Proceedings of the
3rd Biennial Conference on

Molecular and Cellular Biology of the Soybean

July 23-25, 1990
Fisher Theater
Iowa State University
Ames, Iowa

General Information

Meeting Facilities

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

Speaker Information

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

Acknowledgments

The Conference Committee is especially grateful to the following people for their clerical and administrative assistance:

Peter Englin, Personnel Services (Housing)
Tom McCormick, Office of Continuing Education
Deborah Bleile, Departments of Agronomy and Genetics
Janet Gardner, Office of Continuing Education

Schedule of Papers for the
3rd Biennial Conference on
Molecular and Cellular Biology of the Soybean

Monday Afternoon (July 23, 1990) 1300-1615
Building: Fisher Theater, Iowa State Center

1300-1415 - Presiding: Charisse Buising, Pioneer Hi-Bred International

1300 (1)*
ANATOMY AND MORPHOLOGY OF SOMATIC EMBRYOGENESIS FROM SOYBEAN COTYLEDONS.
J.P. Ranch, Pioneer Hi-Bred International; and S.M. Colburn, United AgriSeeds, Champaign, IL

1315 (2)
2,4-D EFFECTS ON SOMATIC EMBRYOGENESIS AND CULTURE-INDUCED VARIATION IN SOYBEAN.
R.C. Shoemaker, USDA-ARS, Iowa State University; L.A. Amberger, Iowa State University; R.G. Palmer, USDA-ARS, Iowa State University; J.A. Bucheim, United AgriSeeds; J.P. Ranch, Pioneer Hi-Bred International

1330 (3)
PLANT REGENERATION *IN VITRO* FROM PRIMARY LEAF NODES OF SOYBEAN (*GLYCINE MAX* L.) SEEDLINGS.
J. Kim, C.E. LaMotte, and E. Hack, Iowa State University

1345 (4)
DIVIDING CALLUS CULTURES OBTAINED FROM SOYBEAN MESOPHYLL PROTOPLASTS.
K. Negaard, R. Isaac, and F. Hoffmann, University of California at Irvine

1400 (5)
PLANT REGENERATION FROM PROTOPLASTS VIA EMBRYOGENESIS IN SOYBEAN.
T. Komatsuda and S. Oka, National Institute of Agrobiological Resources, Japan; X.Z. Zhang, Northeast Agricultural College, PRC; Y. Miyasaka, Nagano Prefectural Agriculture Experiment Station, Japan

1415-1445 Break

1445-1615 - Presiding: Don Lee, University of Nebraska-Lincoln

1445 (6)
IN VITRO SELECTION FOR SOYBEAN LINES TOLERANT TO SALINITY.
P. Srinives, S. Chanprame, and C. Pitakteerabundit, Kasetsart University, Thailand

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

1500 (7)
A CYTOPLASMICALLY INHERITED WRINKLED-LEAF MUTANT IN SOYBEAN.
P.A. Stephens, V.B. Barwale-Zehr, C.D. Nickell, and J.M. Widholm, University of Illinois at Urbana

1515 (8)
EFFECTS OF UNIQUE CYTOPLASMS ON AGRONOMIC AND PHYSIOLOGICAL TRAITS OF SOYBEAN.
S. De Broux, R. Miller, and D.E. Green, Iowa State University; R.C. Shoemaker, USDA-ARS, Iowa State University

1530 (9)
PROGRESS TOWARD THE MOLECULAR CLONING OF THE ACETYL-CoA CARBOXYLASE GENE FROM SOYBEAN.
J. Song and B.J. Nikolau, Iowa State University

1545 (10)
MOLECULAR ATTRIBUTES OF SOYBEAN FLOWER ABSCISISON.
C.D. Dybing and R.N. Reese, USDA-ARS, South Dakota State University

1600 (11)
ONTOGENY AND ULTRASTRUCTURE OF SPONTANEOUS NODULES IN ALFALFA.
P.A. Joshi, G. Caetano-Anolle's, E.T. Graham, and P.M. Greosshoff, Institute of Agriculture and Center for Legume Research, University of Tennessee

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

Tuesday Morning (July 24, 1990) 0900-1200

0900-1030 - Presiding: George Graef, University of Nebraska-Lincoln

0900 (12)
LEAF PROTEIN ELECTROPHORESIS OF WILD TYPE AND MUTANT SOYBEAN (*G. MAX*).
R. Prabhu, L.A. Sayavedra-Soto, and P.M. Gresshoff, Institute of Agriculture and Center for Legume Research, University of Tennessee

0915 (13)
AUTOREGULATION OF NODULATION IN SOYBEAN IS NOT ASSOCIATED WITH MAJOR POLYPEPTIDE CHANGES IN LEAVES.
L.A. Sayavedra-Soto, S.A. Angermuller, and P.M. Gresshoff, Institute of Agriculture and Center for Legume Research, University of Tennessee

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0930 (14)
LOCALIZATION OF mRNA ENCODING FOR VEGETATIVE STORAGE PROTEINS IN SOYBEAN.
J. Huang, Y. Rhee, and P. Staswick, University of Nebraska-Lincoln

0945 (15)
AN ABUNDANT HYDROXYPROLINE-RICH PROTEIN IN SOYBEAN SEED COATS IS AFFECTED BY THE SEED COLOR GENOTYPE.
J.T. Lindstrom and L.O. Vodkin, University of Illinois at Urbana

1000 (16)
POST-TRANSLATIONAL PROCESSING AND DEPOSITION OF KUNITZ TRYPSIN INHIBITOR IN SOYBEANS.
A. Tan-Wilson, A. McGrain, J. Chen, and P. Diaz, State University of New York at Binghamton

1015 (17)
GENE EXPRESSION OF SOYBEAN LIPOXYGENASES IN GERMINATED COTYLEDONS.
D. Shibata, T. Kato, and H. Ohta, Mitsui Plant Biotechnology Research Institute, Japan; K. Tanaka, Kyoto Prefectural University, Japan

1030-1100 Break

1100-1200 - Presiding: Robert Thornburg, Iowa State University

1100 (18)
UREASE PRODUCTION BY *METHYLOBACTERIUM MESOPHILICUM*, A SEED-TRANSMITTED BACTERIUM UBIQUITOUS IN SOYBEAN.
J.M. Dunleavy, USDA-ARS, Cereal and Soybean Improvement Research, Iowa State University

1115 (19)
UREA METABOLISM IN SOYBEAN: A JOINT EFFORT BETWEEN PLANT AND MICROBIAL COMMENSAL?
M.A. Holland and J.C. Polacco, University of Missouri

1130 (20)
GENETIC CRITERIA FOR UREA UTILIZATION IN DEVELOPING SOYBEAN SEEDS.
N.E. Stebbins and J.C. Polacco, University of Missouri

1145 (21)
STUDIES OF CLONED SEQUENCE COMPONENTS OF *M. ARENARIA* DNA.
E. Hiatt, E. Shipe, H. Skorupska, and A. Abbott, Clemson University

1200-1330 Lunch

Tuesday Afternoon (July 24, 1990) 1330-1615

1330-1430 - Presiding: Kathleen Danna, University of Colorado

1330 (22)
PREPARING A GENETIC MAP OF SOYBEAN USING AN *INTRA*-SPECIFIC CROSS.
K.G. Lark, K. Chase, and T. Macalma, University of Utah; L. Mansur, Iowa State University; R. Palmer, USDA-ARS, Iowa State University; J. Weisemann, B. Matthews, USDA-ARS, Beltsville, MD

1345 (23)
COMPARISONS OF MOLECULAR MAPS FROM *MAX-MAX* AND *SOJA-MAX* SOYBEAN CROSSES.
R.C. Shoemaker, USDA-ARS, Iowa State University; T.C. Olson, Iowa State University; K.G. Lark, University of Utah

send letter exchange selected probes 22 / linkage group

1400 (24)
GENETIC MAPPING OF BIOCHEMICALLY DEFINED LOCI IN SOYBEAN USING CLONED GENE PROBES.
J. Antoni Rafalski and Scott Tingey, E.I. du Pont de Nemours and Co (Inc), Wilmington, DE

1415 (25)
RFLP ANALYSIS OF SYMBIOTIC MUTANTS OF SOYBEAN.
D. Landau-Ellis, S.A. Angermuller, L.J. Schuller, L. Sayavedra-Soto, and P.M. Gresshoff, Institute of Agriculture and Center of Legume Research, University of Tennessee

1430-1500 Break

1500-1615 - Presiding: Nevin Young, University of Minnesota

1500 (26)
USING NEAR-ISOGENIC LINES AS A RESOURCE FOR POSITIONING CONVENTIONAL GENETIC MARKERS ON THE SOYBEAN RFLP MAP.
J.E. Specht and G.L. Graef, University of Nebraska-Lincoln

