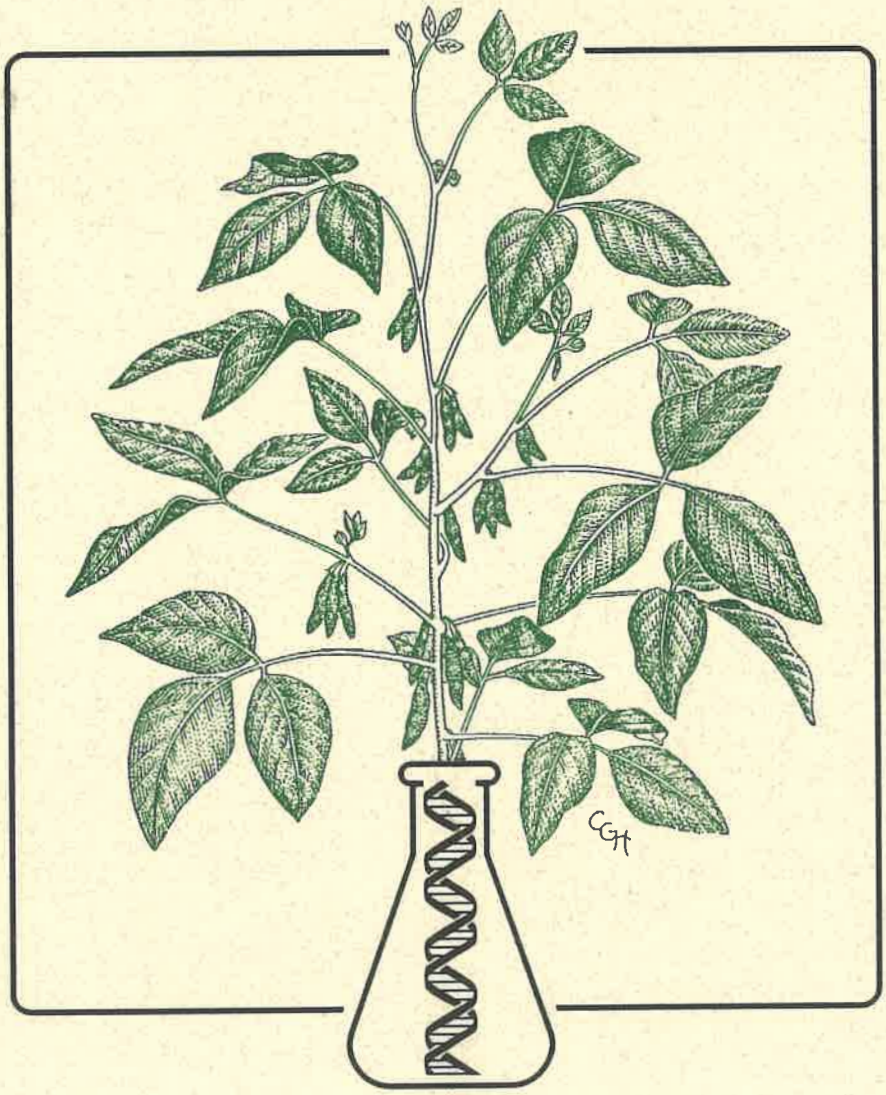
5th BIENNIAL CONFERENCE PROCEEDINGS

Molecular & Cellular Biology of the Soybean



July 25—27, 1994
Georgia Center for Continuing Education
Athens, Georgia

1785
The University of Georgia

GENERAL INFORMATION

Meeting Facilities

All conference activities will take place at the Georgia Center for Continuing Education. All contributed papers will be presented in Master's Hall. The barbecue and social on Monday evening will take place at the university's Flinchum's Phoenix, on the Oconee River. Buses will run regularly between the Georgia Center and the Phoenix for the duration of the barbecue. Tuesday's lunch and invited address will be at the Georgia Center's banquet facility.

Speaker Information

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

Poster Information

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

Acknowledgements

Organizing committee: Mike Adang, Matthew Bailey, Dick Hussey, Neal Stewart, Jr., Wayne Parrott, co-chairman, H. Roger Boerma, co-chairman

Conference coordinators: Margaret Caufield, Norma Reed

Logo design: Carol Hahn *Graphics:* Bill Reeves

Clerical support: Vivienne Sturgill *Computer systems*: Chris Tuttle

Financial support: UGA Office of the Vice President for Research
UGA Center for Soybean Improvement

ORAL PRESENTATIONS

Monday afternoon, July 25, 1994, 1:00-2:45

Genome mapping I, Presiding, H. Roger Boerma, The University of Georgia

1:00-1:05 Orientation, H. Roger Boerma

1:05-1:15 Welcome, Joe L. Key, Vice President for Research, The University of Georgia

1:15-1:30 *1** PAINTING SOYBEAN CHROMOSOMES BY FISH WITH HIGH-COPY DNA PROBES

L. Shi[1], T. Zhu[1], P. Keim[1], M. Morgan[2] and A. Rafalski[2], [1]Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011; [2]DuPont Co., Wilmington, DE 19880

1:30-1:45 *2* ORGANIZATION AND MAPPING OF SOYBEAN TELOMERES

A. Kolchinsky and P.M. Gresshoff, Plant Molecular Genetics and Center for Legume Research, The University of Tennessee, Knoxville, TN 37901

1:45-2:00 *3* QUANTITATIVE TRAIT EVALUATION AND IDENTIFICATION OF QUANTITATIVE TRAIT LOCI IN A RECOMBINANT INBRED SOYBEAN POPULATION

J.H. Orf[1], L.M. Mansur and K.G. Lark[2], [1]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108; [2]Department of Biology, University of Utah, Salt Lake City, UT 84112

2:00-2:15 *4* INTERACTIONS BETWEEN QUANTITATIVE TRAIT LOCI DETECTED IN RECOMBINANT INBRED LINES OF SOYBEAN

K.G. Lark[1] and L. Mansur[1], J. Orf[2], [1]Department of Biology, University of Utah, Salt Lake City, UT 84112; [2]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108

2:15-2:30 *5* ANALYSIS OF LINKAGE BETWEEN SUDDEN DEATH SYNDROME AND CYST NEMATODE RESISTANCE GENES BY MOLECULAR MARKERS IN SOYBEAN

S. Chang, N. Hnetkovsky, P. Gibson, M. Schmidt, and D. Lightfoot, Department of Plant and Soil Science, Molecular Science Program, Southern Illinois University, Carbondale, IL 62901

2:30-2:45 *6* GENE MAPPING IN SOYBEAN WITH PRIMARY TRISOMICS

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

Monday afternoon break, 2:45-3:30

**Numbers refer to the abstracts in this booklet*

Monday afternoon, 3:30-5:30

Genome mapping II, Presiding, Randy Shoemaker, Iowa State University

3:30-3:45 *7* MICROSATELLITE DNA MARKERS FOR GENOME MAPPING AND GENOME IDENTIFICATION

P.B. Cregan[1], J.E. Specht [2], R.C. Shoemaker[3], and A.A. Bhagwat [4], [1]Soybean and Alfalfa Research Lab, USDA-ARS, Beltsville, MD 20705; [2]Department of Agronomy, University of Nebraska, Lincoln, NE 68583; [3]Department of Agronomy, Iowa State University, and USDA-ARS, Ames, IA 50011; [4]Department of Agronomy, University of Maryland, College Park, MD 20742

3:45-4:00 *8* MOLECULAR GENETIC ANALYSIS OF SOYBEAN NODULATION GENES: GENOMIC COPIES AND POSITIONAL CLONING

P.M. Gresshoff, Plant Molecular Genetics and Center for Legume Research, The University of Tennessee, Knoxville, TN 37901

4:00-4:15 *9* RFLP AND RAPD MARKERS LINKED TO GENES AFFECTING SOYBEAN SUDDEN DEATH SYNDROME RESPONSE

N.K. Hnetkovsky, T.W. Doubler, P.T. Gibson, and D.A. Lightfoot, Department of Plant and Soil Science, Molecular Science Program, Southern Illinois University, Carbondale, IL 62901

4:15-4:30 *10* COSEGREGATION OF THE PURPLE-THROAT FLOWER COLOR WITH AN RFLP REPRESENTING DIHYDROFLAVONOL REDUCTASE (DFR) IN SOYBEAN

D.A. Fasoula, P.A. Stephens, C.D. Nickell, and L.O. Vodkin, Plant and Animal Biotechnology Laboratory, Department of Agronomy, University of Illinois, Urbana, IL 61801

4:30-5:00 *11* UPDATE OF SOYBASE, A SOYBEAN GENOME DATABASE

L.L. Lorenzen[1], M. Imsande[1], and R.C. Shoemaker[2], [1]Department of Agronomy, Iowa State University; Ames, IA, and [2]USDA-ARS, Iowa State University, Ames, IA 50011

5:00-5:30 Discussion session: THE FUTURE OF SOYBASE

Randy Shoemaker, Discussion leader

Monday evening, 6:00-10:00

Southern BBQ and Social at Flinchum's Phoenix

Busses will depart regularly between the Georgia Center and the Phoenix.

Tuesday morning, July 26, 8:15-9:45

Genome mapping III, Presiding, Paul Keim, Northern Arizona University

8:15-8:30 *12* SOYBEAN DUPLICATE GENOMES: STRUCTURE AND EVOLUTION
T. Zhu[1], L. Shi[1], J.J. Doyle[2], and P. Keim[1], [1]Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011; [2]L.H. Bailey Hortorium, Cornell University., Ithaca, NY 14853

8:30-8:45 *13* GENOME CONSERVATION AMONG LEGUME GENERA DETECTED WITH DNA MARKERS
S. Boutin[1], T. Olson[2], Z.-H. Yu[3], N. Young[1], R. Shoemaker[2], and E. Vallejos[3], [1]Department of Plant Pathology, University of Minnesota, St. Paul, MN; [2]Department of Agronomy, Iowa State University, Ames, IA; [3]Horticultural Sciences Department, University of Florida, Gainesville, FL.

8:45-9:00 *14* POSITIONING SOYBEAN CLASSICAL MARKERS ON MOLECULAR MARKER MAPS: A CALL FOR A COLLABORATIVE EFFORT AMONG INTERESTED RESEARCHERS
J.E. Specht[1], R.C. Shoemaker[2], and P.B. Cregan[3], [1]University of Nebraska, Lincoln, NE 68583, [2]Department of Agronomy, Iowa State University and USDA-ARS, Ames, IA 50011; [3]USDA-ARS, BARC-West, Beltsville, MD 20705

9:00-9:15 *15* IDENTIFICATION OF CHROMOSOMAL REGIONS ASSOCIATED WITH QTL FOR SEED PROTEIN AND OIL
E.C. Brummer[1], G.L. Graef [2], J. Orf[3], J.R. Wilcox[4,6], A.D. Nickell[5], and R.C. Shoemaker[1,6], [1]Department of Agronomy, Iowa State University, Ames, IA 50011; [2]University of Nebraska; [3]University of Minnesota, [4]Purdue University; [5]Asgrow Seed Company; [6]USDA-ARS

9:15-9:30 *16* RESEARCH ON PRIMARY TRISOMICS IN SOYBEAN
S.J. Xu, R.J. Singh, and T. Hymowitz, Department of Agronomy, University of Illinois, Urbana, IL 61801

9:30-9:45 *17* GENETIC DIVERSITY FOR RFLP MARKERS IN NORTHERN SOYBEAN LINES
T.J. Kisha and B.W. Diers, Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48823

Tuesday morning break & poster session, 9:45-10:30

Poster Session I: Numbers 1P-12P

Tuesday morning, 10:30-11:45

Molecular biology, Presiding, Tony Kinney, DuPont

10:30-10:45 *18* ORGANIZATION AND EVOLUTION OF THE HISTONE H3 GENES IN SOYBEAN (*GLYCINE MAX*)
V. Kanazin and R.C. Shoemaker, Department of Agronomy, Iowa State University, and USDA-ARS, Ames, IA 50011

10:45-11:00 *19* UNDERSTANDING ORGAN-SPECIFIC GENE EXPRESSION IN SOYBEAN
V.P. Sundararaman, M.J. Cho, and L.O. Vodkin, Plant and Animal Biotechnology Laboratory, Department of Agronomy, University of Illinois, Urbana, IL 61801

11:00-11:15 *20* CYTOKININ RESPONSIVE GENES IN *PHASEOLUS* AND *GLYCINE*
K. McDaniel and D.A. Lightfoot, Department of Plant and Soil Science, Molecular Science Program, Southern Illinois University, Carbondale, IL 62901

11:15-11:30 *21* ANALYSIS OF NUCLEAR EFFECTED MITOCHONDRIAL REORGANIZATIONS IN SOYBEAN
C.A. Moeykens and R.C. Shoemaker, Department of Agronomy, Iowa State University, and USDA-ARS, Ames, IA 50011

11:30-11:45 *22* ISOLATION OF TENTATIVE CHOLINEPHOSPHOTRANSFERASE, OLEOYL DESATURASE AND LINOLEOYL DESATURASE CLONES FROM A SOYBEAN GENOMIC LIBRARY
J.E. Cheadle and T.M. Cheesbrough, Department of Biology/Microbiology, Northern Plains Biostress Laboratory, South Dakota State University, Brookings, SD 57007

Tuesday lunch and keynote address, 12:00-1:30 pm

Presiding, Wayne Parrott, The University of Georgia

12:00-1:00 Lunch, Banquet Room

1:00-1:30 *23* SAMUEL BOWEN'S INTRODUCTION OF THE SOYBEAN TO NORTH AMERICA: FLESHING OUT THE SKELETON
T. Hymowitz, member, Georgia Historical Society

Tuesday afternoon, 1:45-3:00

Biochemistry, physiology, & transformation, Presiding, Lila Vodkin, University of Illinois

1:45-2:00 *24* SOYBEAN PHOTOSYNTHETIC SUSPENSION CULTURE AS A MODEL FOR CELL WALL METABOLISM STUDIES

V.V. Lozovaya and J.M. Widholm, Department of Agronomy, Plant and Animal Biotechnology, University of Illinois, Urbana, IL 61801

2:00-2:15 *25* PROGRESS AND PROSPECTS FOR IMPROVEMENT OF SOYBEAN OIL QUALITY

D.F. Hildebrand, W. Liu, L. Peng, R. Torisky, S. Avdiushko, K. Croft, and G.B. Collins, Department of Agronomy, University of Kentucky, Lexington, KY 40546

2:15-2:30 *26* THE MANIPULATION OF FATTY ACID METABOLISM IN DEVELOPING SOYBEANS

A.J. Kinney, K. Stecca, B. Schweiger, K. Ripp, A.M. Campbell, S. Knowlton, W.D. Hitz, DuPont Experimental Station, Wilmington, DE 19880

2:30-2:45 *27* DEVELOPMENT AND REGENERATION OF SOYBEAN (CVS. FAYETTE, CHAPMAN AND RESNICK) SOMATIC EMBRYOS FROM EMBRYOGENIC SUSPENSION CULTURES

B.L. Norris, H. Ma, and J.J. Finer, Department of Agronomy, The Ohio State University, Wooster, OH 44691

2:45-3:00 *28* ANALYSIS OF TRANSGENIC SOYBEAN CONTAINING A SOYBEAN CYTOSOLIC GLUTAMINE SYNTHETASE PROMOTER FUSED TO THE GUS CODING SEQUENCE

T.S. Cheng[1], J.G. Streeter[1], D.-P. Verma[2], and J.J. Finer[1], [1]Department of Agronomy; [2]Department of Molecular Genetics; Ohio State Biotechnology Center, The Ohio State University, Wooster, OH 44691

Tuesday afternoon break & poster session, 3:00-3:45

Poster Session II: Numbers 13P-26P

Tuesday afernoon, 3:45-5:30

Transformation, Presiding, John Finer, The Ohio State University

3:45-4:00 29 TRANSFORMATION OF SOYBEAN WITH BEAN POD MOTTLE VIRUS COAT PROTEIN PRECURSOR GENE *VIA AGROBACTERIUM*

R. Di[1], G.B. Collins[2], and S.A. Ghabrial[1], [1]Department of Plant Pathology; [2]Department of Agronomy, Univ. of Kentucky, Lexington, KY 405046

4:00-4:15 *30* STABLE TRANSFORMATION OF SOYBEAN WITH MAMMALIAN DESATURASE VIA PARTICLE BOMBARDMENT AND SOMATIC EMBRYO CYCLING

R.S. Torisky, W. Liu, D.F. Hildebrand, K.P. McAllister, and G.B. Collins, Department of Agronomy, University of Kentucky, Lexington, KY 40546

4:15-4:30 *31* TRANSFORMATION OF 12 DIFFERENT PLASMIDS INTO SOYBEAN VIA PARTICLE BOMBARDMENT

M. Hadi and J.J. Finer, Department of Agronomy, The Ohio State University, Wooster, OH 44691

4:30-4:45 *32* CHARACTERIZATION OF SOYBEAN TRANSGENIC FOR SYNTHETIC *BACILLUS THURINGIENSIS CRY IA(c)*

C.N. Stewart, Jr.[1], M.J. Adang[2], J.N. All[2], H.R. Boerma[1], and W.A. Parrott[1], [1]Department of Crop and Soil Sciences, and [2]Department of Entomology, The University of Georgia, Athens, GA 30602

4:45-5:00 *33* PRODUCTION OF ELITE ROUNDUP® TOLERANT SOYBEANS USING *AGROBACTERIUM*-MEDIATED TRANSFORMATION AND GLYPHOSATE SELECTION

T.E. Clemente, D.C. Ward, R.J. Rozman, A.R. Howe, P.E. Hunter, P.A. Costello, M.L. Taylor, K.H. Kolacz, S.R. Padgette, and M.A. Hinchee, The Agricultural Group, Monsanto Company, St. Louis, MO 63198

5:00-5:15 *34* DEVELOPMENT OF SOYBEANS WITH A ROUNDUP READY™ GENE

X. Delannay and S.R. Padgette, Monsanto Co., St. Louis, MO 63198

5:15-5:30 *35* OPTIMIZATION OF A HELIUM OUTFLOW GUN (HOG) FOR PLANT TRANSFORMATION

C.N. Stewart, Jr.[1], H.D. Wilde[1], Z.V. Magbanua[1], R.C. Davis[2], and W.A. Parrott[1], [1]Department of Crop and Soil Sciences, and [2]Research Services, The University of Georgia, Athens, GA 30602

Wednesday morning, July 27, 1994, 8:15-9:45

Molecular aspects of plant pathology, Presiding, A.P. Rao-Arelli, University of Missouri

8:15-8:30 *36* HIGH RESOLUTION MAPPING AND RACE SPECIFICITY OF A PARTIAL RESISTANCE GENE FOR SOYBEAN CYST NEMATODE
V. Concibido[1], R. Denny[1], D. Danesh[1], J. Orf[2], and N.D. Young[1], [1]Department of Plant Pathology; [2]Department of Agronomy & Plant Genetics, University of Minnesota, St. Paul, MN 55108

8:30-8:45 *37* INTERRELATIONSHIP BETWEEN TWO ENDOGLUCANASES CLONED FROM THE PHYTOPHATHOGENIC FUNGUS *MACROPHOMINA PHASEOLINA*
R.W. Jones and H. Wang, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

8:45-9:00 *38* ANALYSIS OF SOYBEAN CYST NEMATODE SECRETIONS INVOLVED IN PARASITISM
E.L. Davis[1] and R.S. Hussey[2], [1]Plant Pathology Department, North Carolina State University, Raleigh, NC 27695-7616, and [2]Department of Plant Pathology, The University of Georgia, Athens, GA 30602

9:00-9:15 *39* RFLP MARKERS LINKED TO GENES AFFECTING RESISTANCE TO JAVANESE ROOT-KNOT NEMATODE IN SOYBEAN
J.P. Tamulonis[1], B.M. Luzzi[1], W.A. Parrott[1], R.S. Hussey[2], and H.R. Boerma[1], [1]Department of Crop and Soil Sciences and [2]Department of Plant Pathology, The University of Georgia, Athens, GA 30602

9:15-9:30 *40* IDENTIFICATION OF DNA AMPLIFICATION FINGERPRINTING MARKERS LINKED TO RESISTANCE TO FROG-EYE LEAF SPOT IN SOYBEAN
J. Qui, D.B. Weaver, and S. Tuzun, Auburn University and Alabama Agricultural Experiment Station, Auburn, AL 36849

9:30-9:45 *41* APPROACHES TOWARDS CLONING THE *RPS1* LOCUS OF SOYBEAN
M.K. Bhattacharyya, J. Shi, M. Kraft, R.I. Buzzell[1], and R.A. Dixon, Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK 73402; [1]Agriculture Canada, Harrow, ON Canada N0R 1G0

Wednesday morning break & poster session, 9:45-10:30

Poster Session III: Numbers 27P-36P

Wednesday morning, 10:30-12:00

Molecular aspects of genetic diversity, Presiding, Brian Diers, Michigan State University

10:30-10:45 *42* DIFFERENCES OF ALLELE FREQUENCIES AMONG SOYBEAN CULTIVARS
L.L. Lorenzen and R.C. Shoemaker, Department of Agronomy, Iowa State University, Ames, IA 50011 and USDA-ARS-FCR, Iowa State University, Ames, IA

10:45-11:00 *43* A NEW STATISTICAL APPROACH TO FINGERPRINT ANALYSIS: MULTIDIMENSIONAL SCALING AND NON HIERARCHICAL CLUSTERING
T.E. Carter, Jr. and Z. Gizlice, Department of Crop Science, North Carolina State University, and USDA-ARS, Raleigh, NC 27695-7361

11:00-11:15 *44* RFLP AND COEFFICIENT OF PARENTAGE ANALYSES OF DIVERSITY IN ELITE AND PLANT INTRODUCTION SOYBEAN GENOTYPES
C. Sneller, J. Miles[1], L. Kilgore-Norquest[1], B. Diers[2] and T. Kiesha[2], [1]Department of Agronomy, University of Arkansas, Fayetteville, AR 72701; [2]Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824

11:15-11:45 *45* THE APPLICATION OF GENETIC DIAGNOSTICS TO SOYBEAN BREEDING
S.V. Tingey[1], J.A. Rafalski[1], and M. Morgante, [1]DuPont Ag Biotechnology, P.O. Box 80402, Wilmington, DE 19880; [2]Dipartimento di Produzione Vegetale e Tecnologie Agrarie, Universtia'di Udine, Via delle Scienze 208, I-33100 Udine, Italy

11:45-12:00 *Adjournment & Plans for the 6th Biennial Conference*

POSTER PRESENTATIONS

POSTERS WILL BE SET UP PRIOR TO THE FIRST SESSION AND BE AVAILABLE FOR VIEWING DURING THE ENTIRE MEETING.

