2nd Biennial Conference on Molecular and Cellular Biology of the Soybean

25-27 July 1988

Iowa State University
Ames, Iowa U.S.A.

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 30 minutes after the session is adjourned.

Acknowledgments

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

Robert Benson, Personnel Services
Roberta Kerkove, Department of Agronomy
Tom McCormick, Continuing Education
Eileen Muff, Department of Genetics
Beth Sweeney, Departments of Agronomy and Genetics

SCHEDULE OF PAPERS

FOR THE

2ND BIENNIAL CONFERENCE ON MOLECULAR AND CELLULAR BIOLOGY OF THE SOYBEAN

Monday Afternoon (July 25, 1988) **1300-1615**

Building: Fisher Theater, Iowa State Center

Presiding: Alan G. Atherly, Iowa State University

1300 (1)*

GENETIC ANALYSIS OF THE HOST CONTRIBUTION TO SYMBIOTIC NODULATION
Peter M. Gresshoff, University of Tennessee, Knoxville, TN; and Anne Mathews, Angela C. Delves, and Bernard J. Carroll, Australian National University, Canberra, Australia.

1315 (2)

SUPERNODULATION AND NON-NODULATION MUTANTS OF SOYBEAN
Peter M. Gresshoff[1], Alexander Hansen[2], Anne Mathews[2], Amo Krotzky[1], Jane Olsson[2], Angela C. Delves[2], David A. Day[2], and Bernard Carroll[2], [1]University of Tennessee, Knoxville, TN and [2]Australian National University, Canberra, Australia.

1330 (3)

IN VITRO TRANSLATION AND GENOMIC MAPPING OF SOYBEAN MOSAIC VIRUS RNA
F. Gadani, Enichem SpA, Research and Development, Milan, Italy and L. M. Mansky, D. P. Durand, J. H. Hill, and R. E. Andrews, Iowa State University, Ames, IA.

1345 (4)

A POSSIBLE MOLECULAR MECHANISM OF RESISTANCE TO SOYBEAN MOSAIC VIRUS
R. J. Clem, J. H. Hill, R. E. Andrews, and D. P. Durand, Iowa State University, Ames, IA.

1400 (5)

IN VITRO EXPRESSION OF THE CELLULOSE GENE IN METHYLOBACTERIUM MESOPHILICUM, A SEED-TRANSMITTED BACTERIUM UBIQUITOUS IN SOYBEAN
J. M. Dunleavy, U.S. Department of Agriculture, Agricultural Research Service, Iowa State University, Ames, IA.

BREAK **1415-1445**

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

Presiding: Jack Widholm, University of Illinois **1445-1615**

1445 (6)

USE OF IN VITRO TECHNIQUES TO EVALUATE, SCREEN AND SELECT SOYBEAN GENOTYPES FOR BROWN STEM ROT TOLERANCE

J. Thimmapuram, L. E. Gray, and J. M. Widholm, University of Illinois at Urbana-Champaign, Urbana, IL.

1500 (7)

CAN PARTS OTHER THAN IMMATURE COTYLEDONS REGENERATE SHOOTS OR EMBRYOS IN SUFFICIENT NUMBERS TO BE USEFUL IN A SOYBEAN TRANSFORMATION SYSTEM

JooHag Kim and Clifford E. LaMotte, Iowa State University, Ames, IA.

1515 (8)

SOMATIC HYBRIDISATION OF SOYBEAN WITH GLYCINE CANESCENS

N. Hammatt, M. R. Davey, R. S. Nelson*, A. Lister and E. C. Cocking, University of Nottingham, Nottingham, UK; and *Nickersons Seed Co., Cambridge, UK.

1530 (9)

SHOOT ORGANOGENESIS FROM MATURE SEEDS OF GLYCINE SOJA AND G. MAX

C. Y. Hu, Wm. Paterson College of New Jersey; and G. C. Yin, Soybean Research Institute, Heilongjiang Academy of Agricultural Science, PRC.

1545 (10)

MATURATION AND CONVERSION KINETICS OF SOYBEAN SOMATIC EMBRYOS

J. Buchheim, S. M. Colburn, and J. Ranch, United AgriSeeds, Champaign, IL.

1600 (11)

PLANT REGENERATION FROM MONOCELL CULTURE OF SOYBEAN (GLYCINE MAX)

X. M. Luo, G. L. Zhao, and Y. Z. Liu, Soybean Institute, Gongzuling, Jilin Province, PRC; and L. J. An, M. Y. He, and S. Hao, Northeast Normal University, Changchun, PRC.

Barbecue Picnic and Reception **1830-2100**

South courtyard of the Scheman Building

Tuesday Morning (July 26, 1988) **0900-1200**

Presiding: Joseph Burris, Iowa State University

0900 (12)

SOYBEAN MUTANTS LACKING THE UBIQUITOUS UREASE

J. C. Polacco and Adam Judd, University of Missouri, Columbia, MO; and Silvia Cianzio, Iowa State University, Ames, IA.

0915 (13)

STRUCTURAL ANALYSIS OF A UREASE GENOMIC CLONE
J. D. Griffin and J. C. Polacco, University of Missouri-Columbia, Columbia, MO.

0930 (14)

IDENTIFICATION OF MOLECULAR CLONES WITH SOYBEAN UREASE ISOZYMES
M. A. Holland, O. M. Zaghmout, and J. C. Polacco, University of Missouri, Columbia, MO.

0945 (15)

ISOLATION AND CHARACTERIZATION OF A UREASE-LIKE GENOMIC CLONE
R. S. Torisky and J. C. Polacco, University of Missouri-Columbia, Columbia, MO.