1515 (27)
RFLP MAPPING OF PHYTOPHTHORA RESISTANCE LOCI IN SOYBEAN.
B.W. Diers and L. Mansur, Iowa State University; R.C. Shoemaker, USDA-ARS, Cereal and Soybean Research Improvement Unit, Iowa State University

1530 (28)
APPLICATION OF PULSED-FIELD GEL ELECTROPHORESIS FOR THE PHYSICAL MAPPING OF THE NODULIN REGION OF THE SOYBEAN GENOME.
R.J. Honeycutt, Iowa State University; B.W.S. Sobral, and M. McClelland, California Institute of Biological Research, La Jolla, California; A.G. Atherly, Iowa State University

must look - incomplete

PFGE Gana! et al 1989 MGG 215 395 Cheung et al PM. Biol. 1990 14:5881-888

1545 (29)
ANALYSIS OF THE SOYBEAN GENOME USING PULSED FIELD GEL ELECTROPHORESIS (PFGE).
Roel
R.P. Funke, L.A. Sayavedra-Soto and P.M. Gresshoff, Institute of Agriculture and Center for Legume Research, University of Tennessee

1600 (30) Moved to Monday
USDA PLANT GENOME RESEARCH PROGRAM.
J.P. Miksche, USDA-ARS, Beltsville, MD
Linda Walling
Shirley Sato

Wednesday Morning (July 25, 1990) 0900-1045

0900-1045 - Presiding: Paula Chee, The Upjohn Company

0900 (31)
DAMAGE TO GENOMIC DNA AFTER SEED AGING IN SOYBEAN (*G. MAX*) ✓
J.C. Kamalay and R. Tejwani, Ohio State University

0915 (32)
FLUORESCENT *IN SITU* HYBRIDIZATION WITH SOYBEAN METAPHASE CHROMOSOMES.
M.C. Griffor, L.O. Vodkin, R.J. Singh, and T. Hymowitz, University of Illinois at Urbana ✓

0930 (33)
SUSCEPTIBILITY OF A SOYBEAN CULTIVAR AND DERIVED NODULATION MUTANTS TO AN *AGROBACTERIUM TUMEFACIENS* STRAIN. ✓
J. Bond, R. McDonnell, and P.M. Gresshoff, Institute of Agriculture and Center of Legume Research, University of Tennessee
1 dom gene susc. in Peking
1:1 seg. in bc pop.

0945 (34)
DEVELOPMENT OF SOYBEAN GERMPLASM WITH SUPERIOR REGENERATION CAPACITY AND SUSCEPTIBILITY TO *AGROBACTERIUM*. ✓
M.A. Bailey, W.A. Parrott, and H.R. Boerma, University of Georgia

1000 (35)
TRANSIENT GENE EXPRESSION IN APICAL MERISTEMS MEDIATED BY MICROPROJECTILE BOMBARDMENT. ✓
C.M. Buising, Pioneer Hi-Bred International, Inc. and Iowa State University; R.C. Shoemaker, USDA-ARS, Iowa State University; R.K. Higgins and D.T. Tomes, Pioneer Hi-Bred International, Inc.; R.M. Benbow, Iowa State University

1015 (36)
TRANSGENIC SOYBEAN PLANTS OBTAINED VIA PARTICLE BOMBARDMENT OF EMBRYOGENIC CULTURES.
J.J. Finer, Ohio State University; M.D. McMullen, USDA-ARS, Ohio State University

1030 (37)
INSTRINSIC GUS-LIKE ACTIVITY IN SOYBEAN.
Paula Chee, The Upjohn Company; Ching-yeh Hu, Wm. Paterson College; Paul D. Miller, DNA Plant Technology Corp.

1045 Adjourn to Coffee/Discussions

ANATOMY AND MORPHOLOGY OF SOMATIC EMBRYOGENESIS FROM SOYBEAN COTYLEDONS

Ranch [*], J. P., Colburn, S. M., United AgriSeeds, Champaign IL 61820

[*] current address, Pioneer Hi-Bred, Johnston IA

When whole immature zygotic embryos were cultured on MS + 20 to 180 *u*M 2,4-D, an embryogenic response was invariably associated with the cotyledon whose abaxial surface was in contact with the culture medium. The opposing cotyledon rarely produced somatic embryos. Whole embryos, and cotyledon explants cultured with the abaxial surface to the culture medium, responded qualitatively the same. In contrast, cotyledon explants cultured with the adaxial surface in contact with culture medium responded at a significantly reduced frequency; any response, however, was still confined to the adaxial surface and margin. The difference in response as a function of tissue orientation was significant regardless of 2,4-D concentration or genotype.

In pre-treated cotyledons, the epidermis of the adaxial surface was less differentiated from the cotyledonary mesophyll compared with the epidermis at the abaxial surface. After 5 days culture on initiation medium, cell division was observed at both surfaces regardless of cotyledon orientation. Activity at the surface oriented away from the culture medium was typified by cells which were small, isodiametric, cytoplasmically dense, products of random cell divisions, possessed little to no vacuole, and displayed a prominent nucleus and nucleolus. These cells were typically embryogenic. However, the pattern of cell division at the surface in contact with the culture medium presented with periclinal divisions. These latter cells, over the period of observation, gradually became large, acquired large vacuoles and were not typically embryogenic.

Cell divisions at either surface were initiated in cells just beneath the epidermis. There was no epidermal involvement observed. Embryo formation was not associated with the surface oriented away from the medium. Rather embryo organization was associated exclusively with the adaxial surface. The meristematic tissues organized at the abaxial surface when cotyledons were cultured with the adaxial surface in contact with the culture medium was never observed to produce somatic embryos. Rather, the meristematic tissue became necrotic or produced callus.

2,4-D EFFECTS ON SOMATIC EMBRYOGENESIS AND CULTURE-INDUCED VARIATION IN SOYBEAN

R.C. Shoemaker[1], L.A. Amberger[2], R.G. Palmer[1], J.A. Bucheim[3], and J.P. Ranch[4], [1]USDA-Agricultural Research Service, [2]Iowa State University, Ames, Iowa 50011, [3]United Agriseeds, Champaign, Illinois, 61820 and [4]Pioneer Hi-Bred International Inc., Johnston, Iowa 50131, U.S.A.

The frequency and quality of somatic embryogenic response from immature soybean zygotic embryo cotyledons varied with concentration of 2,4-D. Embryogeny was suppressed at the globular stage by high (200 μm) 2,4-D concentration. Eighty to one hundred percent of the embryos initiated under this high 2,4-D level converted. Only 3% of the families from progeny of plants regenerated under a high 2,4-D concentration exhibited heritable variation. Conversely, at low (22.5 μm) 2,4-D concentrations, morphologically abnormal, cotyledon-stage somatic embryos were formed and less than 10% of these converted. However, over all genotypes, 40% of the families derived from plants regenerated under low 2,4-D concentrations exhibited heritable variation. Possible causes for the effects of 2,4-D on somatic embryo integrity and culture-induced variation are discussed.

PLANT REGENERATION IN VITRO FROM PRIMARY LEAF NODES OF SOYBEAN (GLYCINE MAX L.) SEEDLINGS

JooHag Kim, Clifford E. LaMotte, and Ethan Hack,
Dept. of Botany, Iowa State University, Ames,
Iowa 50011-1020, U.S.A.

A simple and reproducible protocol for regenerating soybean plants from explants derived from 7-day-old seedlings has been developed. Explants, each consisting of the primary leaf node (the unifoliate leaf node), the cotyledonary node, the internode between them, and one cotyledon, were cultured in vitro on a modified Murashige and Skoog (MS) medium containing 3% sucrose, four times the MS inorganic micronutrient level, B5 vitamins, 2 g/l L-proline, 2 mg/l BA, and 0.02 mg/l NAA to induce the formation of adventitious shoots at the primary leaf node. Explants from seedlings of the cultivar Peking produced an average of 34 shoots per explant after 30 days on this medium. Shoot formation was greater when a cotyledon remained attached to the explant and was in contact with medium. Whole plants were developed from the regenerated shoots when they were separated from explants and transferred to B5 medium for more development and to 1/2 B5 medium for rooting.

Proline increased number but decreased length of regenerated shoots, whereas raising micronutrient level increased both shoot number and length and, thus, partly overcame the effect of proline on length. Addition of proline and raising the level of micronutrients enhanced shoot regeneration synergistically. Examining separately the effects of singly omitting, including, raising, and lowering the levels of the seven different micronutrient elements of the original MS medium provides evidence that zinc is the most limiting element for regeneration from explants and that the other six elements, too, are all in less than optimal supply.

Test of GUS activity Transformed shoots have been produced using Agrobacterium-mediated gene transfer and our productive regeneration system, but not yet transgenic plants. Agrobacterium tumefaciens strains A208, A281, and LBA4404, carrying the binary vector pBI121 containing neomycin phosphotransferase and ß-glucuronidase genes were able to transform primary leaf node explants.