Poster session I. *Presenters for posters 1P-12P will be by their posters Tuesday, 9:45-10:30*

*1P*** THE PLANT GENOME DATA AND INFORMATION CENTER
S. McCarthy, A. Kalinski, and S. Ranck, Plant Genome Data and Information Center, National Agricultural Library, USDA/ARS, Beltsville, MD 20705

2P COMPARISON OF RAPD PRODUCTS ON AGAROSE AND DENATURING GRADIENT GELS IN SOYBEAN
T.W. Doubler, and D.A. Lightfoot, Department of Plant and Soil Science, Molecular Science Program, Southern Illinois University, Carbondale, IL 62901

3P ANALYSIS OF LINKED AFLP MARKERS GENERATED BY tec-MAAP FOR GENE MAPPING OF THE SUPERNODULATION *nts* LOCUS
J.E. Padilla[1], G. Caetano-Anollés[2], and P.M. Gresshoff[2], [1]Universidad Nacional Autónoma de México, Cuernavaca 62271, México

4P MOLECULAR MAPPING OF A DAF MARKER IN SOYBEAN (*GLYCINE MAX* L.) USING RECOMBINANT INBRED LINES
R.R. Prabhu[1], K.G. Lark[2], and P.M. Gresshoff[1], [1]Plant Molecular Genetics and Center for Legume Research, University of Tennessee, Knoxville, TN 37901, and [2]Department of Biology, University of Utah, Salt Lake City, UT 84112

5P MOLECULAR ANALYSIS OF AN RFLP MARKER CLOSELY LINKED TO THE SUPERNODULATION LOCUS OF SOYBEAN
D. Landau-Ellis, A.M. Kolchinsky, and P.M. Gresshoff, The University of Tennessee, Knoxville, TN 37901

6P CHARACTERIZATION AND ANALYSIS OF CLONES FROM A PARTIAL SOYBEAN YEAST ARTIFICIAL CHROMOSOME LIBRARY
R.P. Funke, A. Kolchinsky, and P.M. Gresshoff, Plant Molecular Genetics, Institute of Agriculture, The University of Tennessee, Knoxville, TN 37901

7P ISOLATION AND CHARACTERIZATION OF GENOMIC CLONES FOR SOYBEAN CDC2 PROTEIN KINASE
N. Taranenko and P.M. Gresshoff, Plant Molecular Genetics and Center for Legume Research, The University of Tennessee, Knoxville, TN 37901

***Numbers refer to the abstract in the back of this booklet*

8P RFLP MARKERS FOR ENOD2 SHOW MENDELIAN INHERITANCE IN A SOYBEAN F2 POPULATION

F. Ghassemi, D. Landau-Ellis, and P.M. Gresshoff, Plant Molecular Genetics and Center for Legume Research, The University of Tennessee, Knoxville, TN 37901

9P A MAJOR SATELLITE DNA OF SOYBEAN: 92 BASE PAIRS TANDEM REPEAT

A. Kolchinksy and P.M. Gresshoff, Plant Molecular Genetics and Center for Legume Research, The University of Tennessee, Knoxville, TN 37901

10P IDENTIFICATION OF RFLP MARKERS LINKED TO THE *w1* LOCUS AND *ms* LOCI IN *G. MAX*

K.J. Danna, S. Milstrey, and R. Baldivia, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309

11P IDENTIFYING RFLP MARKERS ASSOCIATED WITH COMPONENTS OF INSECT RESISTANCE IN SOYBEAN

B.G. Rector[1], J.N. All[1], W.A. Parrott[2], and H.R. Boerma[2], [1]Department of Entomology and [2]Department of Crop and Soil Sciences, The University of Georgia, Athens, GA 30602

12P IDENTIFYING RFLP MARKERS LINKED TO STRESS TOLERANCE TRAITS IN SOYBEAN

M.A. Bailey[1], D.A. Ashley[1], R.M. Delorme[1], R.S. Hussey[2], W.A. Parrott[1], and H.R. Boerma[1], [1]Department of Crop and Soil Sciences and [2]Department of Plant Pathology, The University of Georgia, Athens, GA 30602; J.W. Burton, T.E. Carter, Jr., T.W. Rufty, R. Wells, Department of Crop Science, North Carolina State University, and USDA-ARS, Raleigh, NC 27695; E.R. Shipe, Department of Agronomy and Soils, Clemson Univ., Clemson, SC 29634

Poster session II. *Presenters for posters 13P-26P will be by their posters Tuesday, 3:00-3:45*

13P CLONING OF SOYBEAN PARTIAL cDNAS HYDROXYMETHYL-GLUTARYL-COENZYME A REDUCTASE

J.J. Wassom, T.K. Cotten, C. Tixier, and C.D. Carter, Department of Plant Science, South Dakota State University, Brookings, SD 57007

14P DETECTION OF CYTOKININ BIOSYNTHESIS ENZYMES IN SOYBEAN

J.K. Ellis and D.A. Lightfoot, Department of Plant and Soil Science, Molecular Science Program, Southern Illinois University, Carbondale, IL 62901

15P GENOMIC ANALYSIS OF ENOD40 CLONE

N.G. Kruchinina and P.M. Gresshoff, Plant Molecular Genetics, Institute of Agriculture, The University of Tennessee, Knoxville, TN 37910

16P EARLY DEVELOPMENT AND MOLECULAR CHARACTERIZATION OF FASCIATED SOYBEAN, *GLYCINE MAX* (L.) MERR.

Y. Tang and H.T. Skorupska, Department of Agronomy and Soils and Department of Biological Sciences, Clemson University, Clemson, SC 29634

17P ISOLATION AND CHARACTERIZATION OF A SOYBEAN cDNA CLONE ENCODING A 100 KD HEAT SHOCK PROTEIN

Y.-R.J. Lee, R.T. Nagao, and J.L. Key. Department of Botany, The University of Georgia, Athens, GA 30602

18P ANALYSIS OF THE OMEGA-3 FATTY ACID DESATURASE IN SOYBEAN GENOTYPES A5 AND A23

J.R. Byrum and B.W. Diers, Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48823

19P DEVELOPMENTAL PATHWAYS OF SOYBEAN MORPHOGENESIS *IN VITRO*

E. Kiss, G. Gyulai, J. Kiss, Z. Jekkel, and L.E. Hesky, Department of Genetics and Plant Breeding, Gödöllő University, Hungary, H-2103

20P CHARACTERIZATION OF A HOMEOBOX-CONTAINING GENE SHOWING ENHANCED EXPRESSION DURING SOYBEAN (*GLYCINE MAX*) SOMATIC EMBRYO DEVELOPMENT

H. Ma and J.J. Finer, Department of Agronomy, The Ohio State University, Wooster, OH 44691

21P COMPARISON OF SOMATIC EMBRYO INDUCTION AND EMBRYO GERMINATION IN SIX VIRGINIA SOYBEAN (*GLYCINE MAX L.)* CULTIVARS

J. Li and E.A. Grabau, Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Poster session III. *Presenters for posters 27P-36P will be by their posters Wednesday, 9:45-10:30*

27P RAPD MARKERS FOR RESISTANCE TO SOYBEAN CYST NEMATODE, *HETEDORA GLYCINES* I., AND MARKER ASSISTED SELECTION
H.T. Skorupska, R. Mahalingam, I.S. Choi, and A. Warner, Department of Agronomy and Soils and Department of Biological Sciences, Clemson University, Clemson, SC 29634

28P RFLP ANALYSIS OF RESISTANCE TO PHOMOPSIS SEED DECAY IN THE SOYBEAN PI417479
G.U. Berger, M.S. Zimmerman, H.C. Minor, and A.P. Rao-Arelli, University of Missouri-Columbia, Columbia, MO 65211

29P GREENHOUSE ASSAY OF SOYBEAN SUDDEN DEATH SYNDROME RESPONSE
J.A. Patrick and D.A. Lightfoot, Department of Plant and Soil Science, Molecular Science Program, Southern Illinois University, Carbondale, IL 62901

30P RAPID N_2 FIXATION INCREASES BIOMASS OF NITRATE-FED SOYBEAN PLANTS
J. Imsande, Department of Agronomy, Iowa State University, Ames, IA 50011

31P PURIFICATION OF BINDING PROTEINS THAT RECOGNIZE OLIGOGLUCOSIDE ELICITORS OF PHYTOALEXIN ACCUMULATION IN SOYBEAN
F. Côté, R. Alba, J.-J. Cheong, and M.G. Hahn, Complex Carbohydrate Research Center, The University of Georgia, Athens, GA 30602

32P FISH ANALYSIS OF rRNA GENES: EXPRESSION AND EVOLUTION
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33P ANALYSIS OF DUPLICATED REGIONS IN THE SOYBEAN GENOME
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NOTES

PAINTING SOYBEAN CHROMOSOMES BY FISH WITH HIGH-COPY DNA PROBES

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Cytogenetic identification of chromosomes has been severely limited by the small size and uniform morphology in soybeans. Pachytene chromosomes and some aneuploid lines proven informative, but their wide spread use has been limited. We have developed fluorescent *in situ* hybridization procedures and molecular probes for the routine cytogenetic identification and for the physical mapping of soybean chromosomes. Chromosome preparation has been achieved by a modification of previous protocols and through the preparation of root-tip protoplasts prior to chromosomes spreading. Our probe technology has focused on highly-repeated DNAs that provide very intense localized hybridization signal. Gene probes that have proven valuable include the rDNA loci (5S and 45S) which are specific to different chromosomes. In addition, we have developed satellite DNA probes for two different sequence families: SB92 and the AC satellite. Both of these are tandemly arranged at multiple chromosomal loci. By using different cloned examples of each family, we have been able to selective label unique subsets of the soybean genome. Double labeling with biotinylated or digoxigenin probes, reveals the overlap between different probes and unique patterns. We are attempting to join the metaphase cytogenetic painting patterns with the genetic map by single-copy FISH and through PCR *in situ* detection of RFLP loci. Cytogenetic identification of soybean chromosomes will eventually allow us to physically separate entire chromosomes containing agronomically important genes.

ORGANIZATION AND MAPPING OF SOYBEAN TELOMERES

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Telomeres of higher organisms consist of short repetitive tandem sequences sometimes interspersed with unique sequences. They are useful for genetic mapping of the ends of linkage groups and for understanding of general organization of telomeres and their inheritance. In this work the organization of soybean telomeres was studied by two approaches.

First, polymorphism and location of restriction sites in telomeric areas were determined by Southern hybridization with plant consensus telomeric repeats. The average size of clusters of telomeric repeats in soybean chromosomes shows significant intervarietal variation and rather narrow distribution within varieties. These clusters are 12-25 kb long in different varieties of *G. max* and wild accessions of *G. soja* and are unstable in the progeny. They vary even within single plants and after cultivation of cells in suspension. However, long range restriction fragments corresponding to the ends of chromosomes are well preserved and show limited variation. In contrast to several other plants studied, no evidence of major satellite DNAs located near telomeres was found.

Second, single telomere-related primers $(TTTAGGG)_4$ and $(CCCTAAA)_4$ were used to drive a PCR-reaction with plant DNA. Under stringent conditions the reaction generates reproducible fingerprints. These fingerprints differed for several leguminous plants and distinguished several lines of soybean and *G. soja*. The polymorphic bands were scored in segregating populations. One band was mapped to the end of linkage group N of the USDA/ARS soybean RFLP map [1]. Another telomere-related polymorphism was mapped on a population of recombinant inbred lines [2]. Several PCR-products generated by single telomeric primers were cloned and sequenced. They are related to telomeric sequences and are expected to provide more molecular markers for the ends of linkage groups.

References

1.Shoemaker, R.C., Olson T.C. (1993) Molecular linkage map of soybean. In: Genetic maps. O'Brien, S., ed. Cold Spring Hrabor Laboratory Press, pp. 6.131-6.138.

2. Lark, K.G., Weisemann, G.M., Matthews, B.F., Palmer, R., Chase, K. and Macalma, T. (1993) A genetic map of soybean (*G. max* L.) using an intraspecific cross of two cultivars: 'Minsoy' and 'Noir 1'. Theor. Appl. Genet. 86: 901-906.

QUANTITATIVE TRAIT EVALUATION AND IDENTIFICATION OF QUANTITATIVE TRAIT LOCI IN A RECOMBINANT INBRED SOYBEAN POPULATION

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A recombinant inbred (RI) soybean population of more than 250 lines from the cross 'Minsoy' (PI 27890) by 'Noir I' (PI 290136) was evaluated for a number of quantitative traits. The quantitative traits evaluated included height, lodging, days to flowering (R1), days to maturity (R8), leaf length, leaf width, yield, seed weight, seed oil and seed protein. Extensive transgressive segregation for the quantitative traits was observed in the population. For example, the parents had a three day difference in maturity while the range in the RI population was 29 days, for plant height the parents varied by 40 cm while the RI population had a range of 93 cm and for yield the parents varied by about 400 kg ha^{-1} while the RI population had a range of over 2300 kg ha^{-1}.

Single quantitative trait loci (QTL) have been identified using about 225 RI lines to establish linkage to RFLP and other qualitative markers. QTL were located on about 2250 cM of the soybean genome comprised of 30 linkage groups. In addition QTL were associated with several unlinked markers. As expected the various traits mapped to several different linkage groups. Primary traits as well as several derived traits have been placed on the map. For example, QTL markers for height and lodging occur on at least 10 different linkage groups; QTL markers for maturity occur on at least 11 linkage groups; whereas QTL for yield are found on at least 7 linkage groups. Not unexpectedly, several related traits map together at some loci but also occur as separate loci. At least 2 QTL for yield are <u>not</u> associated with height or maturity.

The identification of RFLP markers associated with QTL offers the potential use of these markers by geneticists and breeders for genetic studies as well as for breeding improvement. Although single locus markers are important in quantitative traits we believe that many quantitative traits are a result of epistatic interactions.

INTERACTIONS BETWEEN QUANTITATIVE TRAIT LOCI DETECTED IN RECOMBINANT INBREED LINES OF SOYBEAN

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Values for traits of agronomic interest have been measured in a large population of recombinant inbred (RI) plants, derived by single seed descent from crosses between two soybean genotypes, 'Minsoy' (PI 27890) and 'Noir I' (PI 290136). These quantitative traits include height, lodging, flowering date (R1), maturity (R8), seed filling (R8-R1), leaf area, yield, seed weight, seed oil and seed protein. A derived trait, height/lodging, was also analyzed. This trait measures the ability of tall plants to remain upright, as well as the tendency of short plants to lodge. It shows a stronger correlation with lodging than with height, and probably reflects such parameters as stem width and strength. Quantitative trait loci (QTL) have been established by linkage to RFLP and other qualitative markers defining 2250 cM of linkage (comprised in 30 linkage groups), as well as 10 unlinked markers.

Epistatic QTL were identified by two screens: (A) pair-wise comparison of specific alleles in RI plants with extreme phenotypes for quantitative traits; and (B) comparisons of variation between the four genotypes possible within a selected pair of loci. Statistical values were computed from: (A) the binomial distribution; and (B) n-dimensional analysis of variance respectively. Many QTL were found to be espitatic (interacting) with other unlinked QTL (i.e., trait variation which could be correlated with one locus only was observed in the presence of a particular allele at another, unlinked, locus). In some cases, this dependence determined large amounts of variation. Examples include: two pairs of interacting loci for height, each of which determined a variation of about 30% (30cm, or a total of 60cm of height); another interaction which determined 25% of the observed variation for yield (430 Kg ha^{-1}) ; yet a different interaction which determined 27% of the variation in maturity (7.3 days).

Many other interacting pairs of QTL, regulating smaller amounts of trait variation, were detected. These types of interactions explain why crosses between two parents with high trait values more frequently than not yield progeny with lower values. Our results suggest that it may be essential to determine these types of interactions before using marker selection as a guide for breeding programs.

ANALYSIS OF LINKAGE BETWEEN SUDDEN DEATH SYNDROME AND CYST NEMATODE RESISTANCE GENES BY MOLECULAR MARKERS IN SOYBEAN.

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Varieties of soybean resistant to cyst nematode often show partial resistance to sudden death syndrome in the field. We have explored this relationship using molecular markers. Markers linked to *Rhg* genes were examined in recombinant inbred progeny lines of the crosses Essex x Forrest and Pyramid x Douglas which were characterized for SDS response at the $F_{5:7}$ through $F_{5:10}$ in multiple environments and for SCN resistance in greenhouse tests using infested soil (race 3 and race 14). Forest derives SCN resistance from Peking and Pyramid derives SCN resistance from Peking and/or PI88.788.

Chromosomal segments in individual lines were mapped with RFLP and RAPD markers. RFLP Probe A085, 28 cM from *Rhg4* was significantly associated with both SDS resistance (explaining 15% of the DX variability) and SCN resistance. Probe K636 54 cM from Rhg4 was not associated with SCN resistance (as expected) but was strongly associated with SDS resistance (explaining 45 % of DX variability). Such analyses indicate linkage, rather than pleiotropy, between *Rhg4* and genes conditioning SDS resistance though epistasis is apparent.

In Essex x Forrest marker A112 which has previously been mapped within 10 cM of *Rhg1* on linkage group G is neither associated with SCN nor SDS resistance. It may be possible that Essex contains a functional Rhg1 allele since a large number of SCN resistant lines are produced by the Essex x Forrest cross, consistent with a 2 gene model of SCN race 3 resistance.

Marker K455 was associated with SCN resistance but did not map on a linkage group previously reported to carry *Rhg* alleles. In addition a line segregating for SCN resistance was found to be heterogenic for K455. Sublines have been derived and the association between alleles of K455 and SCN resistance will be determined.

