inform 1:289). Current studies are focused on determining the level at which CPT activity is regulated. Northern hybridizations, using a soybean CPT cDNA probe, indicate that the corresponding mRNA is most abundant in early seed development, stage R4 (Fehr, W. 1971, Crop Sci. 11:929). Message for CPT is also present, though less abundant, in mid development seeds (early R6), 7 day old seedlings and expanding trifoliate leaves.

BIOCHEMICAL CHARACTERIZATION OF A SOYBEAN WITH HIGH STEARIC ACID SEED OIL.

Terence Hui and Basil J. Nikolau, Dept. Biochemistry & Biophysics, Iowa State University, Ames, IA 50011.

Soybeans varieties used in commercial crops produce an oil that has an approximate fatty acid content of 12% palmitic acid (16:0), 3% stearic acid (18:0), 22% oleic acid (18:1), 56% linoleic acid (18:2) and 8% linolenic acid (18:3). A number of single gene mutants of soybean have been described that show an altered fatty acid composition phenotype. One of these, A6, is the focus of this study. The seed oil of A6 shows an increase in 18:0 content (27%) and a concomitant decrease in 18:1 content. We have shown that the increase in stearic acid content in A6 is due to a decrease in stearoyl-ACP desaturase activity. We are currently investigating the biochemical basis of the decrease in stearoyl-ACP desaturase in A6.

MOLECULAR CLONING AND CHARACTERIZATION OF 3-METHYLCROTONYL-CoA CARBOXYLASE FROM SOYBEAN

Jianping Song and Basil J. Nikolau, Dept. Biochemistry & Biophysics, Iowa State University, Ames, IA 50011.

Biotin enzymes catalyze key reactions in metabolism. These enzymes have been extensively characterized from animal and microbial sources, but poorly characterized in plants. The biotin prosthetic group is covalently attached to proteins via the side chain of a lysine residue. The amino acid sequence around this unique lysine residue is highly conserved: Ala-Met-Lys-Met-Glu. We have used an oligonucleotide that codes for this amino acid sequence to isolate a soybean genomic clone putatively identified as coding for a biotin protein. The cDNA clone corresponding to this gene was expressed in *E. coli*, as a *TrpE* chimeric protein, which was used as antigen to generate antibodies. The antibodies to the *TrpE* chimeric protein react with a 88 kD biotin containing polypeptide. Furthermore, these antibodies inhibit 3-methylcrotonyl-CoA carboxylase activity. These data demonstrate that we have identified a new plant biotin protein, 3-methylcrotonyl-CoA carboxylase, a biotin enzyme ,previously unknown in the plant kingdom. In animals and bacteria this enzyme is required in leucine catabolism. We are undertaking research to identify the metabolic function of this enzyme in plants.

METABOLIC ORIGINS OF UREA IN SOYBEAN

M.A. Holland and J.C. Polacco, Biochemistry Department,
University of Missouri, Columbia, MO 65211

Urease-negative mutant soybean seeds (*eu3-e1/eu3-e1*) do not accumulate urea when reared on urease-positive (*Eu3/eu3-e1*) plants. However, upon germination there is considerable urea accumulation. We employed non-toxic allopurinol and $\beta$-guanidinoproprionate as inhibitors of ureide synthesis and arginase, respectively. While neither by itself completely blocked accumulation of urea, together they reduced urea to levels observed in wild type seeds without inhibitors. Thus seedling urea appears to have both a ureide and arginine source. It is unlikely, however, that all ureide nitrogen is metabolized via a urea intermediate since $N_2$-fixing *eu3-e1/eu3-e1* plants, and *eu3-e1/eu3-e1* cultures provided a ureide (allantoin) nitrogen source, grew at nearly normal rates. In more mature plants a ureide source for urea was indicted by its increased accumulation in $N_2$-fixing *eu3-e1/eu3-e1* versus $KNO_3$-fed plants. Urease-positive callus accumulated little urea, regardless of the nitrogen source while *eu3-e1/eu3-e1* callus accumulated much more urea when utilizing either arginine or allantoin. A urea-generating allantoicase (allantoate amidinohydrolase), has not been isolated from plants, although it has been demonstrated in bacteria. In contrast, a non-urea generating degradative pathway, from ureides to $NH_4$, has been shown in soybean leaf discs. We are currently testing the idea that urea is generated from ureides in soybean by a commensal bacterium.

*Eu4* IS THE ONLY FUNCTIONAL GENE FOR THE UBIQUITOUS UREASE IN SOYBEAN

R.S. Torisky and J.C. Polacco, Department of Biochemistry, University of Missouri, Columbia, MO 65211

We reported previously that the ubiquitous urease isozyme, which is found in all soybean tissues so far examined (and is the only urease made in maternal tissues) is absent in leaves, roots, seedlings and developing seeds of the *eu4/eu4* genotype (Plant Physiol. 94:681, and R.S. Torisky, unpublished data). Also, we reported that *Eu4* is allelic with or tightly linked to an RFLP detected by a cloned urease gene. We are currently comparing sequence of PCR-derived urease cDNA from different soybean tissues with that of the putative ubiquitous urease gnomic clone, LC4, used to detect the RFLP. In addition, we are attempting to correct the *eu4* lesion in tissue culture by transforming *eu4/eu4* soybean leaf tissue with LC4. We hope with these experiments to confirm (1) that the genomic clone LC4 encodes the ubiquitous urease, and (2) that *Eu4* is the sole ubiquitous urease structural gene.