1000 (16)

POSSIBLE GERMINATION-SPECIFIC LIPOXYGENAGES IN SOYBEAN
T. K. Park and J. C. Polacco, University of Missouri-Columbia, Columbia, MO.

1015 (17)

IMPROVEMENT OF SEED PROTEIN USING SUBUNIT VARIANTS OF 7S GLOBULIN IN SOYBEAN (G. MAX L.)
K. Kitamura, National Agriculture Research Center, Tsukuba; and T. Ogawa, National Chugoku Agricultural Experiment Station, Fukuyama; and N. Kaizuma, Iwate University, Morioka, Japan.

BREAK **1030-1100**

Presiding: James Specht, University of Nebraska **1100-1200**

1100 (18)

SOYBEAN VEGETATIVE STORAGE PROTEIN GENE EXPRESSION: REGULATION BY JASMONIC ACID
Paul Staswick, University of Nebraska, Lincoln, NE.

1115 (19)

GENETIC ANALYSIS OF THREE CHLOROPHYLL-DEFICIENT MUTANTS DERIVED FROM A SOYBEAN POPULATION CONTAINING A TRANSPOSABLE ELEMENT
B. R. Hedges and R. G. Palmer, Iowa State University, Ames, IA.

1130 (20)

SOYBEAN TGM ELEMENTS CONTAIN AN OPEN READING FRAME SIMILAR TO THE MAIZE EN/SPM TRANSPOSABLE ELEMENT
P. R. Rhodes, USDA-ARS, Beltsville, MD; and L. O. Vodkin, University of Illinois, Urbana, IL.

1145 (21)

AN UNSTABLE MUTATION AFFECTING SOYBEAN SEED COAT COLOR
Joel M. Chandlee and Lila O. Vodkin, University of Rhode Island, Kingston, RI and University of Illinois, Urbana, IL.

LUNCH 1200-1330

Tuesday Afternoon (July 26, 1988) 1330-1615

Presiding: Reid G. Palmer, USDA-ARS and Iowa State University

1330 (22)

ANALYSIS OF SOMACLONAL VARIANTS IN SOYBEAN (GLYCINE MAX (L.) MERR.)
L. A. Amberger, R. G. Palmer, and R. C. Shoemaker, USDA-ARS, Iowa State University, Ames, IA.

1345 (23)

TISSUE CULTURE INDUCED VARIATION IN SOYBEANS (GLYCINE MAX (L.) MERR.)
U. B. Zehr and J. M. Widholm, University of Illinois at Urbana-Champaign, Urbana, IL.

1400 (24)

GENOMIC RELATIONSHIPS AMONG NINE OF THE TWELVE WILD PERENNIAL SPECIES (2N=40) OF THE SUBGENUS GLYCINE
T. Hymowitz, R. J. Singh, and K. P. Kollipara, University of Illinois, Urbana, IL.

1415 (25)

THE GENOMIC RELATIONSHIP BETWEEN GLYCINE MAX (L.) MERR. AND G. SOJA SIEB. AND ZUCC. REVEALED BY PACHYTENE CHROMOSOME ANALYSIS
R. J. Singh and T. Hymowitz, University of Illinois, Urbana, IL.

BREAK 1430-1500

Presiding: Paula Chee, The Upjohn Company 1500-1615

1500 (26)

TRANSFORMATION OF PERENNIAL GLYCINE SPECIES BY AGROBACTERIUM RHIZOGENES
B. Jones, E. L. Rech, T. J. Golds, N. Hammatt, B. J. Mulligan, and M. R. Davey, University of Nottingham, Nottingham, UK.

1515 (27)
EXOGENOUS DNA TRANSFER INTO SOYBEAN CELLS AND TISSUE
C. M. Buising, R. C. Shoemaker, M. S. Heath, H. Zhou, A. G. Atherly, and R. M. Benbow, Iowa State University, Ames, IA; and D. T. Tomes, Pioneer Hi-Bred International, Inc., Johnston, IA.

1530 (28)
PROGRESS IN THE DEVELOPMENT OF GENE TRANSFER SYSTEMS FOR SOYBEANS
D. F. Hildebrand, V. Purcell, W. Parrott, D. Bhatt, E. G. Williams and G. B. Collins, University of Kentucky, Lexington, KY.

1545 (29)

SOYBEAN REGENERATION AND INVESTIGATIONS OF AGROBACTERIUM TUMEFACIENS-MEDIATED TRANSFORMATION
B. W. Delzer, J. H. Orf, and D. A. Somers, University of Minnesota, St. Paul, MN.

1600 (30)

GENETICALLY ENGINEERED SOYBEAN
D. E. McCabe, W. F. Swain, B. J. Martinell, W. J. Brill, and P. Christou, Agracetus, Middleton, WI.

Wednesday Morning (July 27, 1988) **0900-1030**

Presiding: Lila O. Vodkin, University of Illinois

0900 (31)

EVALUATION OF CYTOPLASMICALLY INHERITED CHLOROPHYLL-DEFICIENT SOYBEAN MUTANTS
R. C. Shoemaker, J. Logsdon, and P. Close, USDA-ARS, Iowa State University, Ames, IA.

0915 (32)

ORGANIZATION AND EXPRESSION OF THE SOYBEAN MITOCHONDRIAL GENOME
E. A. Grabau, A. J. McCullough, and B. G. Gengenbach, University of Minnesota, St. Paul, MN.

0930 (33)

CHLOROPLAST DNA RESTRICTION SITE POLYMORPHISM IN GLYCINE SUBGENUS SOJA
P. S. Close, P. Keim, and R. C. Shoemaker, Iowa State University, Ames, IA.