GENE MAPPING IN SOYBEAN WITH PRIMARY TRISOMICS

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Primary trisomics ($2n = 2x+1$) are very useful in locating genes on individual chromosomes. In this study, we attempted to associate several seed protein genes with specific chromosomes using soybean primary trisomic series. Triplo 1, 3, 4, 5, 6, 8, 10, 13, 14, and 19 were crossed with seed protein marker stocks for *Ti* (Kunitz trypsin inhibitor), *Le* (seed lectin), *Sp1* (β-amylase), *Lx1* (Lipoxygenase-1), and *Eu* (seed urease). The F_2 seeds from the selfed F_1 trisomic plants were scored for segregation ratios. Of all the genetic markers used, only *Eu* and *Lx1* exhibited the trisomic segregation ratios with triplo 5 and triplo 13, respectively. These results indicated that the *Eu* and *Lx1* loci are on chromosomes 5 and 13, respectively. Thus, chromosome 13 (nucleolus organizer) corresponds to genetic linkage group LG5 and RFLP linkage group L of USDA and LG1 of E.I. du Pont de Nemours and Co. The genetic markers *Ti*, *Le*, and *Sp1* segregated in a disomic fashion with all the primary trisomics indicating their absence on chromosomes 1, 3, 4, 5, 6, 8, 10, 13, 14, and 19. This information is useful in associating cytological map with the genetic and RFLP linkage maps of soybean.

MICROSATELLITE DNA MARKERS FOR GENOME MAPPING AND GENOTYPE IDENTIFICATION.

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Microsatellite or Simple Sequence Repeat (SSR) DNA markers have become the marker of choice by human and other mammalian geneticists. The characteristics of SSR markers that have resulted in their rapid acceptance include their high degree of informativeness, abundance, co-dominant inheritance, high level of reproducibility, and PCR amplification. We and other groups have demonstrated the presence of microsatellites in a number of plant species including soybean, maize, rice, alfalfa, Brassica, mango, avocado, and apple. Among a group of 96 diverse soybean genotypes we have identified microsatellite loci with as many as 29 alleles. In this group of genotypes, Polymorphism Information Content (PIC) values of microsatellite markers ranged from 0.71 to 0.95. These loci are being mapped in a population of 60 F_2 plants from a cross between isolines of the cultivars Clark and Harosoy. A total of 13 conventional morphological and pigmentation, 7 isozyme, 110 RFLP, and 30 microsatellite loci were mapped in this population. Analysis of the mapping data indicated a total of 31 linkage groups. Microsatellite loci mapped to 12 of the groups and were linked to numerous RFLP, morphological, and isozyme markers suggesting random distribution in the soybean genome. Because of their highly informative nature, microsatellite markers function well in distinguishing soybean cultivars. Using seven microsatellite loci only cultivars Lee and Pickett could not be distinguished from one another in a group of 96 genotypes. A recent report indicates that these two genotypes are 93.4% identical. Therefore, the inability to distinguish these genotypes is not surprising.

MOLECULAR GENETIC ANALYSIS OF SOYBEAN NODULATION GENES: GENOMIC COPIES AND POSITIONAL CLONING

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Research in our laboratory has focused on the establishment of genetic techniques which allow the analysis of plant genes involved in early nodulation of soybean. Supernodulation and non-nodulation mutant phenotypes were not correlated with a major molecular change. Accordingly we initiated a positional cloning project in which molecular markers (RFLP, DAF) were placed close to the supernodulation gene (D. Landau-Ellis and G. Caetano-Anollés). Gene transfer was achieved using Agrobacterium rhizogenes strain K599. Transgenic roots and nodules were produced, but no plants (R. Chian). Biolistic transformation of soybean embryos was possible (R. McDonnell). The first YAC cloning and physical mapping in soybean was achieved (R. Funke and A. Kolchinsky). The final goal of positional cloning of a nodulation gene has not been achieved, but significant progress has been made. At present the YAC library of soybean presents about 15% of the genome. The average YAC size is 200 kb, with the largest being 900 kb. Endclones were isolated using the vectorette approach and found to be either unique or repeated DNA (1:1 ratio). Stability of YACs was high with only the occasional clone showing abnormal segregation. About 7% of YAC represent chloroplast DNA. Telomeres and a major satellite of soybean were isolated, sequenced and mapped (A. Kolchinsky). Single primer DNA amplification placed molecular polymorphisms close to the nts locus (G. Caetano-Anollés, J. Padilla). Similar DAF markers were inherited as Mendelian dominant loci, and were mapped on the RIL map of G. Lark (R. Prabhu and G. Lark). One DAF marker was inherited in a maternal fashion and presumably represents organelle DNA. Genetic analysis of the nod139 non-nodulation mutant indicated that the phenotype was controlled by two genes (*rj5* and *rj6*; D. Landau-Ellis). The parent cultivar Bragg already lacks the *rj5* function and was mutated in *rj6* to yield nod139. To understand nodulation further, genomic copies of *cdc2*, *cyclinB* and *enod40* were isolated from a Bragg gene bank (N. Taranenko, J. Deckert and N. Kruchinina). *cdc2* homologous clones exist as at least two copies. One is presumably a pseudogene as it lacks introns. The other gene is similar to the cDNA clone isolated by Verma's laboratory. The *enod40* gene exists in low copy number (perhaps 2 or 3) and does not have an intron. These early mitotic genes are differentially regulated during nodulation and the different nodulation mutants.

RFLP AND RAPD MARKERS LINKED TO GENES AFFECTING SOYBEAN SUDDEN DEATH SYNDROME RESPONSE.

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Four loci affecting soybean sudden death syndrome response, yield, and maturity date have been mapped in Essex x Forrest using 100 RFLP and RAPD markers. pA71a, on linkage group N, is linked to yield under SDS pressure. pA117b and pK636c, on linkage group A, and pK455a, on linkage group C, are associated with SDS leaf symptoms. RAPD marker OPF13b, on linkage group H is associated with maturity date, and RAPD marker OPO05e, on linkage group C, is associated with SDS leaf symptoms.

pK636c, OPO05e, and pK455a have R^2 values of 0.45, 0.24, and 0.16 respectively for SDS response. pA71a has an R^2 value of 0.45 for yield, and OPF13b has a R^2 of 0.21 for maturity date.

pA71a and pA117b, where the Forrest allele is beneficial to SDS response, were only significantly associated in 2 of 4 environments. pA71a is significant only in Cora 1991 and Cora 1992. pA117b is significant only in Villa Ridge 1990 and Pulaski 1991. These inconsistencies indicate that the markers may be linked to environmentally sensitive resistance genes, possibly pathovar specific. K455a and OPF05e, where the Essex allele is beneficial, were consistent across environments. However, K636c was only significant in Cora 1991 and 1992 showing environmental sensitivity of some beneficial Essex alleles. Some markers linked to SDS response in Essex x Forrest are also linked to SDS response in RILs derived from Pyramid x Douglas, quite distinct germplasm.

In order to critically analyze linkage between individual alleles and SDS response near isogenic sublines are being developed from heterogeneic RILs for each of the six markers. Alleles within each subline will be detected with the six markers and the effects of individual alleles on SDS response determined in near isogenic sublines.

Effectiveness of the markers will be further tested by marker assisted selection for 100 SDS resistance line from among 2000 lines per cross. Two crosses between varieties with Forrest and Essex as major genetic contributors and which contrast in SDS response have been made (Resnik x Hartwig and Flyer x Hartwig) and RILs extracted. Resnik and Flyer are derived from Williams x Essex, Hartwig is Forrest^3 x PI437.654. ASAPs and SSRs will be developed to aid with this project.

COSEGREGATION OF THE PURPLE-THROAT FLOWER COLOR WITH AN RFLP REPRESENTING DIHYDROFLAVONOL REDUCTASE (DFR) IN SOYBEAN

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In soybean there are several possible flower colors, including white, purple, near-white, purple-throat, magenta, and pink. At least five genes are known to act epistatically to produce the above colors. The genotype W1W3w4 gives the purple-throat phenotype and the genotype W1w3w4 the near-white phenotype. It is presumed that the different colors represent mutations at different steps of the anthocyanin biosynthetic pathway.

In an effort to investigate the molecular basis of the flower pigmentation in soybean, crosses were made between the Clark flower color isolines. Genomic DNA was isolated from the parents and from segregating generations, followed by restriction with 12 endonucleases. After Southern transfer, the blots were probed with three clones corresponding to three enzymes of the anthocyanin biosynthetic pathway. The three enzymes are phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), and dihydroflavonol reductase (DFR). The objectives of the project were to determine whether any of the known alleles for flower color cosegregate with a particular RFLP pattern, as well as the extent of cosegregation.

Association of the purple-throat flower color with a specific RFLP pattern was first detected when the source of DNA was the Clark purple-throat isoline, derived after successive backcrosses from the original purple-flowered Clark. The RFLP was detected following restriction with HaeIII and probing with DFR. Of a total of 78 segregating individuals, 18 were found to have near-white flowers, the rest being purple-throated, consistent with the 3:1 ratio. In all cases, we observed cosegregation of the purple-throat flower color with a specific RFLP using the DFR probe. These results provide a strong indication that the W3 locus may code for a DFR enzyme or that there is tight linkage between a DFR gene and the W3 locus.

UPDATE OF SOYBASE, A SOYBEAN GENOME DATABASE

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Soybase, a soybean genome database, now contains over 100 megabytes of data. Previously existing classes (topics) have grown in size, and data for new classes is being rapidly assembled. Some of the most recent information to be included in Soybase is information relating to enzymes involved in both nitrogen and fatty acid metabolism, information on nodulins, vegetative storage proteins, and seed storage proteins, as well as information concerning QTL's and sequences. The information regarding metabolic pathways contains an interactive graphical interface, and the QTL information contains connections to the RFLP map graphical displays. A summary of the information in Soybase is as follows, reported on a class-by-class basis:

Class	No. of entries
Linkage Group	84
Probe	350
Locus	470
2Point data	400
Images	200
Sequences	200
Germplasm	~20,000
Gene	300
Allele	500
Trait	100
QTL_Study	35
Colleague	150
Pathology	9
Reactions and Pathways	250
Nodulin	90
Enzyme	230
Metabolites	250
References	~35,400

SOYBEAN DUPLICATE GENOMES: STRUCTURE AND EVOLUTION

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Soybean [*Glycine max* (L.) Merr.] genome organization is complicated by the presence of a high degree of duplication among DNA sequences. An ancestral tetraploidy state has been postulated from which soybean is evolving towards diploidy. We used over 700 genomic clones to examine the copy-number of hypomethylated genomic regions and found that greater than 15% were single-copy. The remaining 85% were duplicate or middle-repetitive sequences. A comparison of single and duplicate copy fractions indicates that about 25% homeologous sequences have been lost during diploidization. Methylation patterns for homeologous regions were compared using *Msp*I and *Hpa*II restriction enzymes. Fifty duplicate genomic regions were examined and in no cases were there major differences in methylation levels. Some low level interspersed methylation was evident in many hypomethylated regions. One example of homeologous loci was comparatively sequenced to examine the level of divergence (pA-199a and pA-199b). These two regions have been previously mapped to unique linkage groups K and J (respectively) and PCR fluorescent *in situ* detection revealed four hybridization foci located at different positions on the metaphase chromosomes. Comparative sequencing revealed that these regions have diverged at about 10% of their nucleotide positions. We compared pA-199 sequence similarity among diploid soybean relatives with the pA-199 homeologous and found that *Neonotonia* was most similar to the pA-199a locus but that *Teramnus* was most similar to pA-199b. While these data suggest an allotetraploid origin for soybean, a relatively ancient autotetraploid event would still explain our observations.

GENOME CONSERVATION AMONG LEGUME GENERA DETECTED WITH DNA MARKERS

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To detect synteny among the genomes of three legume genera (*Vigna, Phaseolus, Glycine*), we compared the linkage order of 173 RFLPs common to at least two of the genera. Extensive linkage conservation was observed between *Vigna* and *Phaseolus*, with some linkage groups conserved virtually intact. Nearly every *Vigna* linkage group corresponded to only one or two *Phaseolus* group(s). The level of genome conservation between the genomes of *Vigna* and *Phaseolus* and that of *Glycine* was lower, but still substantial. In general, *Glycine* linkage groups were mosaics of linkage blocks of *Vigna* and *Phaseolus*. In a few cases, linkage blocks up to 50 cM in length were conserved among all three genera. Generally, only shorter linkage blocks were preserved in *Glycine*. Linear order within conserved linkage blocks of *Vigna* and *Phaseolus* was very similar, with most differences being statistically insignificant. *Glycine* exhibited several genomic duplications, as might be expected from a diplodized tetraploid. This may have complicated parallel mapping in *Glycine* and contributed to the degree of rearrangement that was observed.

POSITIONING SOYBEAN CLASSICAL MARKERS ON MOLECULAR MARKER MAPS: A CALL FOR A COLLABORATIVE EFFORT AMONG INTERESTED RESEARCHERS.

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Our past and current joint research efforts have been directed at the integration of classical markers into soybean molecular maps. Phase I research consisted of a molecular marker comparison of near-isogenic lines (NILs) and their recurrent and donor parents. However, singular assignments of the 70+ classical markers to molecular linkage groups of the "public" RFLP map could not be made due to the retention of nonlinked bits of random donor parent DNA in these BC5-derived NILs. In the Phase II research, a molecular marker characterization of an F2 population segregating for 13 classical and 7 isozyme markers resulted in the exclusive assignment of most of the classical markers, and their respective classical linkage groups (CLGs), to molecular linkage groups (MLGs). However, suitable parents did not exist for the creation of additional multi-marker F2 populations. Also, characterization of classical marker phenotypes is difficult in such populations due to epistasis (trait masking or nonsynchronous expression), and missing F2 data cells lead to imprecise or erroneous linkage estimates. Therefore, for Phase III research, we created 150+ F2 populations, each segregating for just one *single classical locus*, by mating one *hub* parent to 150+ *source* parents, the latter providing the contrasting classical marker allele. Among the 150+ loci are those that govern disease and Rhizobium response, herbicide reaction, plant morphology and developmental ontogeny, sterility, mineral nutrition, pigmentation, and chlorophyll deficiencies. About 50 of the 200 F_2 plants will be homozygous for any given classical marker and *only these 50 plants (or their descendants) need be assayed for molecular marker segregation*. The linkage information obtainable per F2 plant drawn from the homozygous recessive class is as much as *50 times more* (tight linkages) than that per F2 plant drawn from a 9:3:3:1 repulsion phase population, and is no less than *50%* (tight linkage) *to 100%* (loose linkage) of that per F2 plant drawn from a 9:3:3:1 coupling phase population! Simple Chi-square linkage analysis (α=0.05) has the power to detect linkages of $p \leq 0.3$ if the molecular marker segregates in ratio deviating from a 1:2:1 (or 3:1) *within* the 50-plant homozygous recessive class. Recombination values can be estimated using maximum likelihood equations. These F2 populations can serve as a useful resource for the integration of classical markers into the various public/proprietary molecular marker maps. We are therefore willing to share this F2 material with any researcher interested in putting classical markers on a (or their) soybean molecular map.

IDENTIFICATION OF CHROMOSOMAL REGIONS ASSOCIATED WITH QTL FOR SEED PROTEIN AND OIL .

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We have identified a number of RFLP markers associated with quantitative trait loci (QTL) controlling protein and oil content of soybean seeds. RFLP maps were constructed in eight F_2 populations of between 79 and 100 individuals. These maps were aligned with the USDA/ISU genetic map. Field analyses were done on advanced generation seed in 1992 and 1993 at Purdue, Minnesota, and Nebraska. Replicated trials were tested at one location in each year except 1993 in Nebraska where two locations were included. In addition to protein and oil content, various morphological traits, such as height and maturity, were also scored. Correlations of protein and oil content with the various morphological traits showed few consistencies. Neither protein nor oil was consistently correlated with yield. Associations between marker loci and putative QTL were determined using one-way ANOVA with the RFLP marker as a class variable. Epistatic interactions were identified using two-way ANOVA. We considered identification of a marker-QTL locus association in two populations or in each of two years as representing a true QTL. Most populations had between three and six QTL for protein and oil. Generally, the same marker was associated with both traits (as expected from the literature), but some markers were identified which showed association with only one trait. The amount of variation explained by various RFLP markers was usually low: R^2 about 10%. One marker, A109, identified 32% of the variation of both protein and oil in both years in Population 13 from Purdue. The gene action of most QTL was additive, although some loci with dominance and (pseudo) overdominance were also present. Epistasis had a seemingly minor effect. No association of QTL to mapped genes for storage proteins or oil mutants was seen. The results of our analysis identify a number of genomic regions which are important for protein and oil levels in soybean seeds. The data also indicate that, in at least some instances, protein and oil may be selected independently.

RESEARCH ON PRIMARY TRISOMICS IN SOYBEAN

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Primary trisomics are useful cytogenetic tools for locating genes on the chromosomes and for associating linkage groups with the specific chromosomes. The establishment of the first cytological map based on pachytene analysis in soybean [*Glycine max* (L.) Merr.] has provided an incentive to isolate and identify primary trisomics ($2n=41$) in this important crop. By pachytene analysis, 13 primary trisomics of soybean were identified previously. This study provides information on new primary trisomics representing chromosomes 11, 15, and 18 for the first time. The precise identification was based on the total length, arm ratio, and distribution of heterochromatin and euchromatin, and trivalent configuration of chromosomes at the pachynema stage. Currently, we have identified 16 of the possible 20 primary trisomics of soybean. These identified trisomics account for about 83.9% of the total nuclear genome and 86.1% of the total nuclear euchromatin.

GENETIC DIVERSITY FOR RFLP MARKERS IN NORTHERN SOYBEAN LINES

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Soybean germplasm in the U.S. was established from a small number of plant introductions which may be a limitation to yield improvement. This study was conducted to assess the genetic diversity within elite soybean germplasm from the northern U.S. and determine whether breeding efforts have reduced the genetic diversity present in the original PIs. A sample of elite lines including released varieties and breeding lines, and a group of ancestral plant introductions were genotyped at 38 RFLP marker loci. The markers were selected because they were previously found to reveal polymorphisms in elite germplasm. Each line was uniquely identified with the markers. Little diversity for the RFLP markers was lost through breeding. There were only two alleles present in the original PIs that were not present in the elite lines evaluated. Pairs of genetically distant lines can be identified within the elite germplasm. The most distant pair of lines had only 32% of the marker alleles common between them. RFLP genetic distance information could be useful in breeding programs by helping breeders to maximize the genetic diversity between parents crossed in the formation of populations.

ORGANIZATION AND EVOLUTION OF THE HISTONE H3 GENES IN SOYBEAN (*GLYCINE MAX*).

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The subject of this research was the organization of the histone H3 multigene family in soybean (*Glycine max*). The existence of multiple gene variants within a single genome suggests that sequence duplication plays an important role in the evolution of gene function and evolution of the genome itself. Over thirty clones containing histone H3 genes were isolated from a soybean genomic library. Only two lambda phage clones contained more than one gene, indicating dispersed arrangement of histone H3 genes. The majority of isolated clones corresponded to the intronless variant of the gene. These genes were divided into several subfamilies according to their sequence similarity. Several genes were found to contain introns. Three introns were located in the same position within genes, but differed in length and nucleotide sequence. Comparison of protein sequences encoded by intron-containing and intronless gene variants demonstrated that they corresponded to two different classes of the histone H3. Sequence diversity among introns and gene-flanking sequences allowed us to use them as gene-specific probes for mapping individual members of this gene family. Members of the histone H3 gene family have been located on several chromosomes, corresponding to linkage groups N, S. Analysis of the nucleotide sequences of the individual histone H3 genes and nucleotide sequences of contiguous non-genic regions, in conjunction with the information about their chromosomal location, allowed us to infer possible evolutionary relationships within this multigene family.

UNDERSTANDING ORGAN-SPECIFIC GENE EXPRESSION IN SOYBEAN

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In an attempt to understand organ-specific gene expression patterns in soybean, the soybean seed-lectin gene was studied. The 5' and 3' regions of the lectin gene were fused to the coding region of the β-glucuronidase reporter gene. These were cloned into binary vectors and introduced into tobacco and Arabidopsis using Agrobacterium-mediated transformation. Histochemical analysis of transformed tissues showed seed-specific expression of the GUS gene. These results were consistent with the lectin gene expression patterns observed in soybean. All further analyses were conducted only in transformed tobacco tissues. The GUS gene in tobacco transformants was selectively amplified by polymerase chain reaction (PCR) of genomic DNA using specific primers. Southern blot analysis of PCR amplified DNA using α^{32}P-labeled GUS gene as probe confirmed presence of the reporter gene in the transformed plants. Tissues from a plant transformed by a GUS gene under control of a CaMV 35S promoter were also analyzed to facilitate comparison of GUS gene expression patterns under constitutive promoter control and tissue-specific promoter control. More precise gene expression patterns were obtained by Northern blot analysis of transformed tobacco seed RNA using α^{32}P-labeled GUS gene as probe. Further, GUS gene expression levels were also quantified by determining GUS enzyme activity fluorometrically.