DIVIDING CALLUS CULTURES OBTAINED FROM SOYBEAN MESOPHYLL PROTOPLASTS

K. Negaard, R. Isaac, and F. Hoffmann, Department of Developmental and Cell Biology, University of California, Irvine, CA 92717

The regeneration of plants or callus tissue from isolated protoplasts, an important methodological step in the genetic engineering of many plants, has proven to be difficult for *Glycine max*. Numerous attempts have been made at regeneration of plants from explants of soybean, but successful reports have been limited to a few very specialized tissue types. Only one report has demonstrated the regeneration of soybean plants from protoplasts, that of Wei and Xu in 1988 (Plant Cell Reports 7: 348-351), and it, too, is from a specialized tissue, the immature cotyledon. Mature mesophyll from seed-derived plants gives high yields of homogeneous protoplasts after relatively short isolation times, and is a convenient and often most appropriate donor tissue for protoplast isolation. We have isolated mesophyll protoplasts from six cultivars of soybean and successfully cultured the protoplasts from all six cultivars to form protoplast-derived calli. Plants were grown in Phototrons® (Pyraponic Industries, Inc., San Diego, California) with 16 hours of light and 8 hours of dark. Optimal conditions for protoplast isolation used fully expanded leaves of 9 to 22 day old plants in an enzyme solution consisting of 0.1% pectolyase, 0.2% cellulase in 0.5 M mannitol, pH 5.6, all agitated on an orbital shaker at 30 rpm for 2-3 hours (or until protoplasts appeared). Protoplasts were then filtered through cotton, washed, and cultured in 8P media. First divisions were seen after 5-12 days in culture, and the derived callus could be subcultured on solid media after 8 weeks. We have found that the most important factor in isolating viable soybean mesophyll protoplasts is the physiological condition of the donor plant (Negaard and Hoffmann (1989) Biotechniques 7: 808-812).

PLANT REGENERATION FROM PROTOPLASTS VIA EMBRYOGENESIS IN SOYBEAN

T. Komatsuda and S. Oka, National Institute of Agrobiological Resources, Tsukuba, 305 Japan, X.Z. Zhang, Northeast Agricultural College, Harbin, PRC, and Y. Miyasaka, Nagano Prefectural Agriculture Experiment Station, Suzaka, 382 Japan

Protoplasts were isolated from immature cotyledon tissue of cv "Bonminori", because immature soybean cotyledons have a competence to produce somatic embryos. Isolated protoplasts were immobilized within "Gelrite beads" and cultured in a liquid medium containing 2,4-dichloro-phenoxyacetic acid and Benzyladenin. First division of protoplasts were observed three days after the culture initiation. Cell division continued to became small brownish calli consist of small cells with dense cytoplasms.

The "Gelrite beads" having small calli (less than 1 mm in diameter) were washed for three days in a hormone free liquid medium. Then the calli were picked up and transferred onto an embryogenesis medium containing Naphthalenacetic acid to induce somatic embryogenesis.

Somatic embryogenesis occurred from the black, brownish small calli. The somatic embryos were transferred onto a maturation medium containing a high concentration of sucrose. Well-developed somatic embryos were transferred onto a germination medium containing Gibberellic acid. The embryos germinated quickly to develop into plantlets.

IN VITRO SELECTION FOR SOYBEAN LINES TOLERANT TO SALINITY

P. Srinives, S. Chanprame, C. Pitakteerabundit,
Department of Agronomy, Kasetsart University, Kamphaeng Saen,
Nakhon Pathom 73140, THAILAND

Somatic embryogenesis of Thai soybean cultivars can be routinely performed following the method advocated by a number of American scientists. Young cotyledons of the size 0.3 to 0.5 cm in diameter (about 15 days after flowering) were cultured on MS inorganic salts, B-5 vitamin, 15 g/l sucrose and 7.5 mg/l NAA. After 4 weeks, the induced embryoids were removed on to the maturation medium consisting of MS inorganic and organic salts, 100 g/l sucrose and 0.5% (W/V) activated charcoal. The mature embryoids (4-5 weeks old) were then regenerated within 1-3 weeks on the conversion (regeneration) medium, SHGG (SH base medium with 1% sucrose, 0.2% Gelrite). It was found that soybean genotypes with high frequency of embryoid formation could have low frequency of plant regeneration. Supplementation of NaCl in any medium impaired the whole regeneration process. When NaCl was added at the concentration of 0.1, 0.2, 0.3 or 0.4% to the induction medium the rate of embryoid formation was reduced by more than 50%. Yet the induced embryoids were abnormally small and malformed. When normal embryoids from the induction medium were transferred to the maturation medium supplemented with NaCl of the above concentration, they became abnormal and could not regenerate in the SHGG medium. A suspension culture has been established to subject soybean cells to salt stress for more effective selection of tolerant genotypes.

A CYTOPLASMICALLY INHERITED WRINKLED-LEAF MUTANT IN SOYBEAN

P.A. Stephens*, U.B. Barwale-Zehr, C.D. Nickell, and J.M. Widholm
Univ. of Illinois, Urbana.

Previously, a chimeric plant, mostly wrinkled leaved but with normal leaf sectors, was observed in the R_2 generation of a soybean [*Glycine max.* (L.) Merr.] line that had been regenerated using an organogenic plant regeneration system. Progeny were advanced to the R_5 generation by harvesting single plants. Observations made during the growout period indicated that the wrinkled leaf phenotype was not always stable. Occasionally normal R_4 plants would give rise to progeny that included 1 or more wrinkled plants and wrinkled R_4 plants would produce progeny that included normal plants. Chimeric plants having both wrinkled and normal sectors produced chimeric, normal, and wrinkled leaf progeny. This variation pattern continued in the R_5 and R_6 generations. Testing by inoculation from infected plants confirmed that leaf wrinkling was not due to a virus. The objective of this research was to: (i) characterize the mode of inheritance of the wrinkled leaf mutation, and (ii) explain the 'instability' observed for the wrinkled leaf phenotype. When reciprocal crosses between wrinkled leaf plants and the cultivar 'Hack' were evaluated in the F_1, F_2, and F_3 generations, cytoplasmic inheritance for the wrinkled leaf trait was indicated.

EFFECTS OF UNIQUE CYTOPLASMS ON AGRONOMIC AND PHYSIOLOGICAL TRAITS OF SOYBEAN

S. De Broux, R. Miller, D.E. Green, Dept. of Agronomy, Iowa State University, and R.C. Shoemaker, USDA-ARS, Depts. Genetics and Agronomy, Iowa State University

Identifying traits that are affected by the cytoplasmic genomes and elucidating the effect upon these traits by cytoplasmic x nuclear genome interaction might result in making a new source of genetic variation in soybean available to the soybean breeder. Close et al (TAG 77:768-776) identified at least five unique chloroplast genomes based on RFLP analysis of several wild soybean accessions and soybean cultivars. In our study, lines differing in chloroplast DNA were crossed with 'Clark 63' and 'Harosoy 63' cultivars. Reciprocal F_1 and F_2 populations indicated that some cytoplasms have an effect on seed fatty acids and seed oil content. Backcrossing to the BC_5 generation has produced isolines that contain Clark 63 or Harosoy 63 nuclear genomes in cytoplasms of each of the five chloroplast RFLP groups. Spaced BC_5F_1 plants were evaluated for developmental traits, several morphological traits and rate of CO_2 exchange. Preliminary data analyses of isoline pairs have shown significant differences ($Pr>F = 0.05$) in plant size during the vegetative stage, leaflet size at beginning seed filling period, and plant height at physiological maturity. This study will include yield plots of BC_5F_2 and BC_5F_3 families in the next two years.

PROGRESS TOWARD THE MOLECULAR CLONING OF THE ACETYL-CoA CARBOXYLASE GENE FROM SOYBEAN

J. Song and B.J. Nikolau, Dept. Biochem. & Biophys., Iowa State University, Ames, IA 50011.

Acetyl-CoA carboxylase is a biotin-containing enzyme that catalyzes the first reaction in fatty acid biosynthesis, a key regulatory reaction in this pathway. This enzyme has been extensively studied from animals, bacteria and yeast, whereas its characterization from plants is less thorough. As a first step in the characterization of the structure, function and regulation of this enzyme we have undertaken the molecular cloning of the gene that codes this enzyme. All biotin enzymes share a common amino acid sequence, ala-met-lys-met, that defines the biotinylation site of these proteins. Genomic Southern analysis of soybean DNA with a cDNA sequence from tomato that codes for this biotinylation site identified a few, distinct restriction fragments. Therefore, a genomic library constructed from soybean (Glycine max L. Merr. cv Corsoy) was screened with this cDNA as a heterologous probe, resulting in the isolation of two genomic clones. These genomic clones hybridize to a single mRNA of approximately 2kb. These genomic clones are currently being sequenced to identify the structure of the gene products.

MOLECULAR ATTRIBUTES OF SOYBEAN FLOWER ABSCISISON.