0945 (34)

MAPPING THE SOYBEAN (G. MAX) GENOME USING NEAR-ISOGENIC LINES
G. J. Muehlbauer, J. E. Specht, M. A. Thomas-Compton, P. E. Staswick, G. L. Graef, University of Nebraska, Lincoln, NE; and R. L. Bernard, USDA-ARS, University of Illinois, Urbana, IL.

1000 (35)

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) IN TWO (G. MAX) CULTIVARS AND CELL CULTURES PREPARED FROM THESE CULTIVARS

J. Roth, T. Malcalma, and K. G. Lark, University of Utah, Salt Lake City, UT.

1015 (36)

MEASURING GENETIC DIVERSITY IN THE SOYBEANS USING RFLP MARKERS

P. Keim, R. G. Palmer, and R. C. Shoemaker, Iowa State University, Ames, IA.

1030

ADJOURN TO COFFEE/DISCUSSIONS

GENETIC ANALYSIS OF THE HOST CONTRIBUTION TO SYMBIOTIC NODULATION

Peter M. Gresshoff, Plant Molecular Genetics, College of Agriculture, University of Tennessee, Knoxville, TN 37920, Anne Mathews, Angela C. Delves, and Bernard J. Carroll, Botany Department, Australian National University, Canberra, ACT, 2600, Australia

The inheritance of EMS-induced supernodulation and non-nodulation mutants of soybeans cv. Bragg was further investigated (see Carroll et al, 1985b, Plant Physiol. 78, 34-40, Carroll et al, 1986, Plant Science 47, 109-119, Mathews et al, 1987, J. Plant Physiol. 131, 349-361). Supernodulation mutants, which were characterised by nitrate-insensitive nodulation, were found to be altered in their autoregulation of nodulation response, presumably because of a deminished synthesis of an inducible shoot derived inhibitory signal (Gresshoff et al, 1988, J. Plant Physiol, in press). Supernodulation mutants were crossed to assign complementation groups. All mutants, except for nts1116, failed to complement, suggesting that they are alleles in the same genetic complementation group (Delves et al 1988, J. of Heredity, in press). Backcrosses to wild-type, F2 analysis and F3 stability tests confirm our previous report that all tested nts alleles are single recessive mutations and behave in a Mendelian manner (Carroll et al, 1988, TAG, in press). Mutant nts1116, characterised by an intermediate supernodulation phenotype, showed dominance to nts382 (an extreme supernodulation allele), but was recessive to wild-type. Complementation analysis of non-nodulation mutants revealed that nod49 and nod772 were allelic with the naturally occurring rj1 locus. In contrast nod139 was a separate complementation group. The non-nodulation alleles epistatically suppressed supernodulation. We isolated a homozygous double mutant between nts382 and nod49. Mutant nod139 is characterised by an absence of curled root hairs, nodules and subepidermal cell divisions. Nod49 and nod772 in contrast are hair curling and nodulation deficient, but form subepidermal pseudo- infections (lacking infection sites, but showing cell divisions). All three non-nodulation alleles are unlinked to the nts382 supernodulation allele and show simple Mendelian inheritance.

SUPERNODULATION AND NON-NODULATION MUTANTS OF SOYBEAN

Peter M. Gresshoff[1], Alexander Hansen[2], Anne Mathews[2], Arno Krotzky[1], Jane Olsson[2], Angela C. Delves[2], David A. Day[2], and Bernard Carroll[2], [1]Plant Molecular Genetics, College of Agriculture, University of Tennessee, Knoxville, TN 37901-1071 USA, [2]Australian National University, Canberra ACT 2601, Australia

Developmental mutants of Glycine max cv. Bragg are being used to analyse the early steps of the Bradyrhizobium-induced root nodule symbiosis. We have isolated and partially characterised twelve independent supernodulation (nts) (1,2) and three non-nodulation mutants (3). Complementation analysis showed that all mutants were controlled by single mendelian recessives. All supernodulation alleles were placed in the same complementation group (4). Non-nodulation mutations nod49 and nod772 were allelic to the naturally occurring variant rj1, while nod139 was in a different complementation group. Nod139 roots, when inoculated with strain USDA110 (10^{6-7} bacteria per plant), failed to show subepidermal cell divisions (Scd^-), root hair curling (Hac^-) and infection thread formation (Inf^-). Nod 49 and nod772, just like rj1, was Scd^+, Hac^- and Inf^-. Colonisation and attachment was normal. All non-nodulation mutants showed occasional nodulation if inoculated with high titre bacteria (5). Non-nodulation (controlled by either nod49 or nod139) assorted independently from the nts locus and epistatically suppressed the supernodulation phenotype (9:3:4 ratio)(7).

Grafting experiments confirmed that non-nodulation is controlled by the genotype of the root, while supernodulation is controlled through the shoot (6). Cytological analyses of infected roots showed that both actual and pseudo-infections occurred at the same frequency in Bragg wild type and nts382. Bragg roots, however, had most of the cell division events in the first three developmental classes, suggesting a major symbiotic arrest after the first 24-36 hours after inoculation. Mutant nts382, in contrast, showed faster nodulation (i.e. more nodules above the root tip at the time of inoculation than in Bragg and earlier appearance of visible nodules) as well as a pronounced absence of symbiotic arrest at the stage III/IV transition (7).

Split root experiments using Bragg and nts382 grown on low level nitrate (0.5mM KNO_3), which was included to permit the equal development of both root halves, demonstrated that Bragg systemically regulated nodule appearance and growth within 24 hours of the initial inoculation (8). Prior inoculation by 7 days of one root half completely suppressed nodulation on the second root half. Mutant nts382, in contrast, failed to show any suppression, indicating that the supernodulation phenotype acts systemically and that autoregulation of nodulation and nitrate sensitivity can be analysed in split root systems.