Organ-specific expression patterns were also studied in order to identify and isolate genes that have pod-specific expression. To accomplish this, a partial cDNA library was constructed using mRNA isolated from young pods of soybean. Positive clones were identified by PCR, where insert cDNA fragments were selectively amplified from phage DNA using M-13 primers. The phagemids containing the cloned cDNA inserts were excised from the phage vector, and the insert DNA was isolated by restriction digestion of the phagemid DNA. The different inserts thus isolated were radiolabeled and used as probes on RNA blots containing RNA from a good representation of the different organ types in soybean such as young pods, old pods, seed coats, immature cotyledons, leaves, stem and roots. Here, Northern blots are used as an assay to screen for pod-specific gene expression.

CYTOKININ RESPONSIVE GENES IN PHASEOLUS AND GLYCINE.

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Genetic responses to different cytokinin applications have been characterized in soybean and snap bean. Gene response has been analyzed in both cell culture and organized tissue. Two novel cDNAs, L-221 and L-22 have been identified that are cytokinin responsive. These cDNAs are of low abundance and were identified by screening of RNA slot blots of RNA from cytokinin treated callus with random clones from a cDNA library. The genes are present in roots, stems and leaves and are of higher abundance in roots than stems or leaves. Searches of the Genbank database found no homologous sequences to the cDNAs.

In leaf tissue the L-221 mRNA abundance was decreased by zeatin, benzyladenine and thidiazuron as determined by Northern hybridization. S-1 Nuclease Protection Assays revealed that L-221 is down regulated as compared to control 2 hours after treatment. The L221 mRNA was not cytokinin responsive in cytokinin treated callus.

The L-22 mRNA is increased in abundance 4 hours after treatment in a dose dependent fashion by all cytokinin treatments in leaf. In callus L22 mRNA is up regulated by at least one dose and at some time point after cytokinin application. Cytokinin responsive gene expression seems to be transient phenomenon dependent upon the time of cytokinin application, cytokinin concentration and cytokinin chemical structure. The leaf response may be adaptable to a bioassay for cytokinin activity since it is both dose dependent and cytokinin specific.

Callus bioassays have shown that the co-application of zeatin and dihydrozeatin or zeatin and O-glucosylzeatin stimulated explant growth at a lower concentration in soybean than zeatin, dihydrozeatin or O-glucosylzeatin alone. This was not the case in snap bean.

ANALYSIS OF NUCLEAR EFFECTED MITOCHONDRIAL REORGANIZATIONS IN SOYBEAN

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A rearrangement in the mitochondrial genome of a BC5F3 line (IX249) containing an Illini cytoplasm and a Harosoy 63 nucleus was detected with a probe from a phaseolus mitochondrial library. The genomic region encompassing the rearrangement was cloned from an IX249 mitochondrial library as a 6.5 kb Hind III fragment. The 6.5 kb Hind III fragment also hybridized to five other Hind III bands which were present in IX249, Illini, and Harosoy 63. Restriction maps showed that the rearranged fragment was a chimera of two of these fragments. These fragments are currently being sequenced to determine whether the rearranged fragment is the result of homologous recombination across a repeat. To determine when the rearrangement first appears, and to clarify the genetics controlling this genomic rearrangement, the crosses are being repeated. The rearrangement was not found in the Illini x Harosoy 63 F2 or BC1F1 generations. An attempt was also made to eliminate the rearrangement by re-introducing the Illini nucleus into IX249. The rearrangement was absent from the F2 plants from IX249 x Illini crosses as well as the BC1F1 generation.

ISOLATION OF TENTATIVE CHOLINEPHOSPHOTRANSFERASE, OLEOYL DESATURASE AND LINOLEOYL DESATURASE CLONES FROM A SOYBEAN GENOMIC LIBRARY

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Cholinephosphotransferase (CPT), oleoyl desaturase (fad2) and linoleoyl desaturase (fad3) are integral for the production of desaturated fatty acids in soybean seeds. A polymerase chain reaction technique was used to screen samples of a soybean genomic library in an EMBL3 SP6/T7 phage vector. Primer sequences were derived from *S. cerevisiae* CPT cDNA (Hjelmstad, et al., 1990), *Arabidopsis* fad2 cDNA (Lightner, et al., 1994) and soybean fad3 cDNA (Yadav, et al., 1993). Library fractions exhibited multiple PCR products from the fad2 and fad3 primers, possibly indicating the presence of either a desaturase gene family or pseudogenes in the genome. Only a single product was seen with the CPT primers. Positive samples from the preliminary PCR screening were further screened via filter lifts from plates of the phage vector and visualized with biotin labelled probe specific for each clone. Positive plaques from the plates have been recovered, amplified and screened a second time to verify the identity of the phage insert. The CPT, fad2 and fad3 clones will be utilized to obtain future sequence data relevant to the respective genes.

References

Hjelmstad, R.H.; Bell, R.M. (1990) The sn-1,2-diacylglycerol cholinephosphtransferase of *Saccharomyces cerevisiae, Journ. of Biol. Chem.*, **265**: 1755-1764

Lightner, J.; Okuley, J.; Feldmann, K.A.; Yadav, N.S.; Browse, J. (1994) The *Arabidopsis* fad2 gene encodes the enzyme that is essential for cytosolic polyunsaturated lipid synthesis, *Plant Cell*, (in press)

Yadav, N.S.; Wierzbicki, A.; Aegerter, M.; Caster, C.S.; Perez-Grau, L.; Kinney, A.J.; Hitz, W.D.; Booth, J.R.; Schweiger, B.; Stecca, K.L.; Allen, S.M.; Blackwell, M.; Reiter, R.S.; Carlson, T.J.; Russell, S.H.; Feldmann, K.A.; Pierce, J.; Browse, J. (1993) Cloning of higher plant ω-3 fatty acid desaturases, *Plant Physiol.*, **103**: 467-476

SAMUEL BOWEN'S INTRODUCTION OF THE SOYBEAN TO NORTH AMERICA: FLESHING OUT THE SKELTON

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This presentation documents the history of the introduction of the soybean, *Glycine max* (L) Merr., to North America. Samuel Bowen, a native of Thorpe St. Peter, Lincolnshire, England brought soybeans to Savannah, Georgia from London via China in 1764. In 1765, at the request of Samuel Bowen, Henry Youge planted soybeans on his plantation located in Skidaway Island, Georgia. From 1766, Samuel Bowen planted soybeans on his plantation 'Greenwich' located at Thunderbolt, Georgia. A report by Dr. James Mease published in 1804, previously thought to be the oldest literature citation in the U.S. for soybeans, has been traced to the Chinese Vetches (soybeans) sent to America Philosophical Society, Philadelphia by Samuel Bowen in 1769. The soybeans introduced by Samuel Bowen most probably belonged to Maturity Group II.

SOYBEAN PHOTOSYNTHETIC SUSPENSION CULTURES AS A MODEL FOR CELL WALL METABOLISM STUDIES

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Since the cell wall is now known to be very active and to have many functions including, participation in cell growth, differentiation and morphogenesis, responses to pathogens and cell to cell interactions and as a storage site of carbon reserves, among others, it is important to know the changes which occur and the mechanisms which control the modifications in cell wall metabolism.

We have proposed to study cell wall polysaccharide turnover considering them to be the products of photosynthesis in pulse-chase experiments with $^{14}CO_2$ as the original substrate and photoautotrophic and mixotrophic soybean cell suspension cultures as the experimental objects.

Incorporation of radioactivity into the cells, culture medium, starch, cell wall and various wall fractions was estimated at cell division, expansion and stationary stages. Very similar results were observed in young and old cells: cell radioactivity declined (about 35%) and cell wall radioactivity increased (from 20 to 80%) during a 4 day chase. The proportion of starch and cell wall radioactivity changed during cell growth cycle. In the young actively growing cells there was a low level of starch radioactivity (about 15% of that of cell wall). Label incorporation into starch, representing a storage pool of carbon in the cells, increased several times at the stationary phase (and accounted for 60% of that of cell wall).

The Biosynthesis and breakdown of cell wall polysaccharides were most active in young dividing cells. Pectins and hemicellulose were hydrolyzed at different stages of the cell growth cycle, the highest intensity being in young cells. The different response of various carbon metabolic pathways on altered photosynthetic performance was shown in our experiments where increased CO_2 levels activated cell wall polysaccharide synthesis more than starch formation at different phases of cell development. The alteration of monosaccharide composition of wall matrix polysaccharides was also characterized during cell growth cycle. The data obtained permits one to propose photoautotrophic soybean suspension cultures as a good new model to study the role of the cell wall in growth under different environmental conditions. The lack of knowledge in this area hampers progress in the use of genetic engineering to improve plant resistance to different stresses.

PROGRESS AND PROSPECTS FOR IMPROVEMENT OF SOYBEAN OIL QUALITY

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After some initial trials as a forage crop, soybeans were originally developed as an oilseed crop in the US. As the soybean oil industry became established it was realized that it was an excellent source of vegetable protein. Since then soybeans have become the most important source of vegetable protein and oil in the US and the world. The composition of soybean oil is less than ideal due to the relatively high level of saturated fatty acids and unstable polyunsaturated fatty acids. A soybean oil with higher monounsaturated fatty acid levels and lower amounts of the other fatty acids would be superior for many edible purposes. The prospects of facilitating these alterations via genetic engineering appear excellent. Genes which can affect levels of all the major fatty acids have now been cloned and molecular genetic alteration of soybean oil composition has been initiated. Expression of a mammalian delta-9 desaturase in transgenic soybean somatic embryos driven by a seed-specific promoter has resulted in a large reduction in the level of saturated fatty acids. Future additional modifications for different end-uses in the marketplace will be presented.

THE MANIPULATION OF FATTY ACID METABOLISM IN DEVELOPING SOYBEANS

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Our objective is to produce and market high value differentiated plant oils through the genetic manipulation of soybeans. The initial focus has been the production of soybean varieties that yield healthier (lower in saturated fatty acids) and more stable (lower in polyunsaturated fatty acids) edible oils. We have identified low saturate and low polyunsaturate lines of soybean by screening mutagenized populations and are using both traditional and molecular breeding techniques to produce elite seed from these lines. We have also isolated many of the genes involved in fatty acid biosynthesis and modification in developing oilseeds. These genes have been isolated by biochemical (purification of fatty acid biosynthetic enzymes and protein microsequencing), molecular biological (DNA sequence homology) and genetical (tDNA insertional mutagenesis, genetic complementation) techniques. We have reintroduced these genes, in both sense and antisense orientations, under the control of heterologous promoters, into elite soybean lines. In addition to allowing the manipulation of oilseed fatty acid content, these experiments are leading to a deeper understanding of the regulation of fatty acid biosynthesis in plant cells.

DEVELOPMENT AND REGENERATION OF SOYBEAN (CVS. FAYETTE, CHAPMAN AND RESNICK) SOMATIC EMBRYOS FROM EMBRYOGENIC SUSPENSION CULTURES.

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Soybean somatic embryo development and germination was evaluated by testing various maturation and desiccation treatments. Clumps of embryogenic suspension culture tissue, maintained in liquid medium (modified MS salts, B5 vitamins, 6% sucrose, 5 mg/l 2,4-D, 5 mM asparagine) at 28°C, were initially plated on solid media containing MS salts, B5 vitamins, 0.8% Noble agar, and either sucrose, glucose or maltose at levels of 1, 3, 6, and 10% and placed at 28°C. After 4 weeks, the individual embryos were rated for amount and type of development. To test the effects of temperature and solidifying agent on embryo development, desiccation and subsequent germination, embryogenic suspension culture tissues were plated on development medium containing 3, 6 or 12% maltose or sucrose, 0.2% Gelrite or 0.8% Noble agar and placed at 23°C or 27°C. After 4 weeks, individual embryos were separated from the clumps and plated on fresh medium for further maturation. After an additional 4 weeks, the embryos were transferred to dry Petri dishes for 3 days. Desiccated developed embryos were then transferred to a medium containing MS salts, B5 vitamins, 3% sucrose and 0.2% Gelrite for germination. The embryos were weighed before and after the desiccation treatment to measure water loss. Depending on the treatment, 14 to 100% of the embryos produced roots within 7 days following transfer to germination medium. After an additional 3-4 weeks, up to 92% of the embryos formed shoots. Mature embryos appeared green to yellow-green and turned yellow to cream-colored during the desiccation process. Depending on the size and fleshiness of the embryos, desiccation proceeded for 1-7 days . Weight loss varied from approximately 21% to 75%. Embryos that developed for only one month and were not exposed to a desiccation treatment formed shoots at 4% or less.

ANALYSIS OF TRANSGENIC SOYBEAN CONTAINING A SOYBEAN CYTOSOLIC GLUTAMINE SYNTHETASE PROMOTER FUSED TO THE GUS CODING SEQUENCE

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The promoter from a cytosolic glutamine synthetase (GS) gene from soybean was fused to the *E. coli* ß-glucuronidase (GUS) reporter gene and introduced into embryogenic cultures of soybean (*Glycine max* Merr. cv Chapman) via particle bombardment. The expression of the reporter gene was examined at various stages of embryo and plant development using both fluorometric and histochemical GUS assays. Expression of the GUS gene was highest in proliferative embryogenic cultures but declined as the embryos developed. Histochemical GUS assays of roots from regenerated plant tissues indicated localization of GUS activity in the vascular system. Expression of GUS in leaf tissue of transgenic plants was not restricted to the vascular tissue but appeared mottled throughout the leaf lamina. Native GS activity was not affected by the introduction of the additional copy of the glutamine synthetase promoter fused to the GUS gene. Ammonia and nitrate induction of the GS promoter:GUS fusion is currently under investigation using both primary transformants and progeny plants.

TRANSFORMATION OF SOYBEAN WITH BEAN POD MOTTLE VIRUS COAT PROTEIN PRECURSOR GENE *VIA AGROBACTERIUM*

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Transgenic soybean plants were produced using *Agrobacterium*-mediated DNA transfer technique. A construct consisting of the coat protein (CP) precursor gene of bean pod mottle virus (BPMV), a plant comovirus, and the NPT II kanamycin resistance gene was produced. It was delivered into soybean (*Glycine max* cv. Fayette) genome when *Agrobacterium tumefacien* (strain Z707) was cocultivated with wounded soybean cotyledons. Multiple shoots were induced through organogenesis on B5 medium containing BAP and 150 mg/l kanamycin. Critical screening of true transformants relied on the root induction of elongated shoots on B5 medium containing IBA and 50 mg/l kanamycin. Finally, five regenerated plantlets were transferred to soil and acclimatized to the growth chamber condition. Every regenerant soybean plant produced fertile flowers and set seeds. Polymerase chain reaction (PCR) results showed that they all had the BPMV CP precursor gene integrated into the genome. The expression of BPMV CP gene was also demonstrated by reverse transcriptase (RT) PCR and enzyme-linked immunosorbent assay (ELISA).

STABLE TRANSFORMATION OF SOYBEAN WITH MAMMALIAN DESATURASE VIA PARTICLE BOMBARDMENT AND SOMATIC EMBRYO CYCLING

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A modification of soybean somatic embryo culture has greatly improved the efficiency and rapidity of generating uniformly transgenic soybean embryogenic lines. By this modified technique, termed somatic embryo cycling, somatic embryos (1° SE's) are used instead of immature seed cotyledons to initiate new globular somatic embryos (called 2° SE's) on high auxin medium. By this method, somatic embryos yield large numbers of globular embryos in about 4 weeks. By particle bombardment of 1° SEs prior to initiation, with a plasmid (pBI426 from W. Crosby) containing a chimeric ß-glucuronidase /neomycin phosphotransferase (GUS:NPTII) gene, uniformly transformed 2° SE's were recovered which contained both GUS activity and kanamycin resistance. The 2°SE's could be either proliferated in liquid culture, or grown to mature somatic embryos to be germinated, as with standard somatic embryo cultures. We have recently recovered transgenic somatic embryo lines transformed with a plasmid containing GUS:NPTII and a mammalian delta-9 desaturase gene, driven by a seed-specific promoter. These lines contain significantly reduced saturated fatty acid levels in their cotyledons, and significant amounts of a monounsaturated fatty acid palmitoleic acid, which does not normally accumulate in soybean seed cotyledons. We are currently attempting to regenerate plants from desaturase-transformed somatic embryos.

TRANSFORMATION OF 12 DIFFERENT PLASMID INTO SOYBEAN VIA PARTICLE BOMBARDMENT

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Particle bombardment offers a simple method for the physical introduction of DNA into many different plant species. DNAs may be introduced on a single plasmid or on separate plasmids (cotransformation). To investigate some of the limits and properties of cotransformation, 12 different plasmids were introduced into embryogenic suspension culture tissue of soybean (*Glycine max* Merr.) via particle bombardment. The DNAs used for cotransformation included 10 plasmids containing RFLP markers for maize and 2 separate plasmids encoding hygromycin resistance and ß-glucuronidase. Two weeks following bombardment with the 12 different plasmids, suspension culture tissue was placed under hygromycin selection and hygromycin-resistant clones were isolated after an additional five to six weeks. Southern hybridization analysis verified the presence of plasmid DNAs in all hygromycin resistant embryogenic clones analyzed to date. All of the cotransforming plasmids were present in most of the transgenic soybean clones and there was no preferential uptake and integration of any of the plasmids. The copy number of individual plasmids was approximately equal within clones but highly variable between clones. While some clones contained as few as zero to three copies of each plasmid, others clones contained as many as fifteen to twenty copies of each of the 12 different plasmids.

CHARACTERIZATION OF TRANSGENIC SOYBEAN FOR SYNTHETIC *BACILLUS THURINGIENSIS CRYIA(c)*.

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One goal of our research program is to increase insect resistance in soybean. *Bacillus thuringiensis* (*Bt*) is a free-living bacterium that produces delta-endotoxins antibiotic specifically against certain insect taxa. Thus, the use of transgenic plants containing a *Bt* gene offers an attractive solution to widespread insect pest problems that soybean growers confront in the southeastern United States. Previously, we had shown soybean transgenic for a truncated native *Bt* gene effectively controlled feeding by velvetbean caterpillars, a species very susceptible to *Bt*. Currently, soybean cultivar 'Jack' was genetically transformed with a synthetic *Bt* insecticidal crystalline protein gene (*Bt Cry IA(c)*) using microprojectile bombardment. The plasmid pSG3525, which contains a hygromycin phosphotransferase (*HPT*) selectable marker and a truncated synthetic *CryIA(c)* gene that was designed to be codon-optimized in plants, both of which were under the control of the *CaMV 35S* promoter, was coated onto 1µm gold microprojectiles. Soybean somatic embryos were mashed and shot on a dry plate two times using the DuPont PDS1000 He device. The next day embryos were transferred to solidified Murashige and Skoog (MS) medium containing B5 vitamins, 20 mg/L 2,4-D, and 25mg/L hygromycin. Tissue was transferred to fresh medium every three weeks, and after 9 weeks, transferred to liquid Finer and Nagasawa (FN) medium. After acclimating 1 month on FN, tissue was transferred to FN with 25 mg/L hygromycin for final selection. Transgenic embryos were resistant to 50 mg/L hygromycin in FN. Three transgenic lines were selected and transferred to growth regulator-free MS medium with 60 g/L maltose and activated charcoal. After subsequent desiccation embryos were transferred to growth regulator-free MS medium for conversion into plants. DNA blot analysis revealed that all three lines contained the *HPT* gene, but the *Bt* gene was lost in one of the lines. The plasmid was rearranged in the second line, and the third line had two copies of the construct, one of which was rearranged. ELISA demonstrated that the two lines of *Bt* soybean had low levels of *Bt* toxin (~1 ng/mg extractable protein and ~9 ng/mg for the 1-copy and 2-copy lines, respectively). In bioassays, plants regenerated from the *Bt* lines had very strong insecticidal activity against the *Bt*-susceptible *Heliothis virescens* (neither *Bt* line sustained any damage) and strong activity against the relatively *Bt*-resistant *Helicoverpa zea* (only 7.5 % defliation for the 2-copy line and 19% defoliation for the one-copy line). In contrast, control lines sustained from 35% to 50% defoliation.