PRESENCE OF PROANTHOCYANIDIN IN SOYBEAN SEED COAT

Joselyn Todd and Lila Vodkin, Department of Agronomy, University of Illinois, Urbana, IL 61801.

The dominant *I* gene inhibits accumulation of anthocyanin pigments in the epidermal layer of soybean seed coats. Seed coat color is also influenced by the pubescence color alleles (*T*, tawny and *t*, grey). Protein and RNA from cultivars with black (*i,T*) and brown (*i,T*) seed coat are difficult to extract. In order to determine the nature of the interfering plant products, we examined Clark isogenic line seed coat extracts for flavonoids, anthocyanins, and possible proanthocyanidins by thin layer chromatography. In addition to TLC, presence of proanthocyanidin was tested by butanol-HCl assay and 0.5% vanillin assay. Proanthocyanidin binding ability was assessed by an in vitro RNA binding assay and ability to precipitate proteins was determined by radial diffusion assay. Yellow seed coat varieties (*I*) do not accumulate identifiable flavonols, anthocyanins, or proanthocyanins. All colored (*i*) genotypes tested positive for the presence of proanthocyanidins by butanol/HCl and 0.5% vanillin assays. Immature black and brown seed coats (*i,T*) contained significant amounts of procyanidin, a dihydroxylated form of proanthocyanidin. Immature black or brown seed coat extracts (*i,T*) tested positive for the ability to precipitate proteins and bind to RNA. Imperfect black or buff seed coats (*i,t*) contained a pigment which has been punitively identified as propelargonidin, a monohydroxylated proanthocyanidin; seed coat extracts from these genotypes did not have the ability to precipitate protein or bind to RNA. The anthocyandins, cyanidin and delphinidin, were identified in mature black and imperfect black seed coat. Mature brown (*i,T*) and buff (*i,t*) seed coats did not contain anthocyanidins. Black and brown seed coats contained the flavonol quercetin. In summary, seed coats from black or brown (*i,T*) genotypes contain procyanidin which has the ability to precipitate proteins and bind to nucleic acids. Imperfect black or buff seed coats do not contain proanthocyanidins which have these abilities. Thus, the *I* gene not only controls inhibition of anthocyanidins but also proanthocyanidins in soybean seed coat. The *T-t* gene pair determines the types of proanthocyandins present.

# QUANTITATIVE AND QUALITATIVE VARIATION OF CELL WALL PROTEINS IN SOYBEAN LINES WITH ANTHOCYANIN MUTATIONS

Christopher D. Nicholas, Jon T. Lindstrom, J. Scott Schmidt, and
Lila O. Vodkin, Department of Agronomy, University of Illinois,
Urbana, Illinois 61801

The *I* locus controls inhibition of anthocyanin accumulation in the epidermal cells of the soybean seed coat and affects abundance of a seed coat cell wall protein, PRP1. PRP1 is abundant in the developing seed coats of Richland (homozygous *I*, yellow) while it is significantly decreased in the pigmented isogenic mutant T157 (homozygous *i*, imperfect black) (Lindstrom and Vodkin, Plant Cell 3: 561-571,1991). In this project, we examined PRP1 in several cultivars containing alleles of the *I* locus which affect spatial distribution of pigmentation on the seed coat. We also characterized PRP1 in isolines with allelic variants of several other loci involved in seed coat pigmentation, including *T* and *Im*. The *T* gene is pleiotropic and affects both pubescence color and seed coat pigmentation and structure. PRP1 was abundant in the developing seed coats of lines with yellow seed (*I* or $i^{i}$ alleles) regardless of pubescence color, just as in Richland. Likewise, PRP1 was decreased in pigmented seed coats ($i^{k}$ or *i* alleles) with grey (*t*) pubescence, as in T157. However, the seed coat proteins were not extractable from pigmented seed coats with tawny pubescence (*i, T* genotypes) because they have proanthocyanidins that exhibit tannin properties. The dominant *Im* allele inhibits seed coat mottling (irregular patches of pigmentation) that occurs if plants are infected with soybean mosaic virus. PRP1 was 35 kilodaltons in mottled (*im*) isolines and 34 kilodaltons in non-mottled (*Im*) isolines. PRP2, which is expressed later in seed coat development and in the hypocotyl hooks of soybean seedlings, was also smaller in *Im* isolines. In summary, some of the anthocyanin mutations affect the quantity of PRP1, while others correlate with structural changes in developmentally regulated proline rich proteins. We are currently investigating whether PRP1 and PRP2 are linked.