Analysis of yield potential of nts mutants have shown that the intermediate hypernodulation mutant nts1116 produces more seed than Bragg in field tests. Mutant nts382 was found to nodulate better than Bragg on acid soils and in acid solution culture (9). Isotope enrichment studies showed that nts mutants grown on nitrate obtain a larger percentage of their nitrogen from the atmosphere confirming earlier data obtained through acetylene reduction assays. We are presently concentrating on the chemical, biochemical and molecular genetic causes of the here described mutants.

(1) Carroll, B. J., McNeil, D. L. and Gresshoff, P. M. (1985a) Isolation and properties of soybean mutants which nodulate in the presence of high nitrate concentrations. Proc. Natl. Acad. Sci. USA 82, 4162-4166. (2) Carroll, B. J., McNeil, D. L. and Gresshoff, P. M.(1985b) A supernodulation and nitrate tolerant symbiotic (nts) soybean mutant. Plant Physiol. 78, 34-40. (3) Carroll, B. J., McNeil, D. L. and Gresshoff, P. M. (1986) Mutagenesis of soybean (Glycine max [L.] Merr.) and the isolation of non-nodulating mutants. Plant Science 47, 109-119. (4) Delves, A. C., Carroll, B. J. and Gresshoff, P. M. (1988) Genetic analysis and complementation of studies on a number of mutant supernodulating soybean lines. J. of Genetics (in press). (5) Mathews, A., Carroll, B. J. and Gresshoff, P. M. (1987) Characteristisation of non-nodulation mutants of soybean (Glycine max [L.] Merr.): Bradyrhizobium effects and absence of root hair curling. J. Plant Physiol. 131, 349-361. (6) Delves, A. C., Mathews, A., Day, D. A., Carter, A. S., Carroll, B. J. and Gresshoff, P. M. (1986) Regulation of the soybean-Rhizobium symbiosis by shoot and root factors. Plant Physiol. 82, 588-590. (7) Mathews, A. (1987) Ph.D. dissertation, Australian National University, Canberra, Australia. (8) Olsson, J. E., Nakao, P., Bohlool, B. B. and Gresshoff, P. M. (1988) Host genetic control of soybean nodulation in split root systems. Plant Physiol. (submitted). (9) Alva, A. K., Edwards, D. G., Carroll, B. J., Asher, C. J. and Gresshoff, P. M. (1988) Effects of acid soil infertility factors on nodulation and growth of soybean mutants with increased nodulation capacity. Agronomy J. (in press).

IN VITRO TRANSLATION AND GENOMIC MAPPING OF SOYBEAN MOSAIC VIRUS RNA.

F. Gadani,[1] L. M. Mansky,[2] D. P. Durand,[2] J. H. Hill,[3] and R. E. Andrews.[2] EniChem SpA, Research and Development, Milan, Italy,[1] Depts. of Microbiology[2] and Plant Pathology,[3] Iowa State University, Ames, IA 50011.

The genomic RNA of soybean mosaic virus, a member of the potyvirus group, was translated in a wheat germ in vitro system to determine the translational strategy of the virus and to identify the specific location of cistrons on the genome. A broad size range of L-[^{3}H] leucine-labelled polypeptides was observed in SMV RNA-programmed wheat germ extracts, with major bands of estimated molecular weights of 20,000 25,000, 30,000, 33,000, 38,000, 43,000, 49,000, 55,000, 67,000, 70,000, 100,000. Time course experiments revealed that after in vitro translation periods as short as 15 min, the smallest gene products were synthesized, indicating that proteolytic processing is not a likely explanation for the smaller polypeptides observed. To determine the location of the coat protein gene as well as that of other viral cistrons on the genome, partial digestion of the RNA with snake venom phosphodiesterase I is being used, followed by immunoprecipitation analyses of the translation products. Preliminary experiments of hybrid-arrested translation, using a cDNA clone corresponding to 700bp of the 3' proximal end of the SMV genome, together with nucleotide sequence data, has provided evidence that the coat protein gene is located on the 3' portion of the viral RNA.

A POSSIBLE MOLECULAR MECHANISM OF RESISTANCE TO SOYBEAN MOSAIC VIRUS.

R.J. Clem, J.H. Hill*, R.E. Andrews, and D.P. Durand, Departments of Microbiology and Plant Pathology*, Iowa State University, Ames, Iowa 50011.

The soybean (Glycine max) PI96983, which carries the resistance gene RSV conferring resistance to strains G1 to G6 of soybean mosaic virus (SMV), was inoculated at the primary leaf stage with SMV isolate Ia75-16-1 (G2). Also inoculated were the susceptible cultivar Williams and the resistant isoline L78-379, which is an isogenic Williams line containing RSV. The inoculated primary leaves were harvested at 2 day intervals up to 10 days. Trifoliate leaves were also harvested at 10 days in order to monitor the spread of the virus through the plant. The samples were analyzed by SDS-PAGE followed by immunoblotting using monoclonal antibodies specific for SMV capsid protein. Infectivity was determined by local lesion assay. In Williams, capsid protein was first detected at 4 days and increased in concentration over the period tested. Ten day trifoliates also contained capsid protein, and both primary and trifoliate leaves contained infectivity. In PI96983 and L78-379, two proteins which reacted with the monoclonal antibodies but which differed in molecular weight from native capsid protein were detected at 6 days and were seen to increase to 10 days, with no accumulation in the trifoliates. The levels of these proteins were much less (at least 100 fold) than native capsid protein in Williams. Peptide mapping studies are underway to confirm that the two proteins contain native capsid protein sequences. No infectivity was detected in either of the resistant soybeans. These results suggest a molecular mechanism of resistance in which normal capsid protein synthesis is somehow altered, and spread of the virus to other parts of the plant is inhibited.