PRODUCTION OF ELITE ROUNDUP® TOLERANT SOYBEANS USING *AGROBACTERIUM*-MEDIATED TRANSFORMATION AND GLYPHOSATE SELECTION

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Glyphosate selection has been implemented into the soybean/ *Agrobacterium* transformation system. Utilizing elite soybean lines, cotyledon explants were excised from 5 day old seedlings and inoculated with an *Agrobacterium* strain conferring CP4, a Roundup® tolerant EPSP synthase gene, and ß-glucuronidase (GUS). Explants were selected on sublethal levels of glyphosate during shoot initiation and shoot elongation. Elongated shoots were rooted without further selection. The glyphosate employed was inhibitory for the formation of elongated shoots on explants inoculated with an *Agrobacterium* strain conferring only GUS. Progeny analysis demonstrated transmission of the transgenes to the R_1 generation. All plants that survived selection were Roundup® tolerant.

DEVELOPMENT OF SOYBEANS WITH A ROUNDUP READY™ GENE

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Soybean plants tolerant to Roundup® herbicide were developed by particle gun transformation using a plasmid encoding the enzyme EPSP synthase from *Agrobacterium* sp. strain CP4. Progenies of plants derived from transformation were evaluated successively in the greenhouse and in the field for tolerance to Roundup herbicide. Over three years of field trials in multiple locations, the best progeny did not show any yield reduction from treatments with up to 64 oz. of Roundup herbicide per acre at any time from emergence to early pod set. The field experiments also showed that the gene does not cause any agronomic changes to the plants besides the tolerance to Roundup herbicide. Numerous analytical and toxicological studies also demonstrated that the transformation and gene insertion process did not lead to any material differences in the composition or safety of the resulting soybeans relative to conventional soybeans. Commercialization of soybeans with a Roundup Ready™ gene will take place once commercial varieties have been developed through breeding and regulatory acceptance has been obtained.

OPTIMIZATION OF A HELIUM OUTFLOW GUN FOR PLANT GENETIC TRANSFORMATIONS.

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A vacuum-free Particle Inflow Gun (PIG)-like device was locally manufactured and tested for the purpose of genetically transforming plants. The modified PIG, or Helium Outflow Gun (HOG), uses flowing helium to propel microprojectiles and in the shooting chamber. The chamber has a helium intake valve at the top and a purge valve towards the bottom, which serve to displace atmospheric gases with helium prior to bombardment. The helium-filled chamber, like an evacuated chamber, provides minimal aerodynamic drag on microprojectiles. However, there is a vacuum line hookup to allow for bombardment under vacuum, should that be desired. Empirical optimization based upon transient β-glucuronidase activity of bombarded embryogenic cultures and leaf tissues suggests that the critical physical parameters are nozzle type, distance, and shot pressures. We have found that an unmodified Millipore nozzle with a stainless steel screen inserted, relatively low shot pressures (6-9 kPa) and close distances (5-7 cm) provided optimal results to date (blue foci per embryo clump, frequency of stained clumps). Thus far results compare somewhat unfavorably with those of the DuPont PDS 1000 He gun. However, we are in the process of designing and testing nozzles that include a gun barrel. One such apparatus has delivered tunsgten particles, shot at 12 kPa, through an oak leaf and into the lower epidermis. In addition we have designed a Blast Limitation Tube (BLT) that serves to immobilize tissue against the helium gas wave blast, so that tissue may be shot repeatedly and recovered quantitatively. By eliminating the use of a vacuum, the HOG and BLT may simplify the shooting process further, while the use of a helium atmosphere instead of a vacuum may be less stressful to target tissues.

HIGH RESOLUTION MAPPING AND RACE SPECIFICITY OF A PARTIAL RESISTANCE GENE FOR SOYBEAN CYST NEMATODE

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To identify genes associated with soybean cyst nematode (SCN) resistance, we analyzed two segregating soybean F2 populations (M83-15 X M85-1430 and 'Evans' X PI 209332) with restriction fragment length polymorphisms (RFLPs). Between the two populations, we identified 2-4 independent partial resistance loci significantly associated with SCN disease response. One of these loci (K69 cluster), common between both populations, appeared to be a major partial resistance gene located on linkage group G. This locus explained 64% ($P<0.0001$) of total phenotypic variation in Evans X PI 209332 and 35% ($P<0.0001$) in M83-15 X M85-1430. When challenged with three different races of SCN, the K69 resistance locus showed significant differential disease reactions, indicating some race specificity. To further analyze the effects of this partial resistance locus, we developed soybean lines with unique recombinations relative to RFLP markers already mapped to this region. We also developed "single" gene lines containing defined combinations of the three putative resistance loci to assess their race specificity. Experiments, including pulsed field gel analysis of high molecular weight DNA, are underway to construct a high resolution map of the K69 region as a starting point for cloning this major partial resistance gene.

INTERRELATIONSHIP BETWEEN TWO ENDOGLUCANASES CLONED FROM THE PHYTOPATHOGENIC FUNGUS *MACROPHOMINA PHASEOLINA* .

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Endoglucanases cleave internal β,1-4 linkages of cellulose, leading to a rapid loss in structural integrity of the substrate. Soilborne root and stem rot pathogens such as *M. phaseolina* produce highly active endoglucanases during both pathogenic and saprophytic stages of their life cycle. In an effort to better understand the spectrum of endoglucanases produced by *M. phaseolina* we have isolated these genes from a cDNA library. Screening was performed by functional expression in *E. coli*. Two types of endoglucanase genes were isolated. One type has strong homology to endoglucanase III produced by the saprophytic fungus *Trichoderma* , and would be expected to play a role in saprophytic growth of *M. phaseolina* . The second type of endoglucanase clone was unique, showing homology only to one other reported endoglucanase, isolated from a phytopathogenic bacterium. The hydrolytic activity of this pathogen-specific endoglucanase mimics the activity of a plant cell-wall bound endoglucanase, suggesting that it is a cell wall penetration-specific enzyme. It is proposed that *M. phaseolina* has two endoglucanase genes with distinct roles in the fungal life cycle.

ANALYSIS OF SOYBEAN CYST NEMATODE SECRETIONS INVOLVED IN PARASITISM

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The soybean cyst nematode (SCN), *Heterodera glycines*, has evolved a very specialized and complex feeding relationship with soybean. A successful host-parasite relationship requires SCN to elaborately modify several soybean root cells to obtain nourishment essential for their development and reproduction. Second-stage juveniles (J2) penetrate soybean roots and inject secretions from esophageal glands through their stylets into pericycle cells to transform these cells into specialized feeding sites called syncytia. These modified cells become the permanent feeding site for the parasite throughout its life-cycle. Infective J2 have three large and complex esophageal gland cells, one dorsal and two subventrals, where secretory proteins are synthesized and sequestered in secretory granules. Monoclonal antibodies (MAbs) are being used to identify secretory components produced by SCN. Stylet secretions produced in vitro from J2 and homogenates of J2 were used for intrasplenic immunization of mice to produce MAbs. Five MAbs that bound to secretory granules within the subventral glands and one MAb that bound to granules in the dorsal gland of SCN were developed. Three of the five subventral gland MAbs bound to stylet secretions from SCN J2 in immunofluorescence and ELISA assays. Two of the subventral gland MAbs bound to both dorsal and subventral glands in young SCN females. All five subventral gland MAbs bound to the subventral glands of the sugar beet cyst nematode (*H. schachtii*) and one of these MAbs bound to the subventral glands of the tobacco cyst nematode (*Globodera tabacum*). The MAbs are being used to screen a SCN cDNA expression library to isolate genes that encode the secretory proteins. A cDNA clone that has been isolated using the dorsal gland specific MAb is currently being sequenced and characterized. Isolated secretion genes will be compared among different SCN races to determine sequence homologies and differences that may relate to their host specificity on soybean cultivars.

RFLP MARKERS LINKED TO GENES AFFECTING RESISTANCE TO JAVANESE ROOT-KNOT NEMATODE IN SOYBEAN

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Javanese root-knot nematode [*Meloidogyne javanica* (Treub) Chitwood] is becoming a serious pest on soybean [*Glycine max* (L.) Merrill] in the southern USA. Inheritance of resistance in soybean to *M. javanica* (Mj) is quantitative, and heritability estimates range from 0.48 to 0.76. We report the identification and mapping of two quantitative trait loci (QTL) from PI230977 involved in resistance to Mj by means of restriction fragment length polymorphisms (RFLP). To identify these QTL, 321 DNA probes were screened for polymorphisms between the parents. Eighty-four F_2 plants from a cross between a susceptible cultivar (CNS) and a resistant soybean plant introduction (PI230977) were mapped with 82 RFLP. F_3 families derived from these F_2 plants were assayed for Mj galling in the greenhouse. Using MAPMAKER, the 82 markers mapped to 19 linkage groups (9 unlinked) spanning a total of 830 cM (Kosambi function). Most of the markers (87%) mapped to the same linkage groups as the USDA-ARS/ISU soybean RFLP map. The F_2 genotypic classes for each of 82 DNA markers were contrasted with Mj disease response to identify marker loci associated with Mj resistance. Eight RFLP markers were identified to be significantly ($p<0.0001$) associated with Mj resistance. Seven of these mapped to Linkage Group F and one, pA725, mapped to Linkage Group W. Among the markers on Linkage Group F, marker pB212 accounted for the greatest variation (47%), whereas pA725 accounted for 13% of the variation in Mj gall number. Together the markers accounted for 57% of the variation in a multiple regression model. Similar results were obtained when the data was analyzed with MAPMAKER-QTL.

IDENTIFICATION OF DNA AMPLIFICATION FINGERPRINTING MARKERS LINKED TO RESISTANCE TO FROG-EYE LEAF SPOT IN SOYBEAN

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Frog-eye leaf spot disease, caused by Cercospora sojina Hara., is a common foliar disease of soybean (Glycine max L. Merr.) in the southeastern United States. This disease is distributed world-wide and can cause significant yield loss. Four genes for resistance to the pathogen have been identified in several resistant cultivars, however, none of the genes have been characterized or cloned. Our long-term goal is to use a map-based cloning procedure to characterize the resistance gene(s). The objectives of this study were to identify PCR-based DNA amplification fingerprinting (DAF) markers which are tightly linked to the resistance loci. Two pairs of near-isogeneic lines or NILs (86205-2381 and 86205-2259), derived from the cross between a resistant parent (Stonewall) and a susceptible parent (Coker 6738), were used. Genomic DNA of each NIL and the parents was isolated from leaves at 2-trifoliate stage, and was amplified using 52 different primers with arbitrary sequences. The amplicons were separated by electrophoresis in a 5% polyacrylamide gel and silver stained. Three polymorphic DAF bands differentiating the NILs have been identified using two primers. Primer AU-Op2 of sequence 5'-CCGAGCTG-3' generated two major polymorphic bands (approximately 1,150 and 400 bp in length, respectively) that are present in the resistance NILs but absent in the susceptible NILs. Primer AU-8.14 of sequence 5'-GAACGGGT-3' generated one major polymorphic band (approximately 450 bp in length) that is present in the resistant NILs but absent in the susceptible NILs. The identified DNA markers are likely to be linked to frog-eye resistance loci.

APPROACHES TOWARDS CLONING THE *RPS1* LOCUS OF SOYBEAN

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The *Rps1* locus is one of the seven loci that carry genes conferring cultivar specific resistance to *Phytopthora sojae*, a fungal pathogen of soybean. This locus carries 5 genes, namely *Rps1-a*,*Rps1-b*, *Rps1-c*, *Rps1-d* and *Rps1-k*. Assuming the possibility that the product of *Rps1-k* is located in the plasma membrane, a cDNA expression library constructed in *Escherichia coli* was screened with antiserum raised against the purified plasma membrane. After screening 500,000 pfu, 44 classes of cDNAs were obtained. Out of these 44 classes, 11 showed sequence homology to available database entries for annexin, thioredoxin, calcium-dependent protein kinase, lipoxygenase, 70 kD heat-shock protein, vegetative storage protein, clathrin heavy chain, valosin containing protein, phospholipase C, yeast gene *ERG6* and pyruvate kinase. Eight out of these 11 genes encode putative plasma membrane proteins. We are performing RFLP analysis for possible linkage of candidate clones with the *Rps1* locus. In a second approach, we are isolating molecular markers that are linked to the locus. The closest marker is a retrotransposon-like element located 0.9 cM from *Rps1-k*. Once a high resolution map of these markers has been completed, tightly linked markers will be used for map-based cloning of the locus.

DIFFERENCES OF ALLELE FREQUENCIES AMONG SOYBEAN CULTIVARS

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The development of soybean cultivars has been described as "a very long" recurrent selection program, that can be traced back to just 12 ancestral lines. This limited germplasm base, and subsequent cyclic breeding strategy has raised questions concerning the amount of diversity present among soybean cultivars. Despite this apparent lack of diversity, breeders have been able to improve soybean cultivars. A retrospective analysis of soybean cultivar development, from a population view, could potentially identify genomic regions which have contributed to these improvements. Fifty-nine soybean ancestral lines and derived cultivars were analyzed at 94 RFLP loci distributed across the USDA-ARS:RFLP molecular map. After 5+ generations of development, 85% of the RFLP loci had allele frequencies that were not statistically different from the ancestral population from which they were derived. Six loci had allele frequencies which showed linear trends across the generations of development, and 10 loci had allele frequencies which showed linear trends across maturity groups. Some of the loci that showed linear trends have been reported in the literature to be linked to both qualitative (*Rps*1, *Rps*2, *Fap*2, and *Rf*2) and quantitative (oleic acid content) traits. After four generations of development, 10.6% of the genetic diversity present in the ancestral population had been lost. Previous literature had estimated the loss of diversity to be 20%. In summary, from a population view, the 5+ generations of soybean cultivar development has primarily resulted in a "shuffling" of the genome, apparently bringing together different favorable combinations of alleles, rather than converging on one, "ultimate", genotype.

A NEW STATISTICAL APPROACH TO FINGERPRINT ANALYSIS: MULTIDIMENSIONAL SCALING AND NONHIERARCICHAL CLUSTERING

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Molecular biologists often use fingerprint techniques to assess diversity in a collection of genotypes. A general approach is to score genotypes for a number of markers and then develop pairwise estimates of similarity between genotypes, usually defined as the proportion of markers that any two genotypes in the collection have in common. Similarity values between all pairs of genotypes can be summarized in a square matrix resembling a correlation table. The analysis and visual representation of patterns in such a matrix are the subject of this paper. Multidimensional scaling (MDS) and nonhierarchical clustering are proposed as new and attractive approaches in the analysis of similarity data. Multidimensional scaling is an iterative procedure available in SAS that develops a set of coordinates in Euclidean space for each genotype represented by the matrix. By selecting appropriate options in the procedure, a complex 'fingerprint' data set can be summarized in a two-dimensional road map. The map has two distinct empirical advantages over most competitors: 1) the similarity units in the original matrix are retained in the map so that map distances have direct genetic interpretations, and 2) information is spread nearly evenly over the two axes for ease of visual representation. Subjecting the coordinates developed through MDS to the FASTCLUS procedure in SAS identifies groups of related genotypes. Average coordinates for each cluster can be calculated and added to the map for visual interpretation. This iterative nonhierarchical clustering approach eliminates the family tree structure often associated with cluster analysis, providing instead results that are more compatible with a road map approach. The techniques are illustrated using a 258 x 258 similarity matrix developed from pedigree analysis of soybean cultivars. The two dimensional MDS map accounted for 60% of the variation in the similarity matrix and clearly reflected geographical origins of the cultivars. Adding 12 clusters from FASTCLUS to the road map allowed a clear interpretation of soybean breeding history in the USA.

RFLP AND COEFFICIENT OF PARENTAGE ANALYSES OF DIVERSITY IN ELITE AND PLANT INTRODUCTION SOYBEAN POPULATIONS.

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Coefficient of parentage analyses have shown the genetic base of cultivated North American soybean to be quite narrow. The base needs to be broaden to improve gain from selection and reduce genetic vulnerability. Parentage analyses also indicate that northern and southern US cultivars are quite distinct, even though they derive the majority of their parentage from a shared set of ancestors. Thus regional diversity could be increased by making inter-regional crosses as well as crossing with plant introductions that have not been previously used. The objectives of the research to be presented are to compare the diversity between four sets of soybean genotypes via RFLP analyses: northern elite cultivars, northern-adapted plant introductions, southern elite cultivars and southern-adapted plant introductions. In addition, RFLP and coefficient of parentage estimates of diversity and population structures among elite cultivars will be compared and examined.

THE APPLICATION OF GENETIC DIAGNOSTICS TO SOYBEAN BREEDING

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DNA-based diagnostics are now well established as a means to assay diversity at the locus, chromosome, and whole genome level. As technology has advanced, DNA nucleotide sequence-based assays have become easier to use, more efficient at screening for nucleotide polymorphisms, and available to a wider cross-section of the research community. A review of the use of molecular markers in several different areas of genetics and plant breeding will be presented, as well as a discussion about their advantages and limitations. Recent advances in several areas of technology development and laboratory automation will also be presented, including a characterization of simple sequence repeats in soybean.

THE PLANT GENOME DATA AND INFORMATION CENTER

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The Plant Genome Data and Information Center provides outreach and support services for the Plant Genome Research Program of the USDA/ARS. Located at the National Agricultural Library in Beltsville, Maryland, the Center provides access to a variety of information resources and services concerning all aspects of plant genome mapping. The Center regularly produces topical bibliographies and funded project summaries extracted from the AGRICOLA and CRIS Databases, a quarterly newsletter for the Plant Genome Research Program, **Probe**, and guides to using various electronic information resources. The PGDIC staff handles inquiries regarding genome mapping data and procedures, professional contacts, the use of scientific databases and software, and the progress of related research programs (e.g. the Human Genome Research Program). The main product of the Plant Genome Research Program is the Plant Genome Database, which standardizes and cross-references the data from the genome mapping projects of many crop species. Training and support services for PGD users are provided by the PGDIC staff. Demonstrations of the PGD, as well as of the other plant genome-related information resources that are available on the Internet, are provided by the PGDIC staff at scientific meetings.

You are encouraged to visit the PGDIC exhibit for computer demonstrations, informational materials, and to subscribe to the **Probe** Newsletter. For further information, contact the PGDIC staff at the address above. Susan McCarthy, Coordinator, Andrew Kalinski, Molecular Biologist and Information Specialist, Sara Ranck, Graduate Assistant. PHONE: (301)-504-6613, FAX: (301)-504-7098, INTERNET: pgenome@nalusda.gov

COMPARISON OF RAPD PRODUCTS ON AGAROSE AND DENATURING GRADIENT GELS IN SOYBEAN

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Randomly Amplified Polymorphic DNA (RAPD) bands were mapped in 100 recombinant inbred lines derived from a cross between Essex x Forrest. RAPD's were separated by agarose or denaturing gradient polyacrylamide gel electrophoresis (DGGE) in order to detect polymorphic bands which segregated in a mendelian fashion in the recombinant inbred lines. The two systems were compared on the number of bands and polymorphisms produced by each primer. Initial screening of thirteen primers against parental varieties showed a percent polymorphism of 8.33% on agarose gels with 10 bands out of 120 being polymorphic and 8.14% polymorphism on DGGE with 14 bands out of 172 being polymorphic. Although we saw more polymorphic bands on DGGE, several did not segregate as mendelian markers in our recombinant inbred lines. In addition in 2/3 bands which did segregate in a mendelian fashion in these same lines co-mapped with bands scored on Agarose gels. Therefore, Denaturing Gradient Gel Electrophoresis does not greatly increase the efficiency of RAPD mapping in soybean. The accuracy of RAPD-DGGE for estimation of relatedness between varieties is called into question.