HERITABILITY OF GENETIC MARKERS IN SOYBEAN GENERATED BY SHORT TANDEM REPEAT LENGTH POLYMORPHISM

P.B. Cregan, M.S. Akkaya, and A.A. Bhagwat, United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, Maryland 20705-2350 USA

We have recently determined that Short Tandem Repeat (STR) DNA sequences in soybean show high levels of length polymorphism similar to that demonstrated in humans. Because of their apparent abundance, this type of genetic marker may provide a useful supplement to RFLP and RAPD markers in linkage map development. The objective of the work reported here was to determine the heritability of the STR length polymorphisms we have identified in the GenBank sequences SOYHSP176$(AT)_n$, SOYSC514$(AT)_n$, and SOYPRP1$(ATT)_n$. All possible $F_1$ hybrids between the soybean cultivars Amsoy, Williams, Fiskeby V, Tokyo, and Jackson were produced. The $F_1$'s always produced two PCR products in those cases in which the parents were polymorphic. Co-dominant segregation of STR markers at the SOYSC514$(AT)_n$ and SOYPRP1$(ATT)_n$ loci among 98 $F_2$ soybean progeny was demonstrated in a cross of Jackson x Williams soybean. Co-dominant segregation at all three loci was demonstrated in a cross of Williams x Amsoy. In a search for additional STR sequences, a library of Williams soybean DNA was screened for the presence of $(CA/GT)_n$ sequences with n greater than 15. Such sequences, which are very common and have proved an excellent source of STR markers in the human genome, do not appear to be present in soybean. However, $(AT/TA)_n$ and $(ATT/TAA)_n$ STRs will probably provide an abundant source of genetic markers in soybean.

# USE OF A REPEATED SEQUENCE IN MAPPING THE SOYBEAN GENOME

K.G.Lark, K.Chase and T. Malcalma, Department of Biology, University of Utah, Salt Lake City, UT 84112

A genetic map of soybean has been prepared from an interspecific cross of the cultivars Minsoy (P.I. 27.890) and Noir 1 ( P.I. 290.136). The map includes 137 markers in 30 linkage groups and defines 1650 cM of the genome. Another 22 polymorphic markers have remained unlinked. Bcl I was particularly useful in defining RFLP fragments which expanded the map.

A repeated sequence identified by a single probe defined a family of RFLP markers which expanded the map, linking other markers and defining regions of the map for which other markers were not available. The structure of this sequence has been determined and the genetic stability of the loci which it defines is being investigated. The distribution of Bcl I polymorphisms and of the loci defined by the repeated sequence suggests that different types of polymorphisms exist and that these are not uniformly distributed over the genome.

# PARALLEL RFLP MAPPING IN SOYBEAN (*GLYCINE MAX*), MUNGBEAN (*VIGNA RADIATA*), AND COWPEA (*V. UNGUICULATA*)

N. Young*, D. Menancio-Hautea*, C. Fatokun*, L. Kumar*, D. Danesh*, and R. Shoemaker**. *Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota. **USDA/ARS, Department of Agronomy, Iowa State University, Ames, Iowa.

Soybean (*G. max*) and mungbean (*V. radiata*) are both members of the subtribe Phaseolinae in the family, Leguminosae, and both are thought to have their centers of origin in eastern Asia. To estimate the level of chromosome conservation between these species, 80 RFLP clones originally derived from soybean have been mapped in both species. Many of these same RFLP clones have also been mapped in cowpea (*V. unguiculata*), a relative of mungbean with its center of origin in Africa.

More than 90% of the soybean RFLP clones showed clear and prominent signals when probed onto mungbean or cowpea DNA at medium stringency. This indicates that there has been significant sequence conservation between the *Glycine* and *Vigna* genera. As evidence of this, a phylogenetic comparison of more than 240 restriction fragment bands between soybean, mungbean, and cowpea showed approximately 64% sequence conservation between soybean and the *Vigna* species, versus 71% sequence conservation between mungbean and cowpea.

In keeping with the hypothesis that soybean is an allodiploid derived from an ancient tetraploidization, ten soybean clones that map to multiple locations in soybean map to only a single locus in mungbean.

In contrast to the significant level of sequence conservation between *Glycine* and *Vigna*, RFLP marker linkage order was not at all conserved. Of the 15 linkage groups in mungbean (corresponding to 11 chromosomes) and 24 linkage groups in soybean (corresponding to 20 chromosomes), only four segments were found in which linkage group location and order of adjacent markers had been conserved. In two of these cases, only two adjacent markers coincided between mungbean and cowpea. This result differs significantly from the results of parallel mapping between mungbean and cowpea, where most linkage groups consist of linkage blocks that are identical or nearly identical between the two species.

This work was supported by a specific cooperative agreement from the U.S.D.A./A.R.S. Plant Genome Database program. This abstract is published as a contribution of the series of the Minnesota Agricultural Experiment Station on research conducted under Project 015, supported by G.A.R. funds.

# RESTRICTION ENZYME COMPARISON OF HOMEOLOGOUS REGIONS WITHIN AND BETWEEN G. MAX AND G. SOJA

K.M. Polzin and R.C. Shoemaker, USDA/ARS Field Crops Research Unit, Agronomy Hall, Iowa State University, Ames, IA 50011

The USDA/ARS soybean RFLP probe pA71 detects at least nine *Taq*I fragments in Williams. An EMBL3 genomic library of Williams was screened with pA71 (1.4 kb). Twenty-four clones containing seven of the nine *Taq*I fragments were recovered. Restriction maps were prepared for six of the seven clones (14-18 kb) containing different pA71-hybridizing *Taq*I fragments and the region of the clones hybridizing to pA71 was determined by Southern hybridization. Alignment of the restriction maps to each other and to a restriction map of pA71 showed that while several restriction sites present in the probe also were present in each of the clones few restriction sites outside of the hybridizing region were conserved between different clones. Thus, regions suggested to be homeologous by hybridization to the same probe fragment may show significant divergence within less than 5 kb from the homeologous region. However, digestion of genomic DNA from the *Glycine max* and *Glycine soja* parents used to construct the USDA/ARS RFLP map with twelve different enzymes and probing with pA71 indicated that only three enzymes produced a fragment polymorphic between these two cultivars. Thus, although different homeologous regions within a cultivar may have diverged significantly at the restriction enzyme level, the restriction enzyme sites within a particular homeologous region appear to be highly conserved between cultivars.