In vitro EXPRESSION OF THE CELLULOSE GENE IN *Methylobacterium mesophilicum*, A SEED-TRANSMITTED BACTERIUM UBIQUITOUS IN SOYBEAN.

J. M. Dunleavy, U.S. Department of Agriculture, Agricultural Research Service, Department of Plant Pathology, Iowa State University, Ames, IA 50011-1020

Research was undertaken to determine if a soybean-associated bacterium was seed-transmitted. Leaves, immature and mature seeds (5g of each) of 10 soybean cultivars were sampled weekly in the field. Mature seeds were immersed in 75% ethanol for 1 min, then in 0.5% sodium hypochlorite for 15 min before crushing. All samples were crushed in a sterile blender. Bacteria were separated from cellular debris by differential centrifugation and plated on trypticase soy agar. *Methylobacterium mesophilicum*, a nonpathogenic bacterium that produces a pink, water-insoluble pigment, was isolated from leaves, immature seed, and mature seed of all cultivars in all trials in each of 3 years. Bacterial populations were greatest in leaves (35×10^5 per g tissue), intermediate in immature seeds, and low in mature seeds. The bacterium was isolated from leaves taken from one field in each of 25 Iowa counties and from all of 100 soybean plants from a single field. When cultured in trypticase soy broth, *M. mesophilicum* produced an extracellular viscous material that adhered to the cells and formed globular masses in the medium. The material was a polysaccharide with linked glucose units, had a refractive index of 1.55, and was insoluble in water and dilute acid. This suggested that the substance might be cellulose. Material produced by an isolate of *M. mesophilicum* from each of 10 soybean cultivars reacted with cellulase to destroy cellulose globules. Globules were deposited on formvar-coated grids, dried, shadowed with palladium, and examined in the beam of an electron microscope. The globules were composed of cellulose fibers which in turn were composed of fibrils with a mean diam of 223A°. The cellulose of plant cell walls also is composed of fibers and fibrils that are about 250A° in diam. The plant cell wall is a product of the cytoplasm, and the primary cell wall is composed of fibrils of cellulose.

USE OF IN VITRO TECHNIQUES TO EVALUATE, SCREEN AND SELECT SOYBEAN GENOTYPES FOR BROWN STEM ROT TOLERANCE

J. Thimmapuram, L.E. Gray and J.M. Widholm, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Culture filtrates from nine different isolates of Phialophora gregata, the cause of brown stem rot of soybean were used to study the response of resistant and susceptible genotypes of soybeans at the callus level. The same fungus isolates were used to inoculate the soybean plants to evaluate genotype response at the plant level. All isolates showed differential reactions between the resistant and susceptible genotypes. Results from callus studies cannot always be correlated with that at the whole plant level. Culture filtrates are also being used to select for tolerance with organogenic cultures. So far the regenerated plants and their progeny from the selected calli do not show any differences in tolerance as compared with their parents. Culture derived plants of BSR 201 have been screened to study the somaclonal variation for BSR tolerance at both the callus and whole plant level. Callus and seedlings were obtained from the same seeds and tested. Some progeny showed decreased tolerance at both the callus and whole plant level indicating that somaclonal variation for BSR resistance does occur.

Can Parts Other Than Immature Cotyledons Regenerate Shoots or Embryos in Sufficient Numbers To Be Useful in a Soybean Transformation System?
JooHag Kim and Clifford E. LaMotte, Department of Botany, Iowa State University.

We are exploring means to regenerate whole plants other than those using immature cotyledons as starting material. An interpretation of regenerative events ensuing when mature cotyledons are cultured on a medium containing synthetic auxin, and results from experiments using repeatedly subcultured callus from mature cotyledons, will be presented. Attempts to cause hypocotyl slices from young seedlings to regenerate buds, as claimed by Kimball and Bingham in 1973, also will be described.

SOMATIC HYBRIDISATION OF SOYBEAN WITH GLYCINE CANESCENS

N. Hammatt, M.R. Davey, R.S. Nelson*, A. Lister and E.C. Cocking, Plant Genetic Manipulation Group, Department of Botany, University of Nottingham, Nottingham NG7 2RD, UK., and *Nickersons Seed Co., Cambridge Science Park, Milton Rd., Cambridge CB4 4WE, UK.

Dark-grown soybean hypocotyl protoplasts[1] were labelled with fluorescein (green fluorescence), and fused electrically with green, cotyledon protoplasts of G. canescens G1171[2] (red fluorescence). The resulting bifluorescent heterokaryons were isolated employing a flow cytometer, and cultured into cell colonies using agarose-solidified media[2].

Aspartate amino transferase isozyme analysis of parental callus revealed a single soybean band and three proteins specific to G. canescens G1171. Heterokaryon-derived material showed the four parental proteins expected and two extra, hybrid-specific bands. Isozymes from putative hybrid tissue, additional to those expressed by parental material, are commonly accepted as indicators of hybridity[3].

Flow cytometric fluorescence analysis of ethidium bromide-stained nuclei isolated from soybean hypocotyls, G. canescens cotyledons, and morphogenetic, somatic hybrid callus revealed that the latter produced 10% less fluorescence than the sum of parental emissions. This is partly due to a departure from perfect proportionality in fluorescence detection, but also indicates that DNA has been lost from the hybrid genome during the hybridisation procedure.

Buds have been obtained following nine months of regular subculture of heterokaryon-derived tissues on SC2 medium, normally used to regenerate shoots from protoplast-derived callus of G. canescens[2]. The majority of buds were abnormal in morphology. This difficulty may be circumvented once it becomes possible to recover plants from protoplast-derived tissue of soybean, thus facilitating the use of protoplast fusion to enhance gene flow from sexually incompatible wild Glycine species to the cultigen.