ANALYSIS OF LINKED AFLP MARKERS GENERATED BY tec-MAAP FOR GENE MAPPING OF THE SUPERNODULATION *nts* LOCUS

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Nodulation mutants of legumes have been used for the genetic analysis of autoregulation in symbiotic interactions with *(Brady)rhizobium* bacteri. Supernodulating soybeans develop abundant root nodulation which appears to be conditioned by a shoot-derived factor. The *nts* locus segregates as a single recessive Mendelian trait, but the altered gene product or its function are still unknown (1). Although an RFLP was mapped to ≤0.5 cM of *nts* in linkage group H of the USDA-ARS/ISU soybean map (2,3), positional cloning requires detection of additional linked markers to this mutation. Multiple arbitrary amplicon profiling (MAAP) techniques were used to generate band patterns from parental, mutant and recombinant individuals with a number of structured and unstructured oligonucleotides. No polymorphisms were confirmed using RAPD conditions or certain DNA amplification fingerprinting (DAF) schemes. However, 20 tightly-linked AFLPs to the *nts* locus were only detected in complex DAF patterns after pre-digestion of template DNA [template endonuclease-cleaved MAAP or tec-MAAP (4)]. We examined these AFLPs to isolate additional and reliable markers for high-resolution linkage and physical mapping of the *nts* locus. Conversion of confirmed AFLP to sequenced/amplifiable genetic markers (STS, SCAR), will facilitate the localization and subsequent capture of the respective genes from cloned DNA fragments in YAC vectors (see Funke et al., this meeting). We will present results on AFLP purification, cloning and advances in confirmation of their identity, polymorphic nature and genetic linkage. Together with bulk segregant analysis (5), tec-MAAP may accelerate the saturation of specific genomic regions with useful markers, for the study of the soybean genome.

1. Gresshoff, PM (1993). Plant Breed Rev 11: 275-318.
2. Keim et al. (1990), Genetics 126: 735-742.
3. Landau-Ellis et al. (1991), Mol. Gen. Genet. 228: 221-226.
4. Caetano-Anollés et al. (1993), Mol. Gen. Genet. 241: 57-64.
5. Michelmore et al. (1991), Proc. Natl. Acad. Sci. USA 88: 9828-9832

MOLECULAR MAPPING OF A DAF MARKER IN SOYBEAN (*Glycine max* L.) USING RECOMBINANT INBRED LINES

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DNA Amplification Fingerprinting (DAF; 1) was used to map the first DAF marker in the soybean genetic map which was constructed using an intraspecific cross between two *Glycine max* cultivars: 'Minsoy' and 'Noir 1'(2). The mapping population consisted recombinant inbred (RI) lines obtained from a cross between 'Minsoy' and 'Noir 1'. Three DAF markers DAF 220, DAF 233 and DAF 234 mapped at distances ranging from 1.2 to 2.8 cM to existing markers and with LOD scores ranging between 7.4 to 12.5. The three markers mapped to three different linkage groups.

Twenty-five oligonucleotide primers (8 mers) ranging in GC content between 60 - 100%, were used to screen parental DNA from 'Minsoy' and 'Noir 1'. Silver stained polyacrylamide gels showed 16 polymorphic markers between 'Minsoy' and 'Noir 1'. Sixteen percent of the primers tested showed more than one polymorphic marker. All the four DAF markers tested showed a 1:1 segregation ratio in RI lines. Two of the five markers analysed showed a LOD score of <3. Current efforts are aimed at screening the remaining RI lines in order to improve LOD scores.

This work with RI lines was based on an initial study of the inheritance of DAF markers using a F_2 population generated from a cross between *Glycine soja* PI468.397 and *Glycine max* (L.) Merrill line nts382 (3). Fifty percent of the primers that were used gave polymorphic markers in both populations. Except for one marker, the polymorphic markers for 'Minsoy' and 'Noir 1' exhibited different molecular weights than for nts382 and *G. soja*.

References

1. Caetano-Anollés G, Bassam BJ, Gresshoff PM: DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Bio/tech 9: 553-556 (1991).

2. Lark KG, Weisemann JM, Matthews BF, Palmer R, Chase K, Macalma T: A genetic map of soybean (*Glycine max* L.) using an intraspecific cross of two cultivars: 'Minsoy' and 'Noir 1'. Theor Appl Genet 86: 901-906 (1993).

3. Prabhu RR, and Gresshoff PM: Inheritance of polymorphic markers generated by short single oligonucleotide primers using DNA amplification fingerprinting in soybean (in review).

MOLECULAR ANALYSIS OF AN RFLP MARKER CLOSELY LINKED TO THE SUPERNODULATION LOCUS OF SOYBEAN

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The pA-132 probe, detecting a *Dra*I polymorphism between *Glycine max* and *Glycine soja*, was linked to the supernodulation (nts) locus of soybean using RFLP analysis of F2 populations generated from crosses between nts382 X *G. soja* and nts1007 X *G. soja* (Landau-Ellis and Gresshoff, 1992). The marker is positioned on linkage group 'H' and was generated as a random genomic *Pst*I insert (Keim et al. 1990). The insert, however, was not unique and three PstI fragments were detected in the probe. To clarify the question whether the three fragments were contiguous, we analyzed a genomic lambda clone as well as the segregation of the three inserts in an F2 population. Only one (pUTG-132a, a 1700 bp insert) was tightly linked (about 0.3cM) to *nts*, while the other two segregated independently from pUTG-132a as well as from each other (Landau-Ellis and Gresshoff, 1994).

The subclone pUTG-132a was sequenced and primers were designed to amplify this section of DNA. The original polymorphism was explained by an insertion of a 0.8kb fragment in *G. max* flanked by a 31 bp repeat present only once in *G. soja*. Amplifications with the pUTG-132 primers were made from DNA of *G. soja* PI468.397, *G. max* Bragg and its mutant lines, *G. max* Peking, thirty Agrigenetics breeding lines, and twelve soybean ancestral lines. Polymorphism was detected between *G. soja* on one side and all varieties of *G. max* on the other. This same lack of polymorphism was observed with Southern hybridizations of pUTG-132a onto the Agrigenetics lines which were restricted with 12 different restriction enzymes.

Approximately 1200 bp of the 1.7 kb fragment amplified from *G. soja* PI468.397, *G. max* Peking, and the *G. max* lines Bragg, Noir 1 and Minsoy were sequenced, resulting in sequence polymorphisms only between *G. soja*, Peking, and the other *G. max* lines which had identical sequences. The average polymorphism between *G. soja* and *G. max* Bragg comprised one bp out of 50; while the average polymorphism between *G. max* Peking and other *G. max* lines was one in 80 bp.

In conclusion, the marker studied shows remarkable conservation although there is no evidence of its functionality. Several other legumes and non legumes were amplified with the primers for pUTG-132a but only Cowpea (*Vigna unguiculata*) exhibited a faint amplification product.

References:

Keim, P., et al. 1990. Genetics. 126:735-742.
Landau-Ellis, D., and P.M. Gresshoff. 1994. Mol Plant Microbe Interact. In Press.
Landau-Ellis, D., and P.M. Gresshoff. 1992. Mol Plant Microbe Interact. 5:428-429.

CHARACTERIZATION AND ANALYSIS OF CLONES FROM A PARTIAL SOYBEAN YEAST ARTIFICIAL CHROMOSOME LIBRARY

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Our eventual goal is the positional cloning of a segment of soybean DNA that contains an important gene regulating early stages of nodulation. With this in view, we are building a library of soybean DNA in yeast artificial chromosomes. After a few trials we opted for a procedure that includes a single size selection step of partially digested soybean DNA. Clones produced in this way have an average insert size of 150 kb, and range from 50 to 1000 kb.

To assess the stability of soybean YACs, we checked whether their size and integrity were preserved after a known number of generations of growth in selective and rich media. An experiment was designed to mimic what happens during storage and handling of a YAC library as individual clones are streaked out repeatedly and propagated in liquid culture. The soybean YACs did not show visible rearrangements or changes in length. A clone with high repetitive DNA contents was as stable as YACs containing primarily low copy number sequences.

Since the soybean genome consists for 30% of repetitive DNA, we were concerned that chromosome walking using YACs would be too arduous because end clones would hybridize to many unrelated YACs with cognate repetitive sequences. To investigate this, we used the "vectorette" procedure to amplify the ends of three YACs, and hybridized the labelled ends to restricted soybean DNA. Two of the YACs were judged to have high repetitive DNA contents by their intensity of hybridization to soybean DNA. We also used a 200 kb YAC which contained largely single copy sequences .

The ends of soybean YACs do not appear to be enriched for repetitive sequences (which could be the case if a common repetitive element contained an *EcoR*I site) and chromosome walking could be a feasible complement to the preferred objective of landing on a specific large YAC using tightly linked markers.

ISOLATION AND CHARACTERIZATION OF GENOMIC CLONES FOR SOYBEAN CDC2 PROTEIN KINASE

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Early events in *Rhizobium*-leguminous plant interaction leading to nodule organogenesis require initiation of meristematic activity in root cortex (1). This step involves mitotic cell division, which is regulated by a protein complex known as the maturation promoting factor (MPF). The key components of cell-cycle-control machinery are a $p34^{cdc2}$ protein kinase and a cyclin.

Molecular cloning of plant $p34^{cdc2}$ protein kinases provides the basis for mechanistic analysis of the regulation of cell proliferation and research in this area is now of a great interest, although the entire genomic clones for cdc2 have been isolated only from *Arabidopsis thaliana* (2).

The objective of our study is to isolate and characterize soybean genes regulating cell division and use their promoter regions with chimeric reporter genes to analyze their expression during induction of nodulation of soybean.

The lambda GEMII genomic library made of cv. Bragg size-fractionated DNA (10^6 recombinants) was screened by using as a probe soybean cdc2-S6 cDNA clone kindly supplied by Prof. Desh Pal S. Verma (3). Five positive clones containing inserts of interest have been isolated. The restriction map of each insert was constructed by both partial and complete digestion of phage DNA followed by Southern hybridization. Two classes of clones were detected: class 1 but not class 2 has the internal *Xho*I site similar to the soybean cdc2-S6 cDNA clone. This might indicate that genes of class 1 were the genomic source of that cDNA clone.

Both classes of the two isolated soybean cdc2 protein kinase genomic clones are being sequenced. Our preliminary data suggest that the genomic clone of class 2 is truncated at 5'-end of coding region, while the genomic clone of class 1 might represent a full cDNA clone. The entirely sequenced 1.0 kb *Hin*dIII fragment of class 1 clone shows that 5'-end of coding region is interrupted by at least three introns. Intron 2 is located at the same site as that of the *S. pombe* cdc2 gene (4) (intron II) and *A. thaliana* CDC2a gene (intron III), although the lengths and sequences of corresponding introns are diverse. To investigate exon-intron organization of the *G. max* cdc2 genes as well as their 5'-untranslated regions, the complete nucleotide sequences of isolated clones must be performed.

References:

1. Caetano-Anollés G. and Gresshoff P.M. (1991). Ann. Rev. Microbiol. 45, 345-382.
2. Imajuku Y., Hirayama T., Endoh H., and Oka A. (1992). FEBS 304, 73-77.
3. Miao G.H., Hong Z. and Verma D.P.S. (1993). Proc. Natl. Acad. Sci. USA 90, 943-947.
4. Hindley J. and Phear G.A. (1984). Gene 31, 129-134.

RFLP MARKERS FOR ENOD2 SHOW MENDELIAN INHERITANCE IN A SOYBEAN F_2 POPULATION

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ENOD2, an early nodulin gene encoding a hydroxy-proline rich cell wall protein with molecular weight of 75,000, N-75 nodulin (1), is expressed in nodule parenchyma (2). It has been postulated to be involved in the oxygen barrier inside of legume nodules. However there is no evidence for its function , other than the localization of the protein. The purpose of this study is to map this gene to a soybean linkage group using RFLP technology. Parental materials were cultivar Bragg, *Glycine max (L. Merr)*, and the ancestral soybean, *G. soja* , PI468. 397. DNA samples from parents were digested with *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Taq*I, and *Xba*I restriction enzymes. Digested DNA samples were blotted on a Nylon membrane and then hybridized with ^{32}P-labeled ENOD2 probe. Autoradiograph of Southern blot hybridization showed polymorphisms for samples digested with *Eco*RI, *Eco*RV, and *Hin*dIII. In case of the *Eco*RV digestion, polymorphisms consisted of two copies. After detection of polymorphisms in parental materials, DNA samples were isolated from an F_2 population generated by a cross between Bragg and *G. soja*. Following digestion with *Eco*RV, DNA samples were blotted and hybridized with the ENOD2 probe. Banding patterns of 30 F_2 plants on autoradiographs were studied. The results showed a ratio of 8: 17: 5 plants with band patterns of Bragg, heterozygote, and *G. soja*, respectively. A Chi square test revealed no significant differences between this ratio and the expected 1: 2: 1 Mendelian ratio, assumed for a single locus. This study enables us to study cosegregation of this RFLP marker and other molecular markers on the Iowa State University / USDA soybean linkage map to map the ENOD2 gene.

References

1. Franssen, H. J., Nap, J. P., Gloudemans, T., Stiekema, W., van Dam, H., Govers, F., Louwerse, J., van Kammen, A., and Bisseling, T. 1987. Proc. Natl. Acad. Sci. 84: 4495-4499.

2. Allen, T., Raja, S., and Dunn, K. 1991. Molecular Plant-Microbe Interactions. 4: 139-146.

A MAJOR SATELLITE DNA OF SOYBEAN: 92 BASE PAIRS TANDEM REPEAT

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We report the cloning, sequencing and analysis of the major satellite DNA of soybean (*Glycine max*). A prominent fragment of 92 bp and its multimers can be seen when total soybean DNA is digested with some 6-cutters, run in acrylamide gels and stained with silver. The repeat was cloned as several monomers and trimers produced by *Xho*I. The consensus sequence is 92 base pairs long, and real sequences do not fluctuate in length. Their average homology to the consensus sequence is 92%. The 92 bp consensus contains slightly degenerated homologies for several 6-cutters. Therefore many of them (*Xho*I, *Cla*I, *Xba*I and others) generate a typical ladder of 92 bp oligomers. The distribution of bands seems to be random, but the occurrence of sites for different 6-cutters varies widely. There is no obvious correlation between the sequences of the neighboring 92 bp units in cloned trimers. Also, there are no internal repetitive blocks reported for many satellite DNAs from other species. The screening of the EMBL Database showed weak homology of the 92 bp consensus to several genes for tRNA and 5S RNA (all below 60%), but not to any known satellite DNAs. The 92 bp sequence makes about 0.7% of total soybean DNA, or is represented by 8×10^4 copies, or 7 megabases. The hybridization of 92 bp repeat to soybean DNA digested with an enzyme that does not cut the repeat and run on a pulse field gel electrophoresis shows the signal in the compression zone only, well above 500 kb. Therefore, the repeat is organized in giant tandem blocks and there are fewer blocks than chromosomes. The 92 bp is present in identical arrangement and number of copies in the ancestral subspecies *Glycine soja*, in approximately 10 times fewer number of copies in a related species *Vigna sinensis* (cowpea), but not in several other more distant leguminous plants studied.

IDENTIFICATION OF RFLP MARKERS LINKED TO THE *w1* LOCUS AND *ms* LOCI IN *G. MAX*

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Several recessive mutations in *G. max* that specifically affect pollen development have been described by Reid Palmer and his colleagues [reviewed in Graybosch, R.A and Palmer, R.G. (1988) *Amer. J. Bot. 75*:144-156]. For example, plants homozygous for mutations at either the *ms2* or *ms6* locus exhibit degeneration of tetrads as well as aberrant tapetal and anther wall development, resulting in shrunken, distorted anthers, whereas the *ms1* mutation results in complete failure of cytokinesis.

As a first step toward understanding the molecular basis of male-sterility in mutant plants, we have generated F2 populations needed to identify RFLP markers tightly linked to the *ms2*, *ms1*, and *ms6* loci. Crosses are shown in the following table:

Locus	Female Parent	Pollinator
ms2	*G. max* isoline L74-01 (Williams^{6} X T259H) *ms2ms2 w1w1*	*G. soja* PI 468.916 *Ms2Ms2 W1W1*
ms1	*G. max* isoline L74-03 (Clark L6^{6} X T260H) *ms1ms1*	*G. soja* PI 468.916 *Ms1Ms1*
ms6	*G. max* T-295 *ms6ms6 w1w1*	*G. soja* PI 468.916 *Ms6Ms6 W1W1*

Because both *ms1* and *ms6* are linked to the white color marker *w1* in linkage group 8, we have focussed on the L74-01 X PI 468.916 F2 population, which will allow us to assign all three *ms* loci to RFLP linkage groups. As expected, ~25% of this population was male sterile and ~25% had white flowers. In an initial screen of DNA from 24 *w1w1* plants, we determined that RFLP probes pA401 and pK390 are linked to the *w1* locus, with ratios of *G. max*:F1:*G. soja* banding patterns of 11:5:0 and 18:6:0, respectively. We are currently screening DNA from a population of 35 *ms2ms2* plants.

INDENTIFYING MOLECULAR MARKERS ASSOCIATED WITH COMPONENTS OF INSECT RESISTANCE IN SOYBEAN

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Restriction fragment length polymorphisms (RFLPs) in genomic DNA are found between soybean genotypes known to be susceptible and resitant to insect defoliation. The genotypes used in this experiment are Cobb (susceptible) and PI171451, PI227687, and PI229358 (resistant). Correlations are drawn between segregation of RFLPs and segregation of phenotypic expression in F_2-derived lines from susceptible x resistant crosses.

Greenhouse defoliation screening procedures have been developed to separate antibiosis and antixenosis components of insect resistance. The insect used in this experiment is the corn earworm (*Helicoverpa zea* Boddie). Antibiosis tests have shown significant differences between Cobb and the three resistant PIs in the categories of leaf-area defoliated, head capsule size, larval survival rate, and mean and total larval weight. Antixenosis tests have shown significant differences in leaf-area defoliated. Heritibility studies indicate that insect resistance is controlled by three or more genes; some, but not all, of which may be shared by these three PIs.

To date, the four parent genotypes have been screened with 251 RFLP probes. Polymorphism rates between susceptible and resitant genotypes range from 39% to 53% based on DNA restriction by five different endonucleases.

IDENTIFYING RFLP MARKERS LINKED TO STRESS TOLERANCE TRAITS IN SOYBEAN

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Abiotic and biotic stresses are the principal contributors to low profitability of soybean throughout the world, and are of particular consequence in the southern United States. Drought and heat are particularly damaging, and sensitivity to these stresses is compounded in the southern U.S. by acid subsoils with toxic levels of aluminum that restrict root growth. The most damaging biotic stress is that caused by the soybean cyst nematode (SCN) which imposed a 3% yield loss and $115 million loss in the southern states in 1991.

To-date, little progress has been made in breeding for complex traits such as drought, heat, and aluminum tolerance due to the absence of rapid and reliable screening procedures that identify tolerant genotypes. Marker-assisted selection may be useful in this regard. To identify molecular markers linked to stress tolerance traits, RFLP linkage maps are being constructed in populations which segregate for field drought tolerance and aluminum tolerance (Young × PI 416937), rooting and root recovery (Lee74 × PI 416937), SCN tolerance (PI 97100 × Coker 237), and water use efficiency (S100 × Tokyo). Field drought tolerance is measured as drought tolerance index (DTI): DTI = (seed yield in unirrigated subplot ÷ seed yield in irrigated subplot) X 100, and 120 recombinant inbred lines of Young × PI 416937 were planted at 4 locations in 1994 to measure this trait. The same population is being screened for aluminum tolerance using laboratory solution culture methods. Rooting is being assessed in 146 recombinant inbred lines of the Lee74 × PI 416937 population by inverting the soybean plants with a peanut inverter and rating the number of observable roots. Root recovery is being screened with a greenhouse assay which measures root growth after repeated cycles of water stress. Water use efficiency is being estimated in F_2-derived lines of S100 × Tokyo by a greenhouse assay, by carbon isotope discrimination, and by ash content. SCN tolerance is expressed as (seed yield in untreated plots ÷ seed yield in nematicide-treated plots) X 100. Tests for SCN tolerance are being conducted in 1994 at two SCN-infested locations with 120 F_2-derived lines of PI 97100 × Coker 237.

Sufficient polymorphisms have been identified between the parents of these populations to construct linkage maps. The linkage map of PI 97100 × Coker 237 currently has 90 mapped loci in 19 linkage groups. The map of this population is being constructed with all available polymorphisms including heterologous probes derived from genomic or cDNA libraries of mungbean, peanut, and alfalfa. This map and the USDA/ARS-ISU map will provide a framework for constructing well-spaced maps in the other populations of interest.