SATURATING THE SOYBEAN MOLECULAR GENETIC MAP

A. Nickell, T. Olson, L. Lorenzen, Iowa State University, S. Lonning, K. Polzin, and R. Shoemaker, USDA-ARS-FCR, Iowa State University.

The ISU/USDA-ARS soybean molecular genetic map contains 24 linkage groups and covers approximately 3000 cM. The map is composed of RFLP, RAPD, cDNA, isozyme, and conventional markers. We are currently identifying RFLP probes that detect polymorphisms among elite soybean genotypes. These probes, if they present an easily distinguishable polymorphism, are being used to identify a set of 'core' probes and a 'core' map. We are also collecting segregation data from probe/enzyme combinations not previously mapped. The data is being collected from 12 different populations that include interspecific and elite breeding lines. This database will allow us to map and define a large number of loci over a broad range of germplasm. The resulting genetic mapping information is more detailed and universally applicable than that provided by a single population.

## POSITIONING CONVENTIONAL GENETIC MARKERS ON THE SOYBEAN RFLP MAP - A PROGRESS REPORT.

J.E. Specht and R.C. Shoemaker, Univ. of Nebraska - Lincoln, and Iowa State Univ.

A primary objective of our cooperative soybean genome mapping effort is to locate all known conventional genetic markers on the ISU/ARS soybean RFLP map. To attain this goal, we, and the staff in our two labs, are using three convergent approaches.

In the first approach, now nearly complete, DNA was isolated from some 60+ near-isogenic lines (NILs) and recurrent parents (RPs). This DNA, after being cut with various restriction enzymes, was hybridized with the probes used for the ISU RFLP map. A RFLP difference between a given NIL and its RP is suggestive of linkage between the RFLP marker and the introgressed conventional gene. The net result of this NIL effort: a presumptive positioning of some 70+ conventional markers on the RFLP map.

In the second approach, currently underway, two unique $F_2$ populations were created from Clark x Harosoy matings where the parents were multiple-marker NILs. The 60 individuals in each $F_2$ population segregate for 20+ conventional and isozyme markers, with some of these markers representing ten of the known conventional linkage groups. $F_2$ individuals were grown in the 91-92 greenhouse and classified as to conventional and isozyme marker phenotype. Classification of the $F_2$-derived $F_3$ lines in the summer of 1992 will provide a 1:2:1 $F_2$ genotypic segregation. The parallel assay of RFLP marker segregation is underway, using DNA collected from the $F_2$ individuals. DNA will also be collected from each $F_2$-derived $F_3$ line and stored for future mapping uses. The net result of this $F_2$ mapping effort: some well-known conventional markers (and a least ten of the conventional linkage groups) will be definitely positioned on the RFLP map.

A third approach will be initiated this summer. It involves the creation of a 100-individual $F_2$ population, for each known conventional gene, by mating a corresponding genetic stock to a suitable tester parent. Assuming monogenic inheritance, 25 of the 100 $F_2$ individuals are expected to be homozygous for the conventional marker. Only these 25 individuals (or their descendants) would be assayed for molecular markers. Chi-square linkage analysis ($\alpha$=0.05) has the power to detect $\rho \leq 0.3$ linkages between a codominant RFLP marker and the conventional marker. The net result of this approach: an efficient means of eventually positioning nearly all of the conventional markers on a RFLP map.

COMPUTER GENERATED GRAPHICAL GENOTYPES AND DNA MARKER PEDIGREES

S. Boutin*, L. Lorenzen**, R. Shoemaker**, and N. Young*; *Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota; ** Department of Agronomy, Iowa State University, Ames, Iowa

Genomic analysis with DNA markers generates a vast amount of information. This information can be stored in various ways, such as spreadsheets and databases, but these formats can be difficult to utilize effectively. Data displayed graphically allows for the visualization of data and can potentially simplify data interpretation. Linkage maps and pedigrees derived from DNA marker studies can potentially be displayed in a color graphic format. Such graphical images can give a visual overview of the genetic relationships between cultivars.

We are designing software to convert DNA marker data into a color graphic format and give the user the ability to: 1) Draw a graphical genotype for a selected cultivar, 2) Draw a single linkage group for many selected cultivars, 3) Draw a DNA-marker-based pedigree leading to or from a cultivar.

The software allows cultivar comparison on a macro level by comparing whole genomes, as well as a more focused comparison, as in a single linkage group. In a graphical genotype, each portion of the genome is drawn to scale based on genetic distance between markers and the region around each marker is colorized according to the DNA genotype at that locus. For comparisons of single linkage groups for selected cultivars, the linkage group is drawn to scale and colorized based on DNA genotype. Finally, for DNA-marker-based pedigrees, a graphical image for each linkage group is displayed for a selected cultivar, its parents and grandparents.

Interesting genomic regions, such as those that may carry desirable genes, can be highlighted in the graphical genome representations. This should be helpful in breeding programs for selecting cultivars based on genotype and in monitoring the DNA genotypes of progeny individuals.