C. Dean Dybing and R. Neil Reese. USDA-ARS and SD State University, Brookings, SD, USA.

Many soybean flowers are shed before setting seed. The cause of abortion and the effect on seed yield are both unknown. Past work on this project has shown that abortion may be initiated within a day of anthesis, the aborting ovaries are growing slowly but are not starving, and their low growth rate appears causal to abscission. We believe that low ovary growth rate somehow signals cells in the undeveloped abscission zone to produce enzymes for wall-weakening processes that lead to flower shedding. In our initial research on this hypothesis, electrophoretic protein banding patterns were analyzed for samples from abscission zones of flowers obtained near anthesis from the variety IX93-100. The racemes were manipulated to maximize percent set or abortion, and cytokinin was applied to further regulate level of abortion. Denaturing one and two dimensional PAGE of total protein extracts showed that suitable banding patterns were visualized by Coumassie Blue staining using the abscission zone of a single flower. This protein fraction had 5 major and more than 30 minor bands. Spectrophometic scanning at 575 nm showed that cytokinin treatment increased total protein per node more consistently than it increased pod set. Labeling of newly-synthesized protein with S35-methionine is now being tested in racemes cultured in vitro. It is hoped that association of abortion with one or more protein bands may be possible, and that this can lead to studies detailing genetic and physiological controls at the molecular level.

ONTOGENY AND ULTRASTRUCTURE OF SPONTANEOUS NODULES IN ALFALFA.

Priyavadan A. Joshi, Gustavo Caetano-Anollés, Effin T. Graham and Peter M. Gresshoff, Plant Molecular Genetics and Ornamental Horticulture and Landscape Design, Institute of Agriculture and Center for Legume Research, The University of Tennessee, Knoxville, TN 37901-1071, USA

Alfalfa (*Medicago sativa* L.) forms nodules in the absence of *Rhizobium* and combined nitrogen. The ontogeny of spontaneous nodules in alfalfa cv. Vernal was studied in serial longitudinal plastic sections of roots at different stages of growth using light and electron microscopy. These nodules, on uninoculated roots, were white multilobed structures, histologically composed of nodule meristems, cortex, endodermis and a central zone with vascular strands. Early development of nodulation was initiated by anticlinal followed by periclinal divisions of dedifferentiated cells in the inner cortex of the root. These cells formed the nodular meristem from which the nodule developed. Each cell division in the meristem formed one large and one small cell which persisted in the central zone. The large cells were characterized by cytoplasm packed with starch and a large central vacuole. Starch was present only occasionally in the small cells. Cells in the meristem and central zone contained fragmented rough endoplasmic reticulum, numerous free ribosomes, and few organelles. There were no infection tubes or bacteria in spontaneous nodules at any stage of development. The large cells became infected with bacteria in normal nodules of inoculated plants whereas they accumulated starch in spontaneous nodules. Alfalfa plants apparently have an endogenous mechanism for inducing early cell divisions which initiate nodular meristems and ultimately develop bacteria-free nodules. Our results suggest that bacteria and therefore their *nod* genes are not necessarily involved in the ontogeny and morphogenesis of spontaneous and normal nodules in alfalfa.

LEAF PROTEIN ELECTROPHORESIS OF WILD TYPE AND MUTANT SOYBEAN (G.MAX)

R. Prabhu, L. A. Sayavedra-Soto and P. M. Gresshoff, Plant Molecular Genetics, Institute of Agriculture and Center for Legume Research, The University of Tennessee, Knoxville, TN 37901

The reported involvement of the shoot in regulation of the soybean-*Rhizobium* symbiosis (1) led us to the search for any protein differences in leaves of wild type and mutant soybean. A two dimensional polyacrylamide gel analysis was performed on total leaf protein from wild type (*Glycine max* (L.) Merr. cv. Bragg) and mutant lines of soybean at various times after inoculation with *Bradyrhizobium japonicum* USDA110. This is a parallel study to the investigation of *in vitro* translational products by Sayavedra-Soto *et al* (2).

In case of soybean leaves, the protein extraction procedure is about 80% when normalized per ml of extraction buffer added. The total number of polypeptides that could be resolved by silver staining was above 200. Differences in polypeptides in the wild type and mutant lines of soybean due to development and inoculation, and its implication in autoregulation of nodulation will be discussed.

Factors that improved resolution on a 2-D gel were use of a different crosslinker, diacrylpiperizine instead of bis-acrylamide, thorough dialysis of the sample before isoelectric focussing, and use of CHAPS instead of Triton X-100.

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AUTOREGULATION OF NODULATION IN SOYBEAN IS NOT ASSOCIATED WITH MAJOR POLYPEPTIDE CHANGES IN LEAVES.

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Previous studies have demonstrated the involvement of the plant's shoot in the establishment and maintenance of autoregulation of the nodulation system in legumes (1). Total leaf RNA and poly A^+RNA of soybean (*Glycine max* (L.) Merrill) cultivar Bragg and its supernodulating mutant *nts*382 (2) were *in vitro* translated using a wheat germ translation system and [^{35}S]-methionine. Labeled polypeptides were compared by 2-dimensional gel electrophoresis. Whether plants were inoculated with *Bradyrhizobium japonicum* strain USDA110 (nodules appearing after 7-10 days) or grown uninoculated, no "on-off" differences in polypeptide pattern were detected. Differences were limited to changes in relative intensity of some peptides due to inoculation. The translation products of the supernodulator mutant *nts*382 (which lacks the factors controlling nodulation) were similar to those of Bragg during the 25 day experimental period. Developmental differences were clearly resolvable. Total protein analysis by 2-dimensional gel electrophoresis confirmed this result. Translatable mRNA populations, as well as accumulated leaf proteins, are similar for the supernodulation mutant and the wild type in both the inoculated and the uninoculated state. Thus no association of autoregulation of nodulation with a major change in polypeptides was detected.

Special care was taken to eliminate experimental variation by basing all reported data on multiple translations as well as independent isolations. In conclusion: a) Translation of the RNA populations and the resolution of the polypeptide patterns were reproducible in our hands in 6 replications of the experiment, b) The physiological differences for the establishment of nodulation control, which may involve either regulation at the biochemical level (enzyme activation, substrate availability, etc.) or transcriptional/synthesis changes, are too subtle to be detected by the *in vitro* (and total) protein separation procedures, c) At least 5 peptides changing in relative intensity, due to inoculation, were detected and d) We also recognize that artifactual differences due to growth conditions can be easily confused with true responses to the treatments, and care needs to be taken when evaluating polypeptide differences of *in vitro* translated products.

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LOCALIZATION OF mRNA ENCODING FOR VEGETATIVE STORAGE PROTEINS IN SOYBEAN

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Soybean vegetative storage proteins (VSP) accumalate to high levels in young leaves and then decline as they mature. VSP has an important function for the temporary storage of nutrients, particularly nitrogen, for reuse by developing seeds. Two genes, each encoding a different VSP subunit, have been cloned and sequenced. By using in situ hybridization technique, differential expression of the VSP genes has been characterized in leaf tissues at the mRNA level. It has been shown that most of the transcripts of VSP genes are in the bundle sheath and paraveinal mesophyll (PVM) cells in leaves. They are also present in some of the epidermal cells of young leaves. The PVM is a single cell layer network that interconnects the leaf vascular tissues and mediates the transfer of assimilates from the palisade and spongy mesophyll to the phloem. (PVM cells are unique to soybean and a few other legumes.) Expression level and pattern of VSP genes can be modulated by several factors including demand for mobilized leaf reserves (i.e. sink size), plant nitrogen nutrition, treatment with jasmonic acid (JA), and leaf wounding.

AN ABUNDANT HYDROXYPROLINE-RICH PROTEIN IN SOYBEAN SEED COATS IS AFFECTED BY THE SEED COLOR GENOTYPE

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Proteins extracted from developing seed coats of isogenic soybean lines that differ in seed color were compared by SDS-polyacrylamide gel electrophoresis. A 35 kilodalton (kd) protein was abundant in Richland (genotype *I/I*, yellow) and much reduced in T157 (genotype *i/i*, black seed). A novel procedure using polyvinylpolypyrrolidone column chromatography was developed to purify the protein for N-terminal sequencing, amino acid composition, and antibody production. The 35 kd protein is rich in proline and hydroxyproline. A cDNA clone for this protein was isolated from a Richland seed coat cDNA library and sequenced. It is markedly similar to a cDNA isolated from soybean hypocotyls that is a member of the soybean proline rich protein family (SbPRP) (Hong, Nagao, and Key, J. Biol. Chem., 262: 8667-8376, 1987; Averyhard-Fuller, Datta, and Marcus, Proc. Natl. Acad. Sci., USA 85: 1082-1085, 1988). Western blotting confirmed that the levels of saline soluble 35 kd proline rich protein (PRP) are much lower in seed coats from the *i/i* genotype at each stage of seed development. A number of other isogenic pairs that have *I/I* versus *i/i* genotype show the same effect on the 35 kd PRP. The effect of genotype on mRNA levels for the seed coat PRP is currently being investigated.