CLONING OF SOYBEAN PARTIAL cDNAS FOR HYDROXYMETHYL-GLUTARYL-COENZYME A REDUCTASE

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Hydroxymethyl-glutaryl Coenzyme A reductase (HMGR) is involved in cholesterol biosynthesis in animals and in the biosynthesis of isoprenoid compounds and sterols in plant cells, and genes for HMGR have been isolated from several plant species. We have screened soybean cDNA libraries generated from RNA of immature soybean seeds (R4 stage) or from soybean racemes at flowering to identify cDNAs homologous to HMGR genes. Single-stranded raceme cDNAs were selected by hybridization to tomato HMG-1 cDNA immobilized on nylon membrane (Pramanik and Bewley, Plant Molec Biol 20: 31), and selected cDNAs were cloned into λgt10. R4 seed cDNAs were cloned into λZAP and screened by colony hybridization to a 32-bp degenerate oligonucleotide probe for a conserved region of HMGR genes; selected clones were amplified and the phagemid recovered by <u>in vivo</u> excision. Phage extracts containing raceme cDNAs and plasmid preps carrying R4 cDNAs were amplified by PCR using oligonucleotide primers for a 275 bp conserved region of HMGR. Several clones from both cDNA populations produced amplified regions with these HMGR primers. Clones positive for HMGR primer amplification were then amplified using T3 and T7 primers, and a few clones with inserts of ≥0.5 KB are currently being characterized.

DETECTION OF CYTOKININ BIOSYNTHESIS ENZYMES IN SOYBEAN

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Genes encoding enzymes capable of cytokinin metabolism are useful probes of plant development. We have assayed cytokinin biosynthesis in soybean roots and immature seed and strains A281, A208 and LBA4404 of *Agrobacterium tumefaciens*. Briefly, enzymes were extracted and partially purified by ammonium sulphate precipitation and anion exchange chromatography on DEAE-cellulose. Enzyme preparations were incubated with ^{3}H-AMP and ^{14}C-IPP, the substrates of the enzyme encoded by the *ipt* and *tzs* genes. Products were separated by reverse phase HPLC using two distinct buffer systems.

In both plant and bacterial extracts radiolabelled products which co-eluted with zeatin were detected as the major cytokinin product. Soybean root also produced substantial amounts of zeatin riboside whereas soybean embryos produced mainly zeatin. We conclude immature embryos are capable of cytokinin biosynthesis and will provide an enriched source of enzyme for further purification.

Assays of activity from *A. tumefaciens* strain A281 (pTiBo542) consistently produced a compound which co-eluted with dihydrozeatin. We conclude this strain may encode an active zeatin reductase not present in other Agrobacteria.

GENOMIC ANALYSIS OF ENOD40 CLONE.

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ENOD40 is an early nodulin located in the pericycle surrounding the nodule vascular bandle and expressed in association with meristematic activity which is controlled by specific interactions between host plant and symbiotic bacteria within the plant cells (1,3).

Two 20 base primers complimentary to the left and right ends of a cDNA ENOD40 sequence from soybean were synthesized (2). These primers, placed about 500 bp apart on the cDNA sequence were used in the polymerase chain reaction (PCR) to amplify a 520 bp product from genomic soybean DNA suggesting the absence of an intron in this gene region. The PCR product was cloned and used to isolate a genomic lambda clone (14 bp). The lambda clone was subcloned to reveal a 1.0 kb *Hind* III fragment with strong homology to the PCR probe. Sequencing of the clone is in progress. Genomic Southern blots with the PCR product clone revealed two, possibly three copies in agreement with data from H. Kouchi, but contrary to those from T. Bisseling (presumed to be a partial digest)(3).

The expression of ENOD40 during nodule development was analyzed by Northern hybridization using RNA isolated from roots of soybean cv. Bragg as well as supernodulating (nts382) and nonnodulating (nod39, nod49) mutants. The size of the mRNA coding for ENOD40 was estimated to be about 700 bp and the expression was detected 2 days after inoculation. Although the transcript level was much higher in inoculated plants, the presence of respective mRNA was detected in uninfected roots.

References:

1. Kouchi H., and Hata S. (1993). Isolation and characterization of novel nodulin cDNAs representing genes expressed at early stages of soybean nodule development. Mol Gen Genet 238: 106-119.

2. Kruchinina N. and Gresshoff P. M. (1993). Isolation of an ENOD40 clone from a genomic library of *Glycine max* using PCR. Fourth Gatlinburg Symposium. Plant Genome Analysis. Abstracts. P. 23.

3. Yang W., Katinakis P., Hendriks P., Smolders A., Vries F., Spee J., Kammen V., Bisseling T. and Franssen H.(1993). Characterization of GmENOD40, a gene showing novel patterns of cell-specific expression during soybean nodule development. The Plant Journal. 3(4): 573-585.

EARLY DEVELOPMENT AND MOLECULAR CHARACTERIZATION OF FASCIATED SOYBEAN, *Glycine max* (L.) MERR.

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Establishment and maintenance of the apical meristem is critical for plant development and organ differentiation. We investigated early development of apical meristems, from 1 to 20 days after germination (DAG) in normal and fasciated plants. The phyllotaxy of the fasciated plant was changed from alternative to almost whorled arrangement. The fasciated plant showed strong apical dominance, with few or no branches. Scanning electron microscopy study showed that pattern of development of fasciated phenotype is caused by morphological changes of apical meristem detectable as early as 1-2 days after germination. The sequential development of leaf primordia was altered in mutant. Both primordia of single leaves were initiated at the same time, whereas primordia of normal plant were timely and spatially separated in development. The short plastochron of the mutant continues throughout trifoliate leaf initiation. The shorter plastochron of the fasciated plant compared with the normal plant may be attributed to the larger apical meristem size (2-3 times that of the normal plant at 7 DAG). The enlarged size of the apical meristem enables initiation of three or more leaf primordia at the same time giving rise to the whorled arrangement of the leaves on the plant. The change of the size of apical meristem in fasciated phenotype was a prerequisite condition for the subsequent changes in shape of the meristems, which were square- to ridge-like depending upon genetic background of the isolines tested. The temporal and spatial occurrence of cotyledonary axillary buds in normal and mutant was the same, but inhibition of leaf axillary bud primordia was observed in mutant. The overdominance of apical meristem in fasciated mutant caused alteration of floral differentiation. cDNA libraries were developed from imbibed embryos (apical part), leaf tissue at V3 and apical meristems at V4 stage of mutant plant. Parallel mapping experiment with the use of RFLPs and RAPDs will permit assignment of the f locus on the molecular linkage map and examination of the f locus association with transcriptional activity. This study will contribute to elucidation of specificity of genes involved in soybean development as compared to CLAVATA1, FASCIATA1 and FASCIATA2 genes in *Arabidopsis*.

ISOLATION AND CHARACTERIZATION OF A SOYBEAN cDNA CLONE ENCODING A 100 KD HEAT SHOCK PROTEIN

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A cDNA clone encoding a 100-kilodalton heat shock protein (hsp100) of soybean (*Glycin max cv* William 82) has been isolated and sequenced. Genomic DNA blot analysis with this cDNA clone indicates that the corresponding *hsp100* gene family is composed of a low copy number of genes. Transcripts homologous to this cDNA clone were not detected in two-day-old etiolated soybean seedlings grown at 28°C but were induced by elevated temperature treatment at 40°C. Polyclonal antibodies were raised against a synthetic peptide of 15 amino acid residues derived from the 3' end of the open reading frame of this cDNA clone. These polyclonal antibodies reacted with a 100 kD peptide extracted from soybean seedlings incubated at 40°C but not at 28°C. DNA sequence comparison shows that this gene family belongs to an evolutionarially conserved gene family, the *Clp* gene family, found in bacteria, fungi and plants. Based upon the similarity at the amino acid sequence level and the length of the spacer region between two highly conserved nucleotide binding domains, this soybean gene belongs to the *ClpB* subfamily. The phenomenon in which cells can survive an otherwise lethal heat treatment after they are incubated with a permissive heat treatment is referred to as the acquisition of thermotolerance. It had been demonstrated that a yeast ClpB homolog, hsp104, is required for acquisition of thermotolerance. The function of soybean hsp100 is tested by complementation of a yeast *hsp104* deletion mutant in the acquisition of thermotolerance.

ANALYSIS OF THE OMEGA-3 FATTY ACID DESATURASE IN SOYBEAN GENOTYPES A5 AND A23

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Little is known about the molecular basis of mutations in soybean [*Glycine max* (L.) Merr.] genotypes A5 and A23 which result in reduced linolenic acid ($18{:}3^{\omega 3,6,9}$) in the seed and roots. Germplasm with reduced levels of this fatty acid was identified from the cross of the two mutant lines A5 x A23. Reduction of linolenic acid content in soybean oil could enhance flavor stability and reduce the need for chemical hydrogenation. A5 has a major gene controlling reduced linolenic acid, at a locus independent of *fan2*(A23). The locus in A5 is designated *fan*(A5). The objective of this project was to describe the molecular nature of both mutations. DNA from genotypes was analyzed by gel blot hybridization with the cDNA coding for the microsomal ω3 linoleate desaturase to identify polymorphisms. The cDNA strongly hybridized to two DNA fragments in lines wildtype for linolenic acid as opposed to one fragment in the A5 mutant. Seventy F_2 derived lines from a population segregating for alleles of the *fan*(A5) and *fan2*(A23) loci were scored for the presence or absence of the fragment. The absence of the fragment was associated with reduced linolenic acid and accounted for 67% of the variation for linolenic acid in the population. These results suggest that the phenotype of the A5 mutant is a result of a deletion of a microsomal ω3 linoleate desaturase gene. Additionally, the microsomal ω3 linoleate desaturase was genetically mapped in a *G. max*/*G. soja* F_2 derived

DEVELOPMENTAL PATHWAYS OF SOYBEAN MORPHOGENESIS *IN VITRO*

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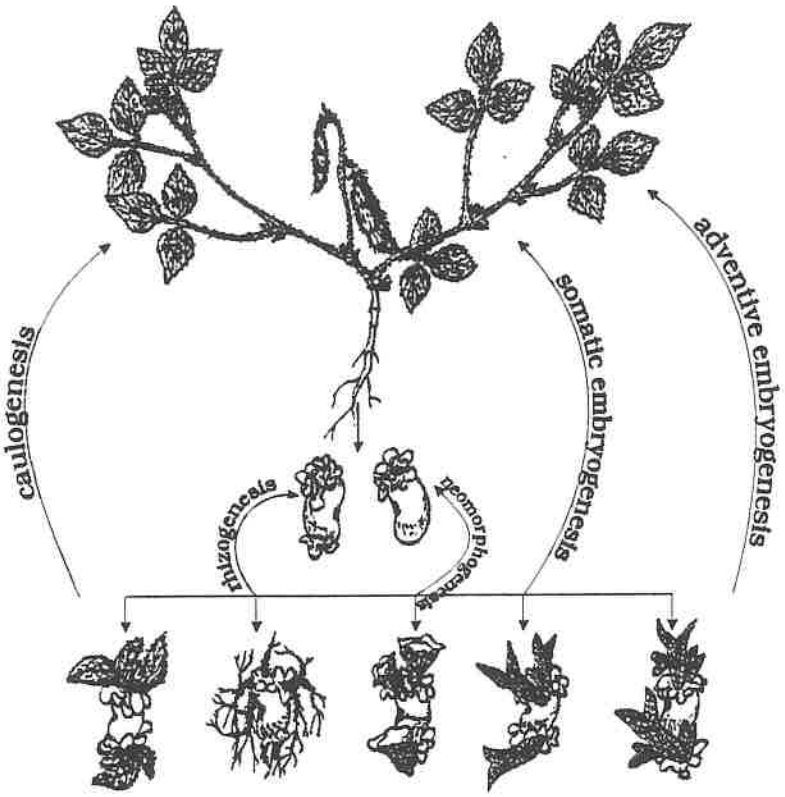
Four different ways of *in vitro* morphogenesis were initiated simultaneously from immature cotyledons of Hungarian soybean cultivar ISZ-15: (1) a *de novo* shoot and root organogenesis, (2) a primary-, and (3) a secondary somatic embryogenesis and (4) an embryo over development (neomorph formation). The characterised embryo abnormalities were presumed to be the reason of plant regeneration difficulties in soybean tissue cultures. In plant tissue culture studies the key point is the successful timing of the de- and redifferentiation cycle of the manipulated plant. In soybean, the dedifferentiation can easily be initiated but the redifferentiation processes have been found to be very tedious. Despite several articles reporting somatic embryogenesis a mass plant regeneration have not been achieved so far. The low frequency of plant regeneration was partly explained by the serious embryo abnormalities occurring during somatic embryogenesis and embryo germination. The different tissue culture techniques provide optimal conditions for one of



these developmental pathways. Nevertheless, simultaneous organogenesis and embryogenesis has been reported for both monocots and the dicot soybean plant. Our results also show that embryogenesis and organogenesis can occur simultaneously in the soybean culture of ISZ-15.

Reference: Gyulai G, E Kiss, Z Horváth, A Csillag, LE Heszky (1994):Alternative pathways of soybean morphogenesis in vitro. Histological and SEM analysis. Plant Cell Tissue Organ Cult (in press)

CHARACTERIZATION OF A HOMEOBOX-CONTAINING GENE SHOWING ENHANCED EXPRESSION DURING SOYBEAN (*GLYCINE MAX*) SOMATIC EMBRYO DEVELOPMENT

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Homeotic genes are key "switches" that control developmental processes. Homeotic genes containing the consensus "homeobox" domain have been identified from a number of organisms including *Drosophila melanogaster*, *Caenorhabditis elegans*, *Homo sapiens*, and *Zea mays*. Although homeotic genes have been demonstrated to be important in embryo development of some insects, amphibians, and mammals, there are no reports of their involvement in plant embryogenesis. Here, we report the isolation and characterization of a cDNA clone for a homeobox-containing gene expressed in somatic and zygotic embryos of soybean. The cDNA (*Sbh1* for soybean homeobox-containing gene) was isolated using maize *Knotted-1* (*Kn1*) cDNA as a heterologous probe. The *Sbh1* cDNA clone is 1515 bp long which is the approximate size of its transcript. The deduced SBH1 protein shares a high amino acid identity with KN1 protein (47.0% overall and 87.5% for the homeodomain). Southern hybridization analysis indicated that *Sbh1* is a member of a small gene family. The expression of *Sbh1* is development- and tissue-specific. Northern hybridization analysis indicated that *Sbh1* transcript was present in early staged somatic embryos, increased prior to cotyledon formation and decreased thereafter. *Sbh1* was weakly expressed in soybean stems and hypocotyls but was not detected in other plant tissues and nonembryogenic materials. *In situ* hybridization analysis showed more localized expression in the root and shoot meristems of the heart-shaped and cotyledon embryos. The procambium, which forms the vascular tissue, also expressed the gene when the embryos developed to the cotyledon stage. The striking similarity of *Sbh1* expression in both zygotic and somatic embryos indicates that somatic embryogenesis can be used as a model for studies on plant embryogenesis. The expression patterns during embryogenesis, the homology with maize *Kn1* gene, and the regulatory nature of homeodomain proteins suggest that the SBH1 protein plays an important role in plant embryo development.

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COMPARISON OF SOMATIC EMBRYO INDUCTION AND EMBRYO GERMINATION IN SIX VIRGINIA SOYBEAN (*GLYCINE MAX* L.) CULTIVARS

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Six widely-grown Virginia soybean cultivars and one old domestic variety 'Peking' have been compared for differences in induction efficiencies of somatic embryogenesis, primary embryo yields, efficiencies of somatic embryo maturation and embryo germination abilities. These studies were undertaken in order to identify optimal commercially important cultivars for recovery of regenerated plants after gene transfer by particle bombardment techniques. The experiments were carried out as Latin Square Design. Since explants consisted of cotyledons obtained from immature seeds, staggered plantings of all soybean cultivars were carried out over four weeks in order to ensure availability of seeds of similar developmental stage at embryo harvest. Results showed that the initiation and the yield of primary somatic embryos were genotype dependent. In contrast, the maturation and germination of embryos were genotype independent. The percentage of cotyledons which showed somatic embryogenesis in Virginia soybeans ranged from 64.07% to 89.00%, with the average number of primary globular embryos per responding cotyledon ranging from 6.11 to 12.98. The cultivar 'Bay' showed both highest somatic embryogenic response and yield of globular embryos from the responding cotyledons among the six Virginia cultivars tested. The cultivar 'Peking' showed very poor initiation frequency (26.12%) and yield of embryos per responding cotyledon (5.65). The germination efficiencies for all seven cultivars ranged from 27.49% to 44.83%.

References:

Finer, J.J. 1988. Apical proliferation of embryogenic tissue of soybean [*Glycine max* (L.) Merrill]. Plant Cell Rep. 7: 238-241.

Parrott, W.A., Williams, E.G., Hildebrand, D.F., and Collins, G.B. 1989. Effect of genotype on somatic embryogenesis from immature cotyledons of soybean. Plant Cell Tissue Organ Cult. 16: 15-21.

SOMACLONE PRODUCTION FROM SOUTHERN SOYBEANS

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Somaclonal variation from soybeans has the potential of providing new and novel sources of germplasm useful for breeding programs. The ability to regenerate large numbers of plants is needed to provide sufficiently large somaclone populations from which to select plants with improved traits. Media modifications have been evaluated to increase the number of embryoids formed on immature cotyledons, and the ability of the embryoids to develop into normal plants. Most media evaluated were Murashige and Skoog based with either 2,4-dichlorophenoxyacetic acid or 1-naphthaleneacetic acid. The rate of embryoid formation varies with the soybean variety and auxin type. Somaclones grown in preliminary field tests appeared phenotypically similar to the variety from which they were derived, with no deleterious mutations evident. Seed from somaclones have been provided to an entomologist for evaluation for soybean looper resistance, and to a nematologist for evaluation for nematode resistance. Greenhouse and laboratory tests are also underway to evaluate for aerial blight resistance and tolerance to aluminum.

INHERITANCE OF THE EMBRYOGENIC PHENOTYPE IN SOYBEAN (cv. TN 4-86) REGENERATED FROM LIQUID SUSPENSION CULTURE

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Liquid suspension cultures of the soybean cultivar TN 4-86 were started from immature cotyledons induced to form somatic embryos. The protocols for initiation were first described by Lazzeri et al. (1985) and modified to the present system by Finer and Nagasawa (1988). The embryogenic culture system involves size selection, sterilization and dissection of immature soybean pods to yield immature seeds in the 4 to 7 mm range. The immature cotyledons are removed and plated on a modified MS medium containing 40mg/l 2,4-D (2,4-dichlorophenoxyacetic acid). This concentration of 2,4-D can stimulate the formation of somatic embryos on the surface of the immature cotyledons in 6 to 12 weeks of culture.
Somatic embryos were induced in the cultivar TN 4-86 using this protocol. Proliferating somatic embryos were removed and placed into liquid suspension culture for further proliferation of the embryos. After a period of establishment, proliferation of embryos in the suspension cultures was achieved. Regeneration from these cultures was attempted . Two plants of TN 4-86 (R_0) were regenerated using this protocol (many abnormal embryos fail to produce normal plants). These two regenerated plants were grown to maturity and found to be fertile and capable of nodulation with *Bradyrhizobium japonicum* USDA 110. Seeds (R_1) of the two regenerants were collected and grown out (18 and 27 seeds, respectively). Immature cotyledons (R_2 tissue) obtained (as described above) from pods of these plants (R_1) were plated on the somatic embryo induction medium to determine if there was a genetic enhancement of embryogenesis in cotyledons from previously regenerated plants. Immature cotyledons from each plant were plated separately. The cotyledons from the R_1 plants represent R_2 tissue(the first segregating generation for any induced heterozygosity). There were distinct differences in the frequency of embryogenesis among the cotyledons from the R_1 plants. A possible genetic model for embryogenesis is proposed.

References

Bailey, M.A.; Boerma, H.R.; Parrott, W.A. 1993. Genotype effects on proliferative embryogenesis and plant regeneration of soybean. *In Vitro* Cell. Dev. Biol. 29P: 102-108.

Finer, J.J.; Nagasawa, A. 1988. Development of an embroygenic suspension culture of soybean. Plant Cell Tissue Organ Cult. 15: 125-136.

Komatsuda, T.; Lee, W.; Oka, S. 1992. Maturation and germination of somatic embryos as affected by sucrose and plant growth regulators in soybeans *Glycine gracilis* Skvortz and *Glycine max* (L.) Merr. Plant Cell Tissue Organ Cult. 28: 103-113.

Lazzeri, P.A.; Hildebrand, D.F.; Collins, G.B. 1985. A procedure for plant regeneration from immature cotyledon tissue of soybean. Plant Mol. Biol. Rep. 3: 160-168.