This work was supported by a specific cooperative agreement from the U.S.D.A./A.R.S. Plant Genome Database program. This abstract is published as a contribution of the series of the Minnesota Agricultural Experiment Station on research conducted under Project 015, supported by G.A.R. funds.

ANALYSIS OF A GENOMIC DNA LIBRARY OF SOYBEAN (G. MAX)

D.D. Meling and T.C. Olson, Department of Agronomy, Iowa State University, Ames, IA 50011

Soybean genomic DNA Pst I fragments that were 500-3000 base pairs (bp) in size had been used to construct the USDA/ARS soybean DNA library. Two hundred sixty of the fragments that had been placed on a molecular genetic RFLP map were amplified using polymerase chain reaction or plasmid isolation and their approximate sizes were determined. The distribution of fragment sizes was found to be skewed such that 900-1500 bp fragments were most frequent. Several fragments were smaller than 500 bp or larger than 3000 bp. There was not a significant difference in size among fragments that have been identified with single, duplicate, or triplicate polymorphic loci in the genome. Several of the fragments were found to have an internal Pst I restriction site.

## ANTISENSE ANALYSIS OF NODULIN GENE FUNCTION

S. Li, R. Kosslak, R. Honeycutt, J. Imsande, and A. G. Atherly, Zoology and Genetics Department, Iowa State University, Ames, IA 50010

The development of an effective nitrogen-fixing symbiosis requires the expression of specific genes present in both the host legume and its bacterial partner. Although, the function of several of the bacterial nodulation genes has been determined, little is known about the role which the plant genes (nodulins) play in the development of nitrogen-fixing root nodules. This is largely due to the lack of specific plant mutants for these genes. Our approach to understanding nodulin gene function is to use antisense genes to create nodulin-specific mutants. We have : (1) constructed an antisense gene vector for the introduction of antisense sequences into legume plants; (2) introduced the ENOD2 (nodulin-75) gene fused to the GUS gene into this vector; (3) developed an *Agrobacterium*mediated transformation procedure for the introduction of these antisense sequences into *Lotus corniculatus*; and (4) established nodulation conditions to evaluate the phenotype of transgenic *L. corniculatus*plants.

We have obtained antisense-containing transgenic plants for the nod-75 gene which show a yellowish phenotype during early stages of development. Southern hybridization has demonstrated the presence of multiple inserts of the antisense construct. To interpret this phenomenon, we have performed acetylene reduction assays on nodulated transgenic plants and determined the amount of nod-75 transcriptional product using northern hybridization. These data are then correlated with the phenotype of the plant.

# A SIMPLE SYSTEM FOR THE PRODUCTION OF TRANSGENIC SOYBEAN CALLI

G. Luo, A. G. Hepburn, and J. M. Widholm, University of Illinois, 1201 West Gregory, Urbana, Illinois 61801.

A system was established to study the expression and processing of engineered protein genes and their products in transgenic soybean callus tissue. A 35S promoter was placed in front of the engineered protein gene construct to ensure its expression in callus tissue. This construct was cloned into the T-region of a binary vector (a disarmed Ti plasmid). This binary vector was then introduced into virulent Agrobacterium strain A281. This Agrobacterium strain thus will produce two different T-strands: one from the wild type Ti plasmid pTiBo542 containing the hormone autotrophy genes and the other from the binary vector containing the engineered protein gene construct. Therefore, th
ere should be a high probability that the two different T-strands can be co-transferred into the same transformed cells. When this Agrobacterium strain was used to inoculate the cotyledons of two-day old germinating seeds, about 80% of the cotyledons produced tumors on B5 medium without hormones 10 days after inoculation, with an average of about three tumors per cotyledon. These calli were then propagated on a medium with growth regulators to stimulate growth and within 4 weeks after inoculation, enough transgenic materials could be obtained for the identification of the co-transformed calli by either PCR or GUS assay and for the analysis of the products from the engineered protein gene construct. GUS assay results indicate that about 16.7% of the tumors were indeed co-expressing the 35S-GUS construct.

MALATE DEHYDROGENASE NULL MUTANTS IN SOYBEAN

Reid G. Palmer and Christine Gietl; USDA ARS FCR, Iowa State University and Technical University of Munich, Germany

Five independent mutations that lacked two of three $NAD^+$-dependent mitochondrial malate dehydrogenase (EC1.1.1.37) enzymes were identified in soybean. All five mutations also were of yellow-green phenotype. These two mutant phenotypes, malate dehydrogenase null and yellow-green plant color cosegregated and were inherited as a single recessive allele. Complete linkage of the malate dehydrogenase null trait and yellow-green plant color trait suggested that the mutations were deletions. Three of the mutations were derived from the progeny of independent germinal revertants in a transposon-tagging study with Genetic Type T322 (w4-m), one mutation occurred in the cultivar Harosoy, and one mutation occurred in the Chinese cultivar Jilin 3 (PI 427099). DNA was isolated and purified from wild type and mutant leaves of six-week-old plants, restricted with EcoRI endonuclease and the fragments separated and analyzed by Southern blotting with cDNA clones encoding mitochondrial and glyoxysomal malate dehydrogenase from watermelon (Citrullus vulgaris Schrad.). In the three wild type isolates the mitochondrial probe hybridized to four EcoRI restriction fragments with sizes of 20, 6, 5.7, and 5.5 kb. In all five mutants the 5.5 kb restriction fragment was missing, but no novel fragment of different size appeared. This indicates that the mutations are similar deletions covering one of the two structural genes encoding mitochondrial malate dehydrogenase of soybean. It is attempted to clone the wild type 5.5 kb restriction fragment and identify this malate dehydrogenase gene. The glyoxysomal probe hybridizes specifically to EcoRI restriction fragments with sizes of 10, 7, and 3.8 kb which are present in all wild type and mutant DNA samples.