POST-TRANSLATIONAL PROCESSING AND DEPOSITION OF KUNITZ TRYPSIN INHIBITOR IN SOYBEANS

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The Ti^a allelic form of the Kunitz soybean trypsin inhibitor appears to be synthesized in precursor form. The precursor form is evident in Western blots of extracts of developing seeds (Glycine max, [L.] Merrill cv. Amsoy 71). The precursor can already inhibit bovine trypsin and appears as an anti-KSTI cross-reacting band with slower electrophoretic mobility compared to mature KSTI in non-denaturing electrophoresis gels. Levels of the precursor form increase to maximum levels during mid-maturation as seed lengths increase to 11 mm. As the seed increases to maximum size and turns yellow, precursor levels decrease while mature KSTI levels increase. The conversion from precursor to mature form can be demonstrated by incubating extracts of developing seeds at pH 4, then following the conversion by electrophoresis and scanning densitomery of the protein-stained gels. Such activity was evident during the period of seed fill and maturation. Conversion was also demonstrated in seeds that have been removed from the pod, pulse-labeled with [14C]-leucine, and incubated in culture medium for two hours. Radioactivity initially found coincident with the precursor was later found with the mature KSTI. The precursor form was found by SDS-PAGE to have a molecular weight larger than the mature form by 1.2 kd, suggesting that the conversion is proteolytic. Immunohistochemical analysis of the dry seed shows that the Kunitz inhibitor is not found in uniform concentrations throughout the cotyledons. The major deposition sites of the Kunitz trypsin inhibitor are toward the abaxial and adaxial sides of the cotyledon. There is less deposition in the central region of each cotyledon. This is the same pattern seen with the Bowman-Birk inhibitors. This is not, however, the pattern seen of distribution of the storage proteins which were found to be concentrated in the abaxial but not the adaxial regions.

GENE EXPRESSION OF SOYBEAN LIPOXYGENASES IN GERMINATED COTYLEDONS

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We found seven distinct lipoxygenases in 3-day-old cotyledons of germinated soybeans, four of which differed from the L-1, L-2 and L-3 enzymes present in dry seeds. Three of these four enzymes were separated on a Mono-S column (designated as LOX-A, LOX-B, LOX-C). They resembled each other and also L-3 in numerous characteristics. These included molecular weights, substrate specificities, pH optima, anomalous inverse dependences of activity on enzyme concentration, and regiospecificity (position of oxygen insertion into substrate). Amino acid sequences of the five tryptic peptides of LOX-A so far analyzed were identical to parts of an L-3 sequence but not to those of L-1 or L-2. Three proteolytic-peptides of LOX-C showed identical sequences to L-3, but not to L-1 or L-2, and two further peptides were similar but not identical to L-3. In spite of this similarity, calcium ion (1 mM) inhibited these separated enzymes to varying extents. A lipoxygenase, designated as LOX-D, was separated on a Mono-Q column from L-1, L-2 and L-3. Preliminary studies indicate that it differs from LOX-A, -B, and -C in exhibiting a normal dependence of activity on enzyme concentration.

To study their gene expression, we screened a cDNA library constructed from 5-day-old cotyledons after germination, and found three distinct cDNA clones. These differed from L-1 and L-2. Of the three cDNAs, two clones, SC514 and SC501, were used to isolate genomic clones. The nucleotide sequence of the SC514 genomic clone was determined. Amino acid sequence of the predicted protein showed that it differed from all lipoxygenases so far sequenced. We are currently sequencing the SC501 genomic clone.

UREASE PRODUCTION BY METHYLOBACTERIUM MESOPHILICUM, A SEED-TRANSMITTED BACTERIUM UBIQUITOUS IN SOYBEAN

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Methylobacterium mesophilicum is a bacterium that is internally seed transmitted and is ubiquitous in leaves of soybean. We have been unable to find soybean plants that are free of this bacterium in 11 years of research. This bacterium is unusual because it produces cellulose in submicroscopic fibrils the same size as those produced in plants. The objective of this research was to determine if M. mesophilicum produces urease, and if so, whether bacterial production of urease might be related to production of this enzyme by plants. Urease is an enzyme that converts urea to ammonia and carbon dioxide. M. mesophilicum was isolated from 25 soybean cultivars plus 25 other plant species, and these isolates were tested for production of urease. All isolates produced urease. Escherichia coli and Curtobacterium plantarum were used as negative controls, and these bacteria did not produce urease. A soybean line that did not produce ubiquitous urease was obtained from J. C. Polacco, and was grown in 1989 with Williams, the ubiquitous urease positive cultivar from which the urease negative line was derived. M. mesophilicum was isolated from leaves of 12 plants that were urease negative, and from leaves of 12 Williams plants. M. mesophilicum was isolated from all urease negative plants and from all Williams plants. There was no significant difference in the number of M. mesophilicum cells isolated from urease negative plants and urease positive Williams plants.

UREA METABOLISM IN SOYBEAN: A JOINT EFFORT BETWEEN PLANT AND MICROBIAL COMMENSAL?

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We have previously described mutations in the structural genes of each of the soybean urease isozymes. These two mutations (eu1-sun and eu4) eliminate the activity of the two enzymes. Upon constructing plants carrying both mutations, we were surprised to find a residual urease activity both in young leaves of the plants and in callus tissue derived from leaf. Furthermore, while both wild type leaf and callus tissues produce the so-called ubiquitous urease isozyme, the low level activity of the double mutant tissues resembled that of the seed-specific isozyme. We have now gathered evidence that the urease activity of the double mutant, which was thought to be a plant activity, is attributable to the presence of a bacterial commensal identified as Methylobacterium mesophilicum. This bacterium, which has been described as universally distributed on soybean, is isolable from all tissues we have examined, including apparently axenic cell cultures. The urease activity of the bacterium is biochemically similar to, but distinquishable from, that of the soybean seed-specific isozyme.

GENETIC CRITERIA FOR UREA UTILIZATION IN DEVELOPING SOYBEAN SEEDS.

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Previous work in this lab has described the roles of several loci that control the expression of the two urease isozymes in soybean plants. eu3-e1 is a tight pleiotropic recessive mutation that effects the loss of activity of both isozymes. Both isozymes are found in wild type developing soybean seeds, but their metabolic roles, if any, during seed development are unknown. We observed that urease negative eu3-e1 homozygotes transport urea to their progeny. Among the progeny, eu3-e1 homozygotes consistently accumulate urea, while their urease positive siblings, heterozygous at eu3-e1, do not accumulate urea. This suggests that one (or both) of the urease isozymes is normally active during seed development.

We are testing the activity of each urease isozyme in developing seeds by:

(1) Further genetic studies comparing urea accumulation by siblings which contain only one or the other of the urease isozymes. (2) Comparing urea utilization in developing cotyledons of our various urease mutants using in vitro culture techniques.

STUDIES OF CLONED SEQUENCE COMPONENTS OF M. ARENARIA DNA

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Meloidogyne arenaria is one of the most economically important nematode species that parasitizes soybean. A diagnostic M. arenaria RFLP probe, denoted pE1.6A, has been shown to carry an interspersed repeated sequence. The EcoR1 clone, taken from a shotgun pUC8 library of total genomic M. arenaria DNA, was found to share sequence homology with soybean and several different plants, a fungus, two insects, and a bird. One of the more interesting aspects of this probe is that it shares homology to as few as one band in some organisms and to as many as seven bands in other organisms. When probed to total genomic M. arenaria DNA, the 1.6kb insert of pE1.6A has homology to as many as 40 bands. A Southern hybridization was conducted by probing pE1.6A with pSR1.1 (a soybean clone containing a portion of the 26S subunit). No hybridization signal was evident in this experiment. However, when soybean RNA was probed with pE1.6A, hybridization with the 26S subunit was confirmed. The cloned insert of pE1.6A has been further restriction digested with Sau3A into four smaller fragments and probed with total M. arenaria RNA. One predominant band, presumably the region coding for the 26S subunit, was observed. The smaller fragments will be sequenced allowing for more thorough characterization of the cloned insert.