[1]Hammatt, N. and Davey, M.R. (1988) In vitro, in press: [2]Hammatt, N. et al. (1987) Plant Sci. 48, 129-135: [3]Schiavo, F.Lo. et al. (1983) In "Isozymes in Plant Genetics and Breeding Part A" (S.D. Tanksley and T.J. Orton eds.), pp. 305-312, Elsevier.

SHOOT ORGANOGENESIS FROM MATURE SEEDS OF *GLYCINE SOJA* AND *G. MAX*

C.Y. Hu. Biology Department, Wm. Paterson College of New Jersey, USA, and G.C. Yin, Soybean Research Institute, Heilongjiang Academy of Agricultural Science, PRC (Present address: Department of Biology, Wm. Paterson College of New Jersey, USA)

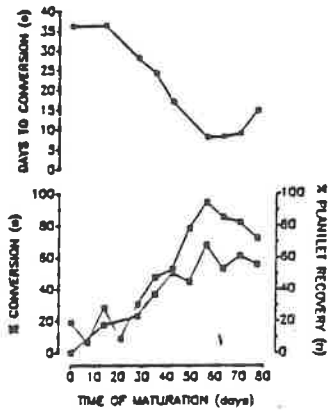
Mature seeds were germinated in agar water for three days. Cotyledonal nodes with the hypocotyl and one-half of the cotyledons severed were cultured in BAP and NAA containing modified MS media. Masses of buds were developed from the cotyledonal axes of about 80% of the explants of *G. soja* and about 15% of the explants of *G. max* cv. Pella in 6 weeks' 25°C incubation. The first 4 weeks of incubation were in darkness followed by 16-h photoperiod of approximately 2000 lux cool white fluorescent lights. Since individual buds evolved from several independent locations of the same axis, they were likely derived from organogenesis instead of through axillary bud proliferation. Bud masses were subcultured.

MATURATION AND CONVERSION KINETICS OF SOYBEAN SOMATIC EMBRYOS

Buchheim, J., Colburn, S. M., and Ranch, J.
United AgriSeeds, Champaign IL 61820.

The maturation of soybean somatic embryos was assayed by evaluating their ability to make the transition to a plantlet through a germination-like process. Somatic embryos of soybean, cv. Gem, were organized from 0.4 mm-0.6 mm long cotyledons cultured on semi-solid MS + 90 *u*M 2,4-D. Colorless, translucent somatic embryos developed upon the adaxial surface of the cotyledons within 4 weeks. Somatic embryos were excised from the cotyledons and matured on semi-solid MS basal medium + 0.5 % activated charcoal + 10% sucrose for periods up to 11 weeks. The somatic embryos were assayed weekly for conversion and plantlet development on hormone-less SH + 1% sucrose. During maturation, the somatic embryos passed through embryological stages resembling zygotic embryogeny. The conversion frequency increased and the conversion days decreased with time on maturation medium (Figure 1). Conversion frequency, velocity and plantlet recovery frequency varied concurrently. After 8 weeks on maturation medium, somatic embryos exhibited vigorous, high-frequency conversion and development to plantlets. Nearly 100% of the embryos matured for 8 weeks converted within 7 days. In contrast, immature somatic embryos converted in 20-35 days with a frequency of 20% or less. Conversion and plant recovery required no exogenous growth regulators. Conversion was characterized by the rapid appearance of a deep green color in the embryo, root formation, and development of the apex and trifoliolate leaves. There was little effect of genotype on conversion velocity or frequency.

FIGURE 1. KINETICS OF EMBRYO CONVERSION AND PLANTLET DEVELOPMENT AS A FUNCTION OF MATURATION PERIOD



Morphological abnormalities appeared in the matured somatic embryos. These morphotypes were classified into mono-, di-, and polycotyledon, fused cotyledon, trumpet, moderately and grossly fasciated, and diaxial fusion. While there was a significant difference in the conversion frequency of different morphotypes, the conversion velocities of the different morphologies were not significantly different.

TABLE 1. EFFECT OF DESICCATION ON CONVERSION OF IMMATURE SOMATIC EMBRYOS

TREATMENT	% VIABILITY	% CONVERSION	CONVERSION DAYS
none	100 ± 0	0	>21
75 % RH, 4 days	100 ± 0	70 ± 28	11.4 (0.91)
75 % RH, 7 days	60 ± 18.9	60 ± 27	8.6 (1.1)
93 % RH, 4 days	50 ± 19.3	20 ± 23	10.0 (0.73)
93 % RH, 7 days	60 ± 18.9	40 ± 27	16.0 (1.1)
40% RH, 4 days	0	—	—
	X^2=58.2*	X^2=27.8*	

Desiccation of immature somatic embryos under controlled humidity regimes resulted in an increased frequency of immature somatic embryo conversion (Table 1). Immature somatic embryos were desiccated under relative humidities of 75% and 93% using H_2SO_4 dilutions. Desiccation significantly increased the embryo conversion frequency and decreased the embryo conversion days compared to untreated somatic embryos.

PLANT REGENERATION FROM MONOCELL CULTURE OF SOYBEAN (GLYCINE MAX)

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Studies on plant regeneration of soybean in monocell culture are reported in this paper. The cotyledons from young pods 20 days after pollination and the hypocotyls from aseptic seedling grown 6-7 days were tested. The cotyledons and the hypocotyls were put onto solid MS medium and SL medium separately, and calli were formed in both of the media. The calli were transferred to fluid media of MS (embryo) (MS+0.5mg/L 2.4-D+5% CM+2% sucrose) and SLA (SL+0.06mg/L picloram+0.1mg/L BA+0.5mg/L 2.4-D+4 kinds of amino acids+3% sucrose) for vibrate culture. The suspention culture cells were obtained from the vibrate culture after twice filtration and cultures. Through divisions, the monocells formed embryonic cell colonies, then formed calli. These calli were transferred to 6 kinds of solid differentiation medium separately. The calli from monocells of cotyledon in soybean Jilin No. 12 were differentiated to form shoots in A_5 medium (MS+0.11mg/L Zeatin+3% sucrose) and the shoots were transferred to MS medium (no hormone). Lastly, intact plant was eventually produced.