## KINETICS OF NODULE INITIATION IN *GLYCINE SOJA*

**Q. Jiang, D.L. Eskew, G. Caetano-Anollés and P.M. Gresshoff**

**Plant Molecular Genetics , Institute of Agriculture and Center for Legume Research, The University of Tennessee, Knoxville, TN37901-1071, USA.**

We have modified the fast clearing and staining method of Truchet et al (1989) for the cytological study of *G. soja* (PI468.397) roots. Markedly curled root hairs (HAC) and cortical cell division foci (CCD) stain dark blue while the cortex stains light blue. The examination of CCDs instead of nodules reduced nodulation assay times to 2-3 d. We studied the kinetics of nodule initiation and development along the entire length of the primary root of *G. soja* over a 15 d period following inoculation with *B. japonicum* USDA110. Development of CCDs beyond stage II was confined to 2 RDU of the root tip (RT) at the time of inoculation, thus feedback regulation (autoregulation) of nodule development in *G. soja* was normal. However, markedly curled root hairs and initiation of CCDs continued down the root. These younger CCDs were tightly controlled and seldom advanced beyond stage I. The number of HACs per 2 mm of root showed a peak up to 4 fold higher in the later portion of the root than in the initial susceptible zone when observed at 6 d post-inoculation. This 'peak' disappeared at 10 d, but was replaced by a new peak further down the root. In contrast to *G. max*, in *G. soja* very few CCDs were observed without associated HAC, that is, there very few pseudoinfections. Our results suggest there is a tight correlation between cortical cell division and root hair curling. The staining method was also used with supernodulating interspecific hybrids between *G. soja* and *G. max*, making it useful for studying plant processes which control nodule development in the genus *Glycine* and also for screening *Bradyrhizobium* mutants for their ability to induce CCDs.

# USING RESTRICTION FRAGMENT LENGTH POLYMORPHISMS TO DESCRIBE ELITE SOYBEAN VARIETIES

D.M. Webb[1], H.J. Jessen[1], J.M. Schupp[2], and P. Keim[2], Pioneer Hi-Bred Int.[1], P.O. Box 1004, Johnston, IA 50131, and Dept. of Biological Sciences[2], Northern Arizona Univ., Flagstaff, AZ 86011

DNA-fingerprinting using restriction-fragment-length polymorphisms (RFLPs) can be used to identify germplasm, estimate relative genetic distances among varieties, and track chromosomal regions by pedigree. We are searching for RFLP probe and restriction enzyme combinations that produce codominant, clearly separated, and relatively frequent polymorphisms among elite soybean varieties; and that represent approximately 90 well-distributed markers on a soybean linkage map. Using these polymorphism criteria, we evaluated 738 public and proprietary probes with five restriction enzymes against 16 elite soybean varieties. We are re-evaluating the best of these probe-enzyme combinations for their cumulative capacity to distinguish unique DNA-fingerprint profiles among several hundred experimental and commercial varieties. To minimize error in our descriptions and comparisons of RFLP profiles, we use stringent laboratory conditions, abundant molecular weight standards, camera-scanned data entry, and computer-assisted band recognition. A standardized DNA-fingerprinting system will enable us to describe the genetic relationships among present and future soybean varieties using one accumulating data base.

## ISOLATION OF MONOSOMIC ALIEN ADDITION LINES IN SOYBEAN

R.J. Singh, K.P. Kollipara, and T. Hymowitz, Department of Agronomy, University of Illinois, Urbana, IL 61801, U.S.A.

Wild perennial relatives of the soybean [*Glycine max* (L.) Merr.] have not been utilized to broaden the genetic base of the crop. This study provides information on production, morphology, cytology and breeding behavior of backcross ($BC_2$ to $BC_4$)-derived fertile plants from soybean ($2n=40$, genome GG) and *G. tomentella* Hayata ($2n=78$, DDEE). The main hurdle was to obtain $BC_1$ plants [(*G. max* cv. Altona, $2n=40$, genome GG) x (*G. tomentella*, PI 483218, $2n=78$, genome DDEE)] → $F_1$, $2n=59$, GDE → colchicine treatment (CT) → $2n=118$, GGDDEE x soybean cv. Clark 63 → $BC_1$, $2n=76$ (expected $2n=79$). Three sterile $BC_2$ plants ($2n=58$, $2n=56$, $2n=55$) were produced. Soybean cv. Clark 63 was always used in the back-crossing programs. The range of chromosome segregation in $BC_3$ plants were $2n=41$ to $2n=52$ and in $BC_4$ was $2n=40$ to 64. A plant with $2n=64$ (hypertriploid) in $BC_4$ originated after fertilization of an unreduced egg ($n=44$) by a normal haploid ($n=20$) male gamete. Morphologically, $BC_3$ and $BC_4$ plants were dissimilar but resembled closely to the soybean cv. Clark 63. We have isolated a series of aneuploid lines, particularly several morphologically distinct monosomic alien addition lines (MAALs) from $BC_3$ to $BC_6$ generations. These materials will facilitate the establishment of cytogenetic and molecular genetic linkage maps of the soybean, and will open up the path to introgress desired traits from wild perennial *Glycine* species to the cultivated soybean.