PROLIFERATING EMBRYO CULTURES ON SOLID MEDIUM AS AN AID FOR SUSPENSION CULTURE ESTABLISHMENT, CULTURE MAINTENANCE, AND SELECTION OF TRANSGENIC CELL LINES

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Proliferating embryo cultures of soybean have been very useful for the recovery of large numbers of somatic embryos and for soybean transformation (Finer & McMullen, 1991). However, such cultures can be difficult to start and tedious to maintain. Proliferation of embryogenic cultures can also occur on solidified MS medium (Finer, 1988; Wright et al., 1991). The use of a solid medium for the proliferation of embryogenic cultures can assist the overall process in several ways. Somatic embryos are induced from zygotic cotyledons placed on MS medium at pH 7.0 supplemented with 40 mg/L of 2,4-D, then transferred to solid medium supplemented with 20 mg/L 2,4-D for 4 weeks prior to transfer to liquid Finer & Nagasawa (1988) medium. This protocol results in a near 100% success rate in the initiation of liquid cultures from every genotype tested. As cultures proliferate more slowly in solid medium than in liquid medium, cultures can be easily maintained on solid medium, requiring only monthly subcultures to fresh medium. It is possible to transfer cultures back and forth between solid and liquid media as necessary. Finally, cultures subjected to microprojectile bombardment may be placed on solid medium for selection of transgenic cell lines. In contrast to the frequent medium replacements that are necessary when a selection agent is used in liquid medium, cell lines being selected on solid medium only require transfer at monthly intervals. After 2 months on solid medium, surviving cell lines can be transferred to liquid medium for a final selection and rapid proliferation prior to plant recovery. Thus far, selection for hygromycin resistance has been far more effective than for geneticin resistance, with 25 mg/L hygromycin being the most effective concentration.

Finer, J.J. 1988. Plant Cell Rep. 7:238-241
Finer, J.J. and M.D. McMullen. 1991. In Vitro Cell. Dev. Biol. 27P:175-182
Finer J.J. and A. Nagasawa. 1988. Plant Cell Tissue Organ Cult. 15:125-136
Wright, M.S., K.L. Launis, et al. 1991. In Vitro Cell. Dev. Biol. 27P: 153-157

HIGH FREQUENCY TRANSFORMATION OF SOYBEAN CALLUS CULTURES

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A reliable protocol for regeneration of soybeans from explants taken from mature cotyledons and embryonic axes of the cultivar Jack has been developed. The majority of the tissue culture-derived plants were fertile and phenotypically normal and third-generation progeny have been obtained. Progeny plants produced yields typical for the cultivar in a field trial.

As reported by other laboratories, *Agrobacterium*-mediated transformation of soybean is infrequent in comparison with standard results for tobacco. False positive indications of transformation have been reported that can often be traced to the presence of live *Agrobacterium tumefaciens* within the regenerated tissue. To facilitate discrimination between GUS expression resulting from contaminating bacteria and that from the inserted gene sequence, we used a vector that contains an intron within the GUS coding sequence (Vancanneyt et al., *Mol. Gen. Genet.* 220:245-250, 1990) driven by the CaMV 35S promoter.

Calli derived from embryonic axes have proven to be amenable to transformation, with frequencies of transformation exceeding 90% (60/64 calli), based on GUS expression from the intron-containing construct. Results from genomic DNA blots support evidence for stable transformation provided by induced resistance to hygromycin and histochemical staining. Regeneration of plants from transformed callus cultures is now underway.

PARTICLE BOMBARDMENT AND AGROBACTERIUM-MEDIATED TRANSFORMATION OF NORTHERN-ADAPTED SOYBEANS

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Embryogenic suspension cultures of several Canadian northern-adapted soybean breeding lines were established following the method of Finer and Nagasawa (1988)[1]. Following particle bombardment with the phosphinothricin resistance gene (*pat*) and either the hygromycin resistance gene (*aphIV*) or the ß-glucuronidase (*uidA*) gene, three different selection approaches were used to recover transgenic lines of X2650-7-2-3 and X2653-4-3-S2-2 (both northern-adapted Natto lines). These included selection for resistance to either hygromycin or phosphinothricin or visual screening for ß-glucuronidase positive colonies. Southern hybridization analysis revealed multiple-copy integration patterns for a majority of the lines although evidence for integration of only a single copy of the hygromycin gene, in at least one line, was obtained. The influence of conditions such as culture medium composition, photoperiod and temperature on embryo conversion and plant recovery from the transgenic cultures was also examined.

Different northern-adapted breeding lines were also screened for the *in vitro* response of cotyledonary nodes and selected lines were used in *Agrobacterium tumefaciens*-mediated transformation experiments. With varying frequency, all lines responded to culture in the presence of 1 mg/L BA with production of multiple shoots at the cotyledonary node. Cotyledonary explants of lines X2653-4-3-S2-2 and OT92-15 were co-cultured with a disarmed *Agrobacterium tumefaciens* strain EHA105 which also carried the binary vector pBI121. Transformants were selected in the presence of 50-100 mg/L kanamycin and resistant tissue was screened for ß-glucuronidase activity. Kanamycin-resistant calli were observed at a frequency of 10-15% of explants, however not all calli exhibited prolonged growth in the presence of kanamycin. In total 2-5% of cultured explants yielded stable, kanamycin-resistant, ß-glucuronidase positive calli. A majority of these calli likely represent transformation events associated with non-organogenic cells since they proliferate in the presence of kanamycin but do not produce organized tissues. In spite of this, several organogenic calli were obtained and thus far one fertile, regenerated plant exhibiting ß-glucuronidase activity has been obtained.

[1]Finer J.J. and A. Nagasawa (1988) Plant Cell Tissue and Organ Culture 15:125-136

RAPD MARKERS FOR RESISTANCE TO SOYBEAN CYST NEMATODE *Heterodera glycines* I. AND MARKER ASSISTED SELECTION

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Breeding efforts to incorporate resistance to soybean cyst nematode (SCN), *H. glycines* I., into elite lines are complicated by the quantitative nature of the trait and the existence of biological races within the nematode. We found putative QTLs for resistance to SCN race 3 on linkage groups A, C, M, P. Ninety $F_{2:3}$ lines from cross of the cultivars 'Peking' x 'Essex', isolines 'NC55' and 'Lee', 'Hartwig' and 'Forrest' were used for transmission, bulk and segregation analyses with RAPD markers. SCN bioassays were performed using the selected populations of race 3. Individuals were scored based on the percentage of cysts (Index of Parasitism) relative to the infection of the standard susceptible cultivar Essex. DNA from homozygous black individuals (ii genotypes) and homozygous yellow individuals (i^1i^1 genotype) were pooled and the two bulks of segregants were screened using 325 decamer primers. A total of 1880 bands were generated, of which 48 bands (2.55%) were polymorphic. Eleven bands were identical in the parental lines and respective bulks. The isolines were screened using 247 primers and 37 primers were polymorphic. Sixteen primers matched the Peking pattern (donor parent) in NC55. Amongst 288 primers tested with Hartwig, Forrest and PI 437654, 28 primers were polymorphic and they generated 18 polymorphic bands which were identical in Hartwig and PI 437654 but different in Forrest and Essex. Additional polymorphisms for screening segregating population were obtained from molecular survey of the Peking gene pool, and the comparisons of race 3 resistant and race 3 susceptible cultivars. The associations between the RAPD markers and IP values were determined by linear and multiple regression analysis using the general linear fixed effects model, PC-SAS version 6.0. We examined 31 combinations of SCN significant RFLP and RAPD markers to identify the marker combination for potential application in molecular marker assisted selection (MAS). The probe A136, which explained 13% of SCN phenotypic variation at p=0.003, and probe A111 when used together, selected Peking molecular type individuals with IP mean value of 26.7 and Essex molecular type individuals with IP mean value of 67.4, p=0.01. These two RFLP probes with RAPD markers S07 and B01 selected Peking molecular type individuals with IP mean value of 17.1 and Essex type individuals with IP mean value of 75.8, p=0.007. The use of two RAPD markers S07 and B01 in the Peking and Essex segregating progeny identified Peking molecular type individuals with IP mean value of 22.3 and Essex molecular type individuals with IP mean value 41.2, p=0.05. Within selected Peking molecular type individuals, 62.5% individuals had IP values less than 10%. Depending on the availability of breeder's resources, marker combination can be chosen and used in selection procedures for SCN resistance. Predictive value of identified markers for SCN should be confirmed in different cross combinations and usefulness of MAS in early generation of SCN resistance breeding program has to be documented. QTL resistance is difficult to select phenotypically and MAS could greatly enhance development of resistant cultivars.

RFLP ANALYSIS OF RESISTANCE TO PHOMOPSIS SEED DECAY IN THE SOYBEAN PI 417479

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Phomopsis seed decay (PSD) caused primarily by the fungus *Phomopsis longicolla* Hobbs is one of the most serious diseases of soybean [*Glycine max* (L.) Merr.] seeds. This disease is endemic in most soybean growing areas of the world. PSD infected seeds have reduced quality and commercial value. Disease incidence is highly dependent on environmental conditions near physiological maturity, making this disease one of the most elusive types. Genetically controlled resistance to PSD was first identified in PI 417479. Resistance in PI 417479 is controlled by two complementary dominant nuclear genes. Resistance is expressed in maternal tissues, delaying disease assessment, including identification of resistant progeny and their use in backcrossing. In this study, we developed F_2 populations from crosses between PI 417479 and each of three susceptible genotypes PI 91113 with 110, AP 350 with 97, and Williams 82 with 104 individuals. PSD incidence was estimated on F_2 individuals using a seed bioassay, which ranged from zero to 100%, zero to 67%, and zero to 80% for the three crosses, respectively. DNA extracted from parents and F_2 progeny is used for standard RFLP analysis. Screening for polymorphism among parents is being conducted using 150 evenly spaced clones from the public soybean molecular map. To this date, 23 out of 44 probes demonstrated to be polymorphic for at least one of the five enzymes tested. Ten probes have banding pattern that separates the resistant PI 417479 from all other susceptibles. Polymorphic probes are going to be screened in the segregating populations.

GREENHOUSE ASSAY OF SOYBEAN SUDDEN DEATH SYNDROME RESPONSE

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A greenhouse assay was set up to evaluate our ability to distinguish resistance to *Fusarium solani* type A among soybean varieties partially resistant to Sudden Death Syndrome (SDS). Two separate assays were conducted to distinguish disease severity between *F. solani* strains 269 and ST90-1. Four varieties, Ripley, Lee, Douglas, and Pyramid, were infected using an oat-based inoculum. SDS symptoms developed 2-3 weeks after inoculation. Plant height, weight, necrotic and chlorotic leaf symptoms were scored. We observed a consistent difference in height between inoculated plants and controls (plants not inoculated); controls being taller. Strain specific differences were observed when the plants were evaluated by the percentage of the chlorotic leaves per plant for each of the two strains. Comparisons showed that the average percentage of chlorosis was less in strain ST90-1 than in strain 269 for varieties Douglas, Ripley, and Lee. However, Pyramid showed greater chlorosis with strain 269. Pyramid and Douglas as well as RILs derived from their cross have previously been reported to show environmental sensitivity in their resistance to SDS in the field. We will attempt to analyze resistance to different strains of *Fusarium solani* type A in progeny lines derived from Pyramid x Douglas.

RAPID N_2 FIXATION INCREASES BIOMASS OF NITRATE-FED SOYBEAN PLANTS

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Nitrogen fixation is an energy intensive process that consumes approximately 10% of a plant's net photosynthetic output. This study was conducted to determine whether rapid N_2 fixation during pod fill affects the harvested biomass of soybean [Glycinemax (L.) Merr.] plants grown hydroponically in the greenhouse under summer sunlight. Accumulation of plant biomass and total N during the R3-->R7 stages was measured on unioculated plants grown on 1.0 mM nitrate, a concentration that did not inhibit nodulation and/or nitrogenase activity appreciably. Plants that relied solely on N_2 fixation during the R3-->R7 stages had the lowest gain in harvested biomass and the lowest percentage gain in biomass. These plants also had the lowest total N content and the lowest percentage gain in total N. On the other hand, plants fixing N_2 rapidly in the presence of 1.0 mM nitrate had the highest harvested biomass and the highest percentage gain in biomass even though their total N content and their percentage gain in N were similar to those of plants provided more than adequate (6.0 mM) nitrate. Thus, under these growth conditions, rapid N_2 fixation in the absence of medium N limited biomass accumulation by providing insufficient N and not by excessive consumption of photosynthate. It is concluded that rapid N_2 fixation in the presence of nitrate concentrations that do not inhibit nodulation and/or nitrogenase activity enhances net photosynthetic output of soybean. How this increased efficiency is accomplished is unknown. Possible interactions between photoinhibition and the zeaxanthin cycle are under investigation.

PURIFICATION OF BINDING PROTEINS THAT RECOGNIZE OLIGOGLUCOSIDE ELICITORS OF PHYTOALEXIN ACCUMULATION IN SOYBEAN

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We are studying the cellular signaling pathway leading to phytoalexin biosynthesis in soybean that is induced by a branched hepta-β-glucoside of fungal origin. Our research has focused on the specific recognition of the hepta-β-glucoside elicitor by binding proteins (EBPs) in soybean cells, and the role of membrane protein phosphorylation in the signal transmission. Total membranes prepared from roots have previously been shown to contain proteinaceous binding site(s) with properties expected of physiological receptors: specificity, saturability, reversibility, and high affinity. Furthermore, the binding affinities of synthetic oligoglucosides correlate with their elicitor activities. The EBPs can be solubilized using the non-ionic detergent, *n*-dodecylsucrose, and will retain their binding affinity (1.8 nM) and specificity for the radiolabeled elicitor. Gel permeation chromatography of solubilized EBPs demonstrate that the bulk of the binding activity is associated with large and very stable detergent-protein micelles. Further purification of the EBPs has been achieved using an affinity column carrying immobilized hepta-β-glucoside elicitor. Active EBPs are eluted from the affinity matrix using low affinity free ligands. Several protein bands are visualized by SDS-PAGE after concentration of the affinity column eluate, Together with the gel filtration data, these results suggest that the EBPs exist as a multimeric protein complex.

FISH ANALYSIS OF rRNA GENES: EXPRESSION AND EVOLUTION

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Ribosomal RNA genes (5S and 45S rRNA) are expressed from tandemly arranged genes and then assembled into ribosomal subunits in the nucleolus. We have determined the number of loci (arrays) and the interphase spatial arrangement of these genes in both soybean and its diploid relative common bean. This information leads to a greater understanding of these genes evolution and their expression. Fluorescent *in situ* hybridization (FISH) using biotinylated and digoxigenin labeled probes allowed us to detect both types of rRNA genes in the same interphase or metaphase nuclei. In contrast to expectations, common bean has multiple 5S (4-5) and 45S (2) rRNA loci while soybean has only a single locus of each. The soybean 5S and 45S loci are on different chromosome with the 5S telomeric and the 45S distal, but not telomeric. In both species, the nucleolus is flanked on its periphery by condensed punctate foci of 45S rDNA. In some cases, 45S rDNA fibers were observed extending into the nucleolus. These less condensed fibers probably represent the active template for 45S rRNA production. Likewise, one or two 5S rDNA foci were observed adjacent to the nucleolus for both species. In common bean, the additional 45S and 5S rDNAs were arranged randomly in the nucleoplasm and distant from the nucleolus. The distribution of the rDNA loci in the interphase nuclei of both species suggests that there is a intimate relationship between spatial distribution and gene activity of rDNAs. It has been previously suggested that rDNA loci would have doubled with tetraploidy and then been reduced to one during "diploidization." However, the presence of multiple rDNA loci in common bean suggests for a more complex soybean rDNA evolution pattern.

ANALYSIS OF DUPLICATED REGIONS OF THE SOYBEAN GENOME.

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The development of the public RFLP molecular genetic map of the soybean genome has demonstrated a high degree of genome duplication. Duplication was examined in detail by characterizing and RFLP mapping members of two multigene families (sle and A071). Homologous loci displayed conservation of the genic sequence and less than 3 Kb of flanking sequence. Non-homologous flanking regions differed in types and distribution of repetitive DNA. RFLP mapping of low-copy probes from the non-homologous regions revealed that copies of these sequences were still genetically linked to sle and A071 loci, suggesting that intra-chromosomal events had altered the distances between sequences. These results also identified five paralogous linkage groups, suggesting that large segmental duplications, in addition to total genome duplications, are responsible for the highly duplicated nature of the soybean genome.

EFFICIENT USE OF RFLPs TO ACCURATELY ESTIMATE RELATIONSHIPS AMONG SOYBEAN VARIETIES

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Plant breeders use genetic-similarity estimates based on DNA patterns to better understand genetic relationships among varieties and to help select parent lines for crosses. We use data from 33 RFLP probes to routinely estimate genetic relationships in soybean [*Glycine max* (L.) Merr.]. These 33 RFLP probes were evaluated in this experiment because their band profiles can be reliably scored when used in 16 two-probe and one single-probe hybridizations (17 total), they represent well-distributed markers around the soybean genome, and they are relatively polymorphic among elite soybean germplasm. We compared 435 genetic-similarity estimates among 30 soybean varieties calculated from data sets of 10, 20, 33, 51, and 109 RFLP probes, respectively. Each smaller set of probes was a subset of all larger sets. Correlations between the genetic-similarity estimates based on 109 probes and smaller sets of probes decreased with decreasing number of probes. However, we believe data from the selected set of 33 probes is adequate for accurate genetic-similarity estimates used in soybean breeding while minimizing the necessary lab work.

MOLECULAR ANALYSIS OF ORGANELLE GENOME INHERITANCE IN SOYBEAN

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Restriction endonuclease digestion analysis and Southern blotting was used to determine the mode of inheritance of mitochondrial and chloroplast DNAs in soybean. F_1 hybrids for sixty different parental combinations were generated from parents that had distinct organelle DNA restriction fragment patterns. The crosses produced 174 F_1 hybrids for a molecular analysis of organelle genome content. Multiple samples of leaf tissue from F_1 plants were also examined to determine if F_1 plants might be organellar mosaics. In addition, approximately 200 F_2 or F_4 progeny from these crosses were examined for their mitochondrial DNA type. Only organellar DNA from the female parent was detected in all plant tissue that was examined. These results confirm with a high degree of statistical certainty (99%) that paternal transmission occurs at a very low or negligible level (less than 3%) in soybeans.

CYTOPLASMIC DIVERSITY IN SOYBEAN, *GLYCINE MAX* (L.) MERR USING TWO SOYBEAN MITOCHONDRIAL PROBES

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Analysis of mitochondrial DNA for restriction fragment length polymorphisms (RFLPs) has previously been used to distinguish four cytoplasmic patterns in *Glycine max* (L.) Merr. This classification was derived from results with a 2.3 kb *Hin*dIII probe isolated from 'Williams 82'. This unique fragment site has only been seen in cultivars derived from the maternal parent 'Lincoln' which is a subset of the 'Mandarin' cultivar. In this study, 204 soybean cultivars from the United States Department of Agriculture's (USDA's) collection of old domestic varieties were analyzed in search of additional diversity. This collection is the source of modern domestic soybean cultivars and has been classified using the 2.3 kb *Hin*dIII fragment as well as the gene for the mitochondrial ATPase subunit 6 as probes. The results using these probes has confirmed a lack of diversity at the cytoplasmic level. Possible mechanisms for the generation of the polymorphism in the 'Lincoln' mitochondrial genome include insertions, deletions, or rearrangements. Cloning and sequencing of the flanking sequences may help to elucidate the origin of the 'Lincoln' polymorphism. A restriction map will be constructed around the 2.3 kb probe. *Hin*dIII-*Pst*I flanking regions of 0.8kb and 1.7 kb will be used as probes. Sequencing of this region will also allow further investigation of a potential protein-encoding gene.

References:

Sission, V.A., Brim, C.A., and Levings, C.S. III. 1978. Characterization of cytoplasmic diversity in soybean by restriction endonuclease analysis. Crop Sci. 18:991-996

Grabau, E.A., Davis, W.H., and Gengenbach, B.G. 1989. Restriction fragment length polymorphisms in a subclass of the 'Mandarin' cytoplasm. Crop Sci. 29:1554-1559.

Grabau, E.A., Davis, W.H., Phelps, N.D., and Genenbach, B.G. 1992. Classification of soybean cultivars based on mitochondrial DNA restriction fragment length polymorphisms. Crop Sci. 32:271-274.