PREPARING A GENETIC MAP OF SOYBEAN USING AN INTRA-SPECIFIC CROSS

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Two crosses between the soybean (Glycine max L.) plant introductions PI 27890 ('Minsoy') and PI 290136 ('Noir 1') were carried out at ISU and the segregating progenies used to prepare a genetic map using RFLP, morphological, and isozyme markers. The most recent cross was also used by L. Mansur to prepare $F_{2:5}$ and $F_{2:6}$ families and 300 F_7-derived recombinant inbred lines to evaluate segregation for quantitative traits. Approximately 150 DNA probes have been characterized as RFLP markers in these crosses. Thirty of these were isolated in Beltsville, 20 are markers that have been used by R. Shoemaker et al. to prepare an inter-specific genetic map using G. soja and G. max. The remaining 100 were isolated at Utah. One-hundred and three of these markers have been tested in Utah against DNA isolated from plants of one or the other of the two intra specific crosses between the same PI's. Of these, 74 are in linkage groups. They define ca. 300cM in the earlier cross, with 25 markers in 9 linkage groups, and 590cM in the more recent cross with 53 markers in 19 linkage groups. Thirty markers so far relate linkage groups between the two crosses. At present, 13 linkage groups are related by common RFLP markers to linkage groups within the Soja-Max map prepared by Shoemaker et al. Five morphological and four isozymes markers scored by R. Palmer at ISU are being mapped in the most recent cross. The F_2-derived families have been used by L. Mansur at ISU to evaluate segregation for quantitative traits in order to map quantitative trait loci (QTL) using the RFLP, isozyme and morphological markers in the genetic map. So far genetic variation occurs for internode length, leaf area, lodging, canopy height, seed weight, seed protein and oil content and days to flowering and maturity. The F2-derived families will be used to do a preliminary search for linkages between the markers and QTLs. Putative linkages will be further investigated using the recombinant inbred lines.

COMPARISONS OF MOLECULAR MAPS FROM MAX-MAX AND SOJA-MAX SOYBEAN CROSSES

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Two public genetic maps are being constructed for soybean, using RFLP markers. The two crosses used in the construction of these maps are NOIR I X Minsoy and PI 468916 X A81-356022. Integration of these two maps has allowed us to draw some preliminary conclusions about the degree to which soybean genetic maps can vary depending upon genotypic background of the parents. In both crosses recombination frequency varied between linked markers and a higher than expected frequency of marker 'clustering' was observed. Data to date indicate that the order of markers does not vary, whereas linkage distances between markers does. These data suggest that two different maps, prepared using different parents, but the same RFLP markers, may contract (link) or expand (unlink) different regions of the genome, thus making an integrated molecular/ classical map a much more powerful genetic tool. The variation in recombination distance which occurs between different crosses is a valuable and perhaps necessary tool in constructing a genetic map of soybean. This type of information will provide a better understanding of soybean genome organization and gene interactions affecting quantitative traits.

GENETIC MAPPING OF BIOCHEMICALLY DEFINED LOCI IN SOYBEAN USING CLONED GENE PROBES.

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The genetics of soybean, one of the major U.S. and world crops, is relatively underdeveloped in comparison with other crop plants like corn, tomato or wheat. Classical genetic map of soybean is composed of 17 linkage groups, many of them composed of only two markers. To partly remedy this we created a genetic map the soybean genome using RFLP markers. Over three thousand low copy number soybean clones , derived form a Pst1 genomic library created in a phagemid vector, were used to identify polymorphisms between Bonus, a cultivar of *Glycine max* and PI 81762, an accession of *Glycine soja* , a wild relative, using five restriction enzymes. The diverse parents were chosen to maximize the number of identified polymorphisms. About 550 polymorphic probes were subsequently mapped in a population of 68 F2 individuals and F2-derived F3 families, segregating from a cross of Bonus X PI 81762. The map has an average resolution of 7 cM, with the largest distances between markers about 40 cM. The total length of the map is 2700 cM, in 23 linkage group. Further experiments will be required to assign linkage groups to the 20 soybean chromosomes.The detailed genetic map of soybean will be used to map agronomically important genes.

To further enrich the map, we used available cloned soybean genes to map many biochemically defined loci Cloned soybean genes, donated to us for this purpose, were screened for polymorphisms using thirteen six-cutter restriction enzymes. Most genes hybridized to more then one genetic locus, as expected in an allotetraploid organism. In many cases more then one polymorphism was found, enabling us to map multiple loci. One of the mapped loci is the 5S rRNA gene cluster, which can also be identified easily by in situ hybridization.Thus it will be possible to assign one of the RFLP-mapped linkage groups to cytogenetically identifiable chromosome.Polymorphisms identified using agronomically important genes will be useful in directly following introgression experiments without having to rely on phenotypic expression of the trait.

RFLP ANALYSIS OF SYMBIOTIC MUTANTS OF SOYBEAN

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The objective of this study is to determine linkage of soybean (*Glycine max* (L.) Merr.) nodulation phenotype to RFLP markers. Four soybean nodulation mutants were included: *nts*382 and *nts*1007 are supernodulators (1), *nod*49 and *nod*139 are nonnodulators (2). Each of the four mutants were originally isolated from M2 and M3 populations of ethyl methanesulfonate mutagenized seeds of a *G. max* cultivar Bragg. This common origin resulted in a high level of homology among Bragg and the four mutants. A more diverse population was needed in order to detect RFLP linkage (4).

The genus *Glycine* subgenus *Soja* consists of two species, the cultivated soybean (*Glycine max* (L.) Merr.) and the wild annual soybean (*Glycine soja* Sieb. and Zucc.). In order to obtain a more diverse population, crosses were made between Bragg, the four mutants, and a *G. soja* plant introduction (PI 468.397; courtesy of Dr. Gary Stacey, University of Tennessee). Linkage analysis is being conducted on the segregating F2 populations resulting from these crosses.

Iowa State University supplied 120 plasmid clones (courtesy of Drs. Paul Keim and Randy Shoemaker) which detect polymorphisms between their experimental line A81-356022 (*G. max*) and PI 468.916 (*G. soja*). These probes make up a large portion of a linkage map which covers 30 linkage groups, 168 loci, and 1800 centimorgans. Given the genomic size of soybean as 1.8×10^9 bp (3), this represents about 10 Mb DNA per locus.

Some of these probes did not detect polymorphisms between the Bragg genotypes and PI 468.397. At this time we have seen 17 out of 30 discernable RFLPs between *G. max* and *G. soja*; these are located on 13 of the 30 linkage groups. None of these RFLP patterns have shown a difference among Bragg, *nts*1007, *nts*382, *nod*139, and *nod*49, emphasizing the common origin of these symbiotic mutants.

F1 plants, from each cross, were confirmed by plant morphology, nodulation phenotype, flower color, and seed size and color. Heterozygous RFLP patterns were also detected for F1 plants when using the probe pK19 and restriction endonucleases *Eco*RI and *Hin*dIII.

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USING NEAR-ISOGENIC LINES AS A RESOURCE FOR POSITIONING CONVENTIONAL GENETIC MARKERS ON THE SOYBEAN RFLP MAP.

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About 4% of the genome of a near-isogenic line (NIL), when derived from the BC5 progeny of an initial recurrent parent (RP) x donor parent (DP) cross, will consist of DNA that has been retained from the DP. About 2/3 of this DP-derived DNA will be located in a chromosomal segment surrounding the introgressed marker. This segment will almost certainly contain molecular marker loci (i.e., isozymes and RFLPs). Consequently, if a DP-derived molecular allele is detected in an NIL (during the course of a comparative allelic analysis of the NIL with its RP and DP), linkage between the molecular marker and the introgressed gene can be presumed.

A theoretical basis for the NIL gene-mapping technique has been established (*Crop Sci.* 28:729-735). Its utility in detecting presumptive linkages was demonstrated for both isozyme loci (*Crop Sci.* 29:1548-1553) and RFLP loci (*Theor. Appl. Genet.* manuscript accepted pending revision).

The existence of RP/DP allelic contrasts at molecular loci are necessary if the NIL gene-mapping technique is to detect presumptive linkages. Observations that have been made to date indicate that, with respect to any given NIL (i.e., any given introgressed gene), RP/DP allelic contrasts can be expected to occur on average in about one-third of the available molecular loci.

The current soybean NIL collection encompasses NILs derived from the introgression of some 60+ genes from various DPs into one or both of the RPs "Clark" and "Harosoy". The public RFLP map is currently comprised of about 200+ RFLP loci scatted throughout about 2000+ cM of recombination distance. If the NIL gene-mapping technique were to be applied to any given NIL, one could expect an average of 66 of 200 RFLP loci to have an RP/DP allelic contrast. In turn, about 2 to 3 (4%) of these 66 should, on average, exhibit a presumptive linkage to an introgressed marker. These data suggest that NIL gene-mapping would be a worthwhile endeavor.

The current brevity of the soybean conventional marker linkage map is due to the lack of systematic means of sorting markers into the 20 potential linkage groups. The NIL gene-mapping technique can offer a means of partially accomplishing this. Presumptive linkage data, when examined *in toto*, could "position" the 60+ introgressed genes on the RFLP map. Although this data would not always be unambiguous, it would, like a crossword puzzle, provoke additional genetic analyses of soybean genome.