# A TENTATIVE RELATIONSHIP BETWEEN GENETIC & PHYSICAL DISTANCE IN THE SOYBEAN GENOME

X. Duan, R. Shoemaker[1] and A. G. Atherly, Department of Zoology and Genetics, [1] USDA/ARS, Iowa State University, Ames, USA

The relationship between genetic and physical distance in the soybean genome is of considerable interest for eventual marker-based cloning of genes. We are attempting to establish this relationship by utilizing pulse-field gel electrophoresis technology. By using successive hybridizations with closely linked genetically mapped probes we can physically link RFLP probes to the same restriction fragment.

In this procedure, intact soybean genomic DNA is obtained from embedded leaf protoplasts and digested in agarose gels with both rare cutting enzymes and frequent cutting enzymes. This is followed by separation of the DNA fragments using a contour-clamped homogeneous field (CHEF) electrophoresis apparatus. The size range of the resulting fragments is from 20 kilobases to 2 megabases, as estimated by internal lambda ladders or yeast chromosome molecular weight markers. Pairs of closely-linked RFLP markers whose genetic distances range from 0 to 2 cM are used to estimate the relationship between physical distance and genetic distance. Closely linked RFLP markers have been found on the same DNA fragment, thus revealing the minimum physical distance between these two markers. The RFLP marker probes that we are using are scattered amongst the different linkage groups. Preliminary results indicate that the relationship between physical and genetic distance is not constant throughout the genome. The minimum distance between two closely linked genetic markers, that we have observed, is about 150,000 bp. However, our estimation of genetic distance between two RFLP markers is limited since only 60 $F_2$ progeny were taken to measure genetic distance, thus our estimation of genetic distance has a margin of error of $\pm$ 1 cM.

LENGTH POLYMORPHISM OF SHORT TANDEM REPEAT DNA SEQUENCES IN SOYBEAN

M.S. Akkaya and P.B. Cregan, United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, Maryland 20705-2350 USA

The presence of length polymorphism of Short Tandem Repeat (STR) DNA sequences [also referred to as microsatellites and Simple Sequence Repeats (SSRs)] is demonstrated for the first time in plants. The multi-allelic length polymorphisms of STRs are a result of variation in the number (n) of repeat units in STR sequences such as $(CA)_n$, $(AT)_n$, and $(ATT)_n$. By a search of soybean DNA sequences in GenBank, $(AT)_{14}$, $(AT)_{15}$, and $(ATT)_{19}$ STR loci were identified. Polymerase Chain Reaction (PCR) primers to the conserved sequences flanking the STRs were synthesized. PCR amplification of these loci using DNA from a diverse group of 43 homozygous soybean genotypes in the presence of a radiolabeled nucleotide and subsequent electrophoresis of the PCR products was performed. Length polymorphism of the PCR products produced by the the 43 genotypes was demonstrated. From 6 to 8 alleles were detected at each locus. By sequencing two different alleles at each locus, the source of length variations was shown to be only due to changes in "n". The abundance (6 potentially polymorphic STRs in 200 kbp soybean sequence from GenBank database) and the multi-allelic nature of the STR loci suggest that may serve as a good source of genetic markers for establishing genetic linkage maps and for DNA fingerprinting in plants.

# CHARACTERIZATION OF A cDNA CLONE FOR SOYBEAN CHOLINEPHOSPHOTRANSFERASE

M. Meyer, T. Wang, T. Cheesbrough, Biology/Microbiology Department, South Dakota State University, Brookings, SD

CDP-choline:1,2-diacylglycerol cholinephosphotransferase (CPT), IUB# 2.7.8.2, catalyzes the transfer of phosphorylcholine to diacylglycerol making phosphatidylcholine. This is the final step in the nucleotide pathway for the synthesis of phosphatidylcholine (Cho,S., Cheesbrough,T., 1990, Plant Physiol., 93:72 ). In soybeans, oleoyl-phosphatidylcholine is the substrate for formation of linoleoyl- and linolenoyl-phosphatidylcholine by sequential desaturation. The reaction catalyzed by CPT appears to be freely reversible in developing oil seeds, such as those of soybean (Slack, C. et al., 1985, Biochem. Biophys. Acta, 833: 435). Reversal of the CPT reaction generates polyunsaturated diacylglycerides used in the formation of triglycerides. Therefore, CPT is part of three pathways in developing soybean seeds: 1) synthesis of phosphatidylcholine, 2) synthesis of polyunsaturated fatty acids, 3) synthesis of polyunsaturated triglycerides.

A cDNA clone for CPT was obtained by PCR amplification of inserts from a soybean seed cDNA library. The library was generated in λ-ZAP using RNA isolated from developing seeds, stage R4.5 (Fehr, W. 1971, Crop. Sci. 11:929). Primers were synthesized based on the sequence of the CPT gene from *Saccharomyces cerevisiae* (Hjelmstad, R. and Bell, R., 1990, J. Biol. Chem. 265:1755). Amplified CPT cDNA was cloned into a pCR1000 vector (Invitrogen). The cDNA clone (pSCPT1) has an apparent size of 1.5 Kb.