SOYBEAN MUTANTS LACKING THE UBIQUITOUS UREASE

J.C. Polacco, Adam Judd and Silvia Cianzio; Biochemistry Department; University of Missouri; Columbia, MO 65211 (JCP, AJ) and Agronomy Department; Iowa State Univ., Ames, IA 50011 (S.C.)

We reported earlier the recovery of two classes of soybean urease mutants. Class I mutants lack the embryo-specific urease while class II mutants lack the activities of both urease isozymes, the embryo-specific and the ubiquitous ureases. We report here the recovery of two true-breeding mutants which represent the third phenotypic class: normal levels of embryo-specific urease and little or no ubiquitous urease. The lack of ubiquitous urease is expressed in leaves and in callus cultures derived from leaf and shoot tip. These mutant callus cultures cannot utilize urea as sole nitrogen source and they are resistant to toxic levels of urea added to maintenance medium. One mutant has much higher tissue culture urease than expected from its leaf activity. Genetic analyses of these mutants are underway.

STRUCTURAL ANALYSIS OF A UREASE GENOMIC CLONE

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Our laboratory is interested in the molecular basis of the developmental- and tissue- specific expression of two urease isozymes in soybean. A 10 kb genomic clone which contains sequences coding for peptides similar to a published jackbean urease amino acid sequence was isolated previously in our laboratory. That clone does not represent a full-length coding sequence; it lacks the 5' end of the coding sequence and the promoter region. We have recovered additional, overlapping clones from a second Charon 4A genomic library. The longest of these extends an additional 10 kb in the 5' direction. Sequence analysis of portions of these clones has located sequences which code for peptides which are 90% similar to the N-terminal and C-terminal peptides of the jackbean urease. Putative control sequences are located immediately 5' of the N-terminal peptide coding sequence. This clone is being used as an initial tool to study urease isozyme expression. The clone also detects an RFLP, which can be used to map it genetically.

IDENTIFICATION OF MOLECULAR CLONES WITH SOYBEAN UREASE ISOZYMES

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The two urease isozymes of soybean are developmentally regulated and are expressed in a tissue-specific manner. Our genetic analysis shows that at least three loci condition this expression and we hypothesize that two loci represent structural genes for urease. Screens of soybean genomic libraries with probes for urease have yielded two distinct but related DNA clones. We have demonstrated that one of these encodes a urease.

Two experimental approaches were taken to identify the molecular clones with the isozyme loci. First, we compared in different tissues the sensitivities of the cloned regions of the genome to treatment with DNaseI. Second, we examined expression of urease clones introduced by electroporation into soybean protoplasts. The suitability of these methods in answering questions of clone identity will be discussed in the light of our results on the urease isozyme system.

ISOLATION AND CHARACTERIZATION OF A UREASE-LIKE GENOMIC CLONE

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The two soybean urease isozymes are believed to be encoded by separate structural genes. Urease genomic clone E15 (11 kb, EcoRI ends) had been previously isolated in our laboratory. The objective of this work was to isolate genomic clones of a second urease locus.

P19 is a HindIII fragment of E15 which encodes the 68 C-terminal amino acids of urease. It hybridizes, in addition to the E15 11 kb fragment, a polymorphic EcoRI fragment (2.3 or 2.8 kb) on genomic blots of a number of cultivars. P19 was used to screen an EcoRI-derived genomic library (in Charon 4A) and one of 60 hybridizing plaques was chosen for further study because (1) it showed a reduced hybridization signal to an E15 subclone 2 kb 3' from P19, and (2) it contained the 2.3 kb EcoRI fragment. This fragment was subcloned as pRI1. Since pRT1 maps to one end of clone RT37, and since it was the only subclone of RT37 which hybridized either P19 or embryo urease cDNA, clones overlapping RT37 in the 5' direction were sought.

Overlapping genomic clone RT38 was isolated from an AluI-HaeIII library. RT38 contains a 5.5 kb EcoRI fragment (pRT4), immediately upstream (5') of pRT1. However, only at low stringency (53°C) does pRT4 hybridize to the E15 fragment containing the translational start site. At high stringency (65°C) pRT4 hybridizes to sequences at least 4 kb upstream of the E15 urease translational start site. Partial sequence data for pRT1 and pRT4 has revealed some ORFs in pRT4 in the expected orientation for urease, but has not as yet revealed recognizable urease ORF. Alternative explanations on the nature of the RT38 locus will be presented.

POSSIBLE GERMINATION-SPECIFIC LIPOXYGENAGES IN SOYBEAN

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Lipoxygenase (LOX), which catalyzes the oxygenation of 1,4-cis,cis-pentadiene fatty acids (e.g. linoleic acid and linolenic acid) to conjugated hydroperoxides, is a monomeric, non-heme iron protein with a M.W. of approximately 100,000. Three LOX isozymes are synthesized during embryo development. The notion that LOX's have a role in germination is based on the observation that LOX activities increase during germination. Whether the increase of LOX activity(ies) upon germination is caused by activation of one or more of the three preexisting LOX's (LOX1, LOX2 and LOX3) or by synthesis or activation of LOX isozymes is not yet clear.