RFLP MAPPING OF PHYTOPHTHORA RESISTANCE LOCI IN SOYBEAN

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Phytophthora root rot caused by *Phytophthora megasperma* Drechs. f. sp. *glycinea* Kuan and Erwin is one of the most serious diseases of soybean [*Glycine max* (L.) Merr.]. Six loci with alleles that give race specific resistance of soybean to phytophthora have been reported. Only one of these loci, *Rps1*, previously has been genetically mapped. The objective of this study is to map the resistance loci using RFLP markers. The mapping is being conducted using a series of Williams near-isogenic lines with each isoline having a phytophthora resistance allele backcrossed into it. The isolines were screened with approximately 150 mapped RFLP markers. At least one marker that was polymorphic between each isoline and the recurrent parent was found. Each polymorphic marker has a probability of approximately 0.65 of being linked to the disease resistance locus. Linkage tests between the polymorphic RFLP markers and each of the six *Rps* loci are being conducted in F2 populations. Linkage has been detected between RFLP markers and *Rps1*, *Rps2*, and *Rps5*. Linkage tests are in progress between RFLP markers and *Rps3*, *Rps4*, *Rps6*.

APPLICATION OF PULSED-FIELD GEL ELECTROPHORESIS FOR THE PHYSCIAL MAPPING OF THE NODULIN REGION OF THE SOYBEAN GENOME

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Several soybean genes have been cloned that encode nodule-specific proteins (nodulins) expressed during symbiosis. The linkage of these genes has not been established due to a lack of mutants for classical genetic studies and the lack of sensitive in situ hybridization techniques for single copy probes in soybean. The technique of pulsed-field gel electrophoresis (PFE) has been used to study chromosomal regions and entire genomes of several organisms. PFE can be used to separate large DNA fragments for constructing yeast artifical chromosome (YAC) libraries, chromosome walking, determining physical equivalents of genetic distances, and linking of RFLP maps. We are using PFE to generate a physical map of the nodulin gene region(s) of the soybean genome. Here we present techniques for the isolation of intact high molecular weight DNA in embedded soybean protoplasts, the identification of restriction enzymes for generating large DNA fragments, and the conditions for separating large DNA fragments using transverse alternating-field electrophoresis (TAFE) and pulsed-field gradient electrophoresis. MluI, NaeI, and SfiI generate DNA fragments ranging from 50 kb to 1000 kb. The majority of fragments generated with ApaI, KpnI, NarI, NotI, and SmaI are less than 500 kb. Complete digestion was confirmed by Southern hybridization using single copy clones; hybridization bands as large as 750 kb have been detected for some nodulins.

ANALYSIS OF THE SOYBEAN GENOME USING PULSED FIELD GEL ELECTROPHORESIS (PFGE)

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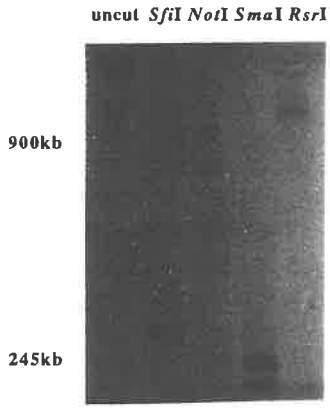
Introduction. Pulsed field gel electrophoresis (PFGE) is used widely in the analysis of the genomes of mammals and microorganisms, and recently a procedure for its application to a higher plant (tomato) was published (1,2). This requires the preparation of unsheared chromosomal DNA, digestion of the DNA with rare-hitting restriction enzymes and, following electrophoresis, the transfer of large DNA fragments to nylon membranes for probing with clones from a genomic library. The technique is being developed in this laboratory as part of a reverse genetics strategy (3) to locate and characterize the genes responsible for the super- and non-nodulating phenotypes of previously isolated soybean mutants (4).

Methods. To obtain intact chromosomal DNA of soybean, protoplasts were prepared from young trifoliate leaves and mixed with an equal volume of LMP agarose (INCERT, FMC) before being pipetted into a prechilled mold. Plug-embedded cells were lysed with 0.5M EDTA, 1% sarkosyl and mg/ml proteinase K and the plugs were equilibrated at length with TE buffer prior to restriction digestion using 10 units enzyme/µg DNA. Plugs were loaded directly onto the gel and running conditions were typically a 60s pulse for 15h followed by 90s for 9h, 200V in 0.5xTBE (CHEF apparatus, BIORAD). DNA was UV-nicked after staining with ethidium bromide and vacuum blotted onto onto nylon membranes (ZETAPROBE, BIORAD). Probes (kindly supplied by Dr. Randy Shoemaker, Iowa St. Univ.) were labelled to high specific activity using the hexanucleotide random priming method.

Results. The autoradiograph shows that this procedure can be used with soybean to produce unsheared DNA embedded in agarose blocks (as demonstrated by hybridization of probe to position of the well in the undigested lane). Restrictability of the embedded DNA is proved by hybridization of the same probe to DNA fragments in the size range 100 to 400 kb produced by the enzymes *SmaI* and *SfiI*. We plan to check the utility of thetechnique in long range mapping of defined regions of the soybean genome, and to investigate the correlation between physical distance along the DNA and distances on the genetic map of soybean.



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USDA PLANT GENOME RESEARCH PROGRAM

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The program will be national in scope with strong international implications and will require maximum input, cooperation, and planning across the entire scientific community. The ARS has been assigned lead responsibility for the program and a coordinating committee cochaired by a representative from the Cooperative State Research Service (CSRS) has been appointed and will serve to enhance and ensure planning and cooperation across ARS, CSRS, the entire university system and industry. There will be networking and coordination with plant genome activities of the National Institutes of Health, National Science Foundation, Department of Energy, Forest Service, other Federal agencies, the agricultural industry, and the international arena.

Agricultural biotechnology advancement with plant commodities can occur only after genes and related gene functions have been determined. The USDA Plant Genome Program will be focused on real world problems and will consider the factors given below:

--Removal of heavy metals to improve water quality.
--Increase efficiency of nitrogen use by plants to improve efficiency of fertilizer application.
--Drought and heat tolerance necessary to meet current and future climate conditions.
--Develop new economic plants for new and more market niches.
--Develop new non-food uses for agricultural commodities.
--Determine natural plant products to serve as herbicides and pesticides.
--Develop disease and pest resistant plants.
--Efficiently search germplasm for genes to transfer.

The effort will target desirable genes, effect molecular gene transfer, and determine the associated metabolism to yield desired genetic expression in important plant commodities and tree species. As lead agency, ARS will be responsible for establishing and effecting a logical sequence of information development to get products in the market place. It will be a science driven program operated through competitive grants that will be open to scientists from private industry, universities, and government laboratories.

DAMAGE TO GENOMIC DNA AFTER SEED AGING IN SOYBEAN (G.MAX)

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There are two questions being asked in this project: Is the genetic heritage of soybean seed maintained during long-term storage? Do lesions in the genetic material have an impact on seed viability? Research into DNA sequence alterations in aged soybean seeds has initially focused on the condition of DNA sequences following 30 years of storage. DNAs from single soybean (Glycine max L.) seeds from the 1958 harvest of the cultivars 'Henry' and 'Ross' were assayed for "nicks" via denaturing agarose gel electrophoresis and by in vitro assays of DNA pol I primer sites. It appears that there is an extensive accumulation of single-stranded "nicks" in both axis and cotyledonary DNA from 30 year old seed and that the level of DNA damage is roughly equivalent in both viable and inviable aged seeds. Using a quantitative assay of the ability of genomic DNAs to serve as templates in the polymerase chain reaction (PCR), we are currently attempting to determine the level of DNA sequence deterioration of specific regions within and around one of the structural genes for glycinin (Gy-1). It should eventually be possible to determine if the nicks in seed DNA are occurring randomly or if the deterioration is somehow "directed" to specific chromosomal regions. In addition, access to the DNA fragments which constitute a molecular "linkage map" for soybean (from Dr. R. Shoemaker at Iowa State) allow us to examine the level of sequence detioration in distinct linkage groups. Comparisons of the DNA integrity in dry seed, following imbibition, and at several stages during early germination have revealed that the rates of DNA repair are reduced in aged vs. fresh seeds.

FLUORESCENT *IN SITU* HYBRIDIZATION WITH SOYBEAN METAPHASE CHROMOSOMES

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In recent years, fluorescent probes have proven to be sensitive detection methods to locate genes in human chromosomes by *in situ* hybridization. We have investigated the use of fluorescent probes to localize repeated DNA sequences to metaphase chromosome spreads from soybean root tips. The specific DNA probe was a 1.05 kb internal fragment of a soybean gene encoding the 18s ribosomal RNA subunit. The rDNA probe was labeled either by nick-translation with biotin-11-dUTP or by terminal addition of poly T tracts. Detection was accomplished with fluorescein-labeled avidin. The signal was then amplified with a biotinylated anti-avidin antibody followed by a final layer of fluorescein-labeled avidin. In the case of poly T tailing, a Bio-Bridge molecule (Enzo Diagnostics, Inc., N.Y.) consisting of biotinylated oligo A tracts was hybridized to the probe prior to subsequent fluorescein detection. On metaphase chromosomes from a standard diploid cultivar, the rDNA probe exhibits distinct yellow fluorescent signals on two of the forty metaphase chromosomes that have been counterstained with either propidium iodide or DAPI fluorochromes. From earlier analyses on soybean pachytene chromosomes (Singh and Hymowitz, TAG 76: 705-711), it was observed that chromosome 13 is closely associated with the nucleolar organizer region. We demonstrate that fluorescent signals using the rDNA probe are found on three metaphase chromosomes from plants that are known to be trisomic for chromosome 13.