## PATTERNS OF $^{14}C$-PHOTOSYNTHATE MOVEMENT DURING EARLY OVULE AND EMBRYO DEVELOPMENT IN SOYBEAN

M.A. Chamberlin and H.T. Horner, Bessey Microscopy Facility and Department of Botany, and R.G. Palmer, Departments of Genetics and Agronomy, Iowa State University, Ames, Iowa 50011

In the first 21 days post-fertilization (zygote to heart-shaped embryo stages), the ovule and embryo undergo rapid growth and development. During this growth period nutritional requirements of all these tissues are high. Utilizing $^{14}CO_2$, via photosynthesis and autoradiographic techniques, we show that the flow of labelled carbon into the ovular tissues and embryo of soybean is temporally and spatially regulated. From the zygote through globular stages of embryo development, labelled carbon accumulates in a bipolar manner in the integumentary tissue opposite the micropylar and chalazal poles of the embryo sac. As the zygote and endosperm subsequently divide, labelled carbon accumulates within the young embryo and cellular endosperm. At the heart-shaped embryo stage the greatest accumulation of label still is at the chalazal and micropylar ends of the embryo sac, but it is no longer exclusive to these poles. $^{14}C$ is now abundant along the lateral interface between the embryo sac and integuments. Cellularization of the endosperm in this region acts as a common apoplast channeling labelled carbon from the adjacent integumentary tissue into the embryo sac. Autoradiographic evidence for carbon flow into the embryo sac can be directly correlated with ultrastructural and morphological changes in the ovular and endosperm tissues enclosing the embryo.

RFLP ANALYSIS OF NEAR ISOGENIC LINES DIFFERING AT THE *Rps*1 LOCUS

M.K. Bhattacharyya, M.L. Kraft and R.A. Dixon, Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402 and R.I. Buzzell, Agriculture Canada, Research Station, Harrow, Ontario Canada NOR 1GO.

Phytophthora root and stem rot of soybean is caused by *Phytophthora megasperma* f.sp. *glycinea*. Growing of resistant cultivars is considered to be one of the best methods of controlling this disease. To date a total of thirteen genes in seven different loci are reported to confer cultivar specific resistance of soybean to twenty-five physiological races of *Phytophthora megasperma* f.sp. *glycinea*. Of these seven loci, the *Rps1* locus carries five different alleles viz. *Rps1-a*, *Rps1-b*, *Rps1-c*, *Rps1-d* and *Rps1-k*. A series of near isogenic lines differing at the *Rps1* locus is presently available. Recently, the *Rps1* locus has been mapped to the soybean RFLP map (Diers *et al.*, 1992; Crop Sci. **32**: 377-383). As a first step to study this locus through chromosomal walking experiments, we are evaluating 14, 8 and 11 pairs of near isogenic lines, differing at the *Rps1-a*, *Rps1-c* and *Rps1-k* alleles respectively, for the flanking regions around the *Rps1* locus using the RFLP markers that were identified for the *Rps1-k* allele. Analysis of these lines using an RFLP marker (pA280) that mapped 6 cM from the *Rps1* locus (Diers *et al.*, 1992) indicated that the recurrent parents used to develop these near isogenic lines can be grouped into four classes based on their polymorphism patterns for this marker. Among the 33 pairs of near isogenic lines only 13 pairs showed polymorphism for pA280 indicating that no recombination between the *Rps1* locus and pA280 marker occurred in the development of these isolines. Of these 13 lines, 8 carry the *Rps1-a* allele, 2 carry *Rps1-c* and 3 carry *Rps1-k*. Recombination between the *Rps1* locus and pA280 marker was, however, recorded in the development of 3 near isogenic lines, two of which carry *Rps1-a* and one of which carries *Rps1-k* .

# AGRONOMIC CHARACTERISTICS THAT IDENTIFY HIGH YIELD, HIGH PROTEIN SOYBEAN GENOTYPES

Jon Imsande, Department of Agronomy, Iowa State University, Ames, IA.

Seed yield and seed protein content are both heritable traits; however, breeding trials frequently reveal a negative correlation between the two traits. The objectives of this study were to measure and calculate various N-dependent growth characteristics of soybean [Glycine max (L.) Merr.] and to determine their relation to seed yield and seed protein. Well-nodulated plants, each fixing approximately 180 mg N during pod fill, were grown hydroponically in a growth chamber with or without nitrate-N during pod fill. Twenty growth-yield characteristics, including yield, harvest index, N harvest index, and Kjeldahl analysis of $N_2$ fixation were measured or calculated for each of the 384 plants examined. The highest seed yields, approximately 10 g plant$^{-1}$, and approximately 560 mg plant$^{-1}$, were obtained when well-nodulated plants were provided some fertilizer-N during pod fill. Except for N content (%) of the dried plant, correlations between each pair of the 20 N-dependent growth-yield characteristics were generally positive. In the absence of fertilizer-N during pod fill, however, N content (%) of the seeds did not correlate with either harvest index or N harvest index, suggesting that insufficient N during pod fill interferes with the orderly mobilization of foliar-N to the developing seeds. New physiological parameters (seed yield merit, N yield merit, and merit of genotype) are proposed to the identification of genetic lines that produce both a high seed yield and high seed protein content.