SUSCEPTIBILITY OF A SOYBEAN CULTIVAR AND DERIVED NODULATION MUTANTS TO AN *AGROBACTERIUM TUMEFACIENS* STRAIN.

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Experiments were conducted to determine the susceptibility of soybean cultivar Bragg and derived nodulation mutants to *Agrobacterium tumefaciens*. Susceptability of different species and even cultivars to agrobacterium varies greatly. Also soybean is not naturaly susceptable to *Agrobacterium(2)*. Therefore these experiments are preliminary to genetic transformation of these soybean lines. The study compared the susceptibility of cv Peking which has been successfully transformed (3) to cv Bragg and the derived nodulation mutants *nts*382, *nod*49 and *nod*139 both *in vivo* and *in vitro*. The plants and tissues were inoculated with A208, which is the background strain upon which our engineered vector ASE9749 is based. Scoring of susceptibility *in vivo* was based on visual gall formation and positive nopaline assays. Susceptibility *in vitro* was based on positive nopaline assays (4) alone.

Results

(*In vivo*)		# positive	
Cultivar	visual gall formation	nopaline assay	%
Peking	64/64	8/8	100%
Bragg	35/64	35/35	55%
*nod*139	32/66	27/64	42%
*nod*49	18/64	17/64	26%
*nts*382	17/66	13/66	19%

(*In vitro*)	#nopaline positive		#nopaline positive	
Cultivar	hypocotyl culture	%	cotyledon culture	%
Peking	4/20	20%	10/17	59%
Bragg	0/40	0%	5/50	10%
*nod*139	0/20	0%	0/20	0%
*nod*49	1/20	5%	0/20	0%
*nts*382	0/20	0%	0/50	0%

Discussion *In vivo* and *in vitro* cultivar Bragg and its deriveds mutants *nts*382, *nod*49 and *nod*139 show significantly lower levels of susceptability compared to cv Peking. This result mirrors past work on soybean /*Agrobacterium* susceptability (1) and suggests *Agrobacterium* based transformation of Bragg and its mutants may be difficult.

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DEVELOPMENT OF SOYBEAN GERMPLASM WITH SUPERIOR REGENERATION CAPACITY AND SUSCEPTIBILITY TO *AGROBACTERIUM*

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The development of *Agrobacterium*-mediated genetic transformation systems for soybean has been difficult. However, limited success has been obtained with Peking, which is the one genotype that has been consistently identified as susceptible to *Agrobacterium*. Nevertheless, the existence of a genotype susceptible to *Agrobacterium* indicates the potential to transfer this trait into other genotypes, especially ones with higher regeneration capacities than that of Peking.

Century, PI 417138, PI 417210, and Thomas were selected as genotypes not susceptible to *Agrobacterium* and crossed with Peking. Century and the two PI's were selected because they are among the genotypes identified so far as having relatively high regeneration capacities, while Thomas was selected because it is a cultivar adapted to the southern U.S. All F_1 hybrids formed galls that were comparable to those formed on Peking. Each F_1 was backcrossed to Peking, and the progeny evaluated for degree of galling response from replicated inoculations on each plant. A 1:1 segregation for gall formation was observed in the BC_1 generation, suggesting that susceptibility to *Agrobacterium* is conditioned by one dominant gene. This hypothesis is currently being tested by evaluating the response to *Agrobacterium* inoculations in F_2 populations.

Efforts are also underway to develop soybean lines with higher regeneration capacities than those currently available. The two PI's mentioned previously, which originate from Japan and one of which has been classified as *G. gracilis*, were crossed with Century. Starting with the F_3 generation, individuals were selected based on their ability to regenerate and convert into plants. Thus far, at least one F_4 line has been obtained with a regeneration capacity higher than that of its parents. After several backcrosses to Century, these lines are expected to be useful in particle gun-mediated transformation.

Finally, an attempt is in progress to introgress regeneration capacity into southern germplasm, since its regeneration capacity is considerably less than that of northern germplasm. Towards this end, Manchu, believed to be the main ancestral source of regeneration capacity in northern germplasm, was crossed with Thomas. The F_4 progeny with the highest regeneration capacity are being backcrossed to Thomas, and high-regenerating progeny from this backcross will in turn be crossed with progeny from the Peking x Thomas cross, potentially resulting in germplasm adapted to the southern U.S. which will regenerate from culture and be susceptible to *Agrobacterium*. As the cross of Manchu x Thomas is essentially a cross between a regenerator and a non-regenerator, segregation among the progeny is expected to give insight into the genetic control of regeneration via somatic embryogenesis.

Transient Gene Expression in Apical Meristems mediated by Microprojectile Bombardment

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The apical meristem has become a promising target for the introduction of foreign DNA into plants. In this study, embryonic axes were excised from mature seed and treated on Murashige and Skoog (MS) medium with or without the cytokinin, 6-benzylaminopurine (BAP). Cytokinin treatment promoted growth of multiple axillary meristems and inhibited the primary apical meristem. Embryos were examined histologically to follow the structural development of the meristems over time. Meristems treated with BAP lagged in structural development by approximately 24 hours behind non-BAP treated meristems. In addition, a lag of several hours was observed for the initiation of DNA replication of meristems treated with BAP by incorporation of [^{3}H] thymidine. Transient expression of beta-glucuronidase (GUS) was investigated by microprojectile bombardment of meristems at various times after imbibition and culture. Bombardment from 3 to 9 times per sample indicated consistently high GUS expression levels in the apical meristem. Histochemical staining revealed blue cells in the apical dome area and quantitative fluorometric analysis showed GUS accounted for up to $1X10^{-3}$% of total protein in some samples. Some axillary shoots show stable GUS expression after 14 to 28 days using cytochemical staining. However, progeny of putatively transformed plants have been evaluated by enzyme expression, PCR and Southern analysis with no recovery of stable transformants.

TRANSGENIC SOYBEAN PLANTS OBTAINED VIA PARTICLE BOMBARDMENT OF EMBRYOGENIC CULTURES

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Embryogenic suspension cultures of soybean (*Glycine max*) were subjected to particle bombardment, where high density particles carrying DNA were accelerated towards the embryogenic plant cells. Following penetration of the plant cells by the particles, the DNA disassociates from the particles and can then be expressed. Transient expression of a scorable marker gene (β-glucuronidase (GUS)) was initially used to optimize bombardment parameters. Following optimization, soybean cells were bombarded with particles coated with DNA encoding for either hygromycin resistance or both hygromycin resistance and GUS. One to two weeks following bombardment, embryogenic soybean cells were placed under hygromycin selection. Four to six weeks following bombardment, lobes of green, hygromycin-resistant tissue could be seen protruding from clumps of brown/black hygromycin-sensitive tissue. Individual clumps which contained green lobes were placed in fresh liquid medium containing hygromycin. Cultures which proliferated from single clumps of tissue represent independent clones. For embryo development, clumps of proliferating transgenic embryogenic material were transferred to a medium containing 3% maltose for one month. Following a short embryo desiccation treatment, individual embryos were transferred to germination medium. Transgenic soybean plants were obtained 5 months following bombardment. Southern hybridization analyses confirmed the presence of the hygromycin resistance gene in all selected embryogenic soybean tissue and regenerated plants tested to date. Embryogenic tissues transformed with the plasmid construction containing both hygromycin resistance and GUS displayed GUS activity as either solid positive, solid negative or sectored. Copy number and integration patterns in transgenic clones were quite variable. Regenerated T0 plants were fertile and the introduced DNAs were transmitted to T1 progeny plants.

INTRINSIC GUS-LIKE ACTIVITY IN SOYBEAN

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Soybean (*Glycine max* L. Merr. cv. Pella and Williams 82) tissues were surface sterilized and intrinsic GUS-like activity was determined aseptically by a histochemical staining (X-GLU) method. Immature soybean pods at different stages of development showed positive staining reactions, particularly the pod walls, seed coats, endosperms and embryos. Generally, the vascular bundle tissue showed a higher intrinsic GUS-like activity than the surrounding tissues. Germinating mature seeds showed an increase in staining reaction during the first three days, then rapidly decreased after the fourth day of germination and reached undetectable levels by the tenth day of germination. Most of the excised embryos (heart to early cotyledon stages) showed no or undetectable staining activities after one to two days cultured on B5 medium, particularly the smaller embryos. Only a few larger embryos infrequently showed some traces of blue spots by the fifth and seventh day of culture. Results obtained from qualitative fluorometric GUS assays on seed of all stages of development are in agreement with the histochemical staining method. Enzyme(s) responsible for such intrinsic GUS-like activities in soybean is antigenically dissimilar to *E. coli beta*-glucuronidase.