In this research, we show direct evidence that the increased LOX activities in germinating soybean are caused by at least two new lipoxygenases. The evidence consists mainly in finding new isoelectric points (pI's) for the induced enzymes and their continued synthesis in germinating seeds lacking LOX1 (lx1), LOX2 (lx2) or LOX3 (lx3) activity. Two "new LOX's" are active at least from 2 DAG to 5 DAG and are more enriched in the hypocotyl and radicle than in remainder of the seedling. The "new LOX's" might be synthesized after germination since cycloheximide-treated seedlings showed a decreased level of activity staining compared to the untreatred control seedling.

The following criteria indicate that the enzymes induced upon germination are LOX's: 1. They are not inhibited by KCN. 2. They are inhibited by the LOX inhibitors, n-propyl gallate and salicyl hydroxamic acid. 3. They are not active on peroxidase substrate. 4. They are recognized by polyclonal anti-LOX1.

IMPROVEMENT OF SEED PROTEIN USING SUBUNIT VARIANTS OF 7S GLOBULIN IN SOYBEAN (*G. max* L.)

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Keburi and Mo-shi-dou Gong 503 were characterized by the absence of α-subunit and low quantitative levels of both α- and β-subunits of 7S globulin, respectively. In order to gather these characteristics into a genotype, we made a cross Keburi X F_1 (Oodate No. 1 X Mo-shi-dou Gong 503). Genetically fixed 7S-low lines were developed from the cross. The 7S-low lines have such properties as lacking α -subunit and decreasing both α- and β-subunits. Ten 7S-low lines and forty ordinary varieties were examined on the respective contents of 7S and 11S globulins by single radial immunodiffusion with anti-7S and -11S sera, respectively, as well as total protein contents and amino acid compositions of the matured seeds. The 7S-low lines showed only half 7S globulin contents of those in the ordinary varieties and, on the contrary, about 15 % higher levels of 11S globulin contents than those of the ordinary ones. But, no deleterious effects were observed on total protein contents and protein body-development despite of the marked modification of protein composition in the 7S-low lines. High negative correlation was found between 7S and 11S globulin contents ($r = -0.84$), and the calculated absolute value of the slope of the regression line was slightly larger than 1.0. The results suggested that 11S globulin might be overproduced to compensate for the decrease of 7S globulin keeping normal levels of total protein content in the seeds of the 7S-low lines. The mean value of sulfur-containing amino acid contents in the seeds of four selected 7S-low lines was about 20 % higher than that of ordinary varieties.

SOYBEAN VEGETATIVE STORAGE PROTEIN GENE EXPRESSION: REGULATION BY JASMONIC ACID

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Soybeans contain a glycoprotein with a temporary storage function in vegetative tissues. The vegetative storage protein (VSP) has been found in all tissues examined except seeds. It is abundant in young leaves, stored in vacuoles and degraded during seed development. The resulting amino acids or metabolites derived from them are presumably transported to seeds to support protein synthesis. VSP mRNA levels are highly regulated in leaves during plant development and sink strength plays an important role in regulation. Transcript levels decline during periods of vegetative and reproductive tissue growth, when the demand for mobilized reserves from the leaves by these tissues is high, and increase when demand is low. Depodding, or blocking phloem transport increases VSP mRNA levels by about 100-fold in 24 hr. Application of jasmonic acid to leaves increases transcript levels several fold within 2 hr and may be the endogenous signal regulating expression of the VSP genes in leaves.

GENETIC ANALYSIS OF THREE CHLOROPHYLL-DEFICIENT MUTANTS DERIVED FROM A SOYBEAN POPULATION CONTAINING A TRANSPOSABLE ELEMENT.

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A soybean line, the Asgrow mutable, contains a putative transposable element at the W4 locus. Progeny of plants from this line that were revertant at the W4 locus were scored for new mutations. Two of 1,936 families contained green, chimeric, and chlorophyll-deficient plants, while a third family was segregating for green and chlorophyll-deficient plants. Each of these mutants had a different yellow-green plant color and all of the chlorophyll-deficient lines lacked the same mitochondrial malate dehydrogenase (MDH). Reciprocal crosses of the chlorophyll-deficient lines indicated that the three mutants were allelic. In the F2 progeny of crosses of the three chlorophyll-deficient lines by Harosoy-W4, all yellow-green plants lacked an MDH band and all green plants had wild type MDH. This suggested that the mutations could be deletions and/or the absence of the MDH has a pleiotropic effect on plant color. If the mutations were deletions, they were not large enough to affect the transmission of the aberrant chromosome. The segregation ratio of green to yellow-green plants in the F2 was not significantly different from a 3:1 ratio. The MDH and "pale-green" loci were not linked to the W4 locus. Selfed progeny of the three mutants were stable; that is, no reversion to wild type has been observed. Therefore, the mutations are not due to the presence of an active element at a linked locus. Genetic tests to determine if a responsive non-autonomous element is at the MDH locus are being conducted. The F2 progeny also contained a low frequency of plants with an MDH band that had a reduced mobility on a starch gel. These plants have been saved for further study.

SOYBEAN TGM ELEMENTS CONTAIN AN OPEN READING FRAME SIMILAR TO THE MAIZE EN/SPM TRANSPOSABLE ELEMENT

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We have compared the organization of six Tgm elements that were selected from a genomic library of soybean DNA on the basis of hybridization with subcloned regions of Tgm1 (transposon, Glycine max) from the seed lectin gene. Three major conclusions were drawn from restriction mapping, cross-Southern hybridization patterns, and partial sequencing of the clones. (1) Many of the Tgm-related elements are much larger than Tgm1 and ranged in size from 1.6 kilobase pairs to greater than 12 kilobase pairs. (2) The elements form a family of deletion derivatives whose termini are all defined by a series of palindromic repeats similar to Tgm1. (3) Most significantly, a 39% amino acid homology exists between a 1 kilobase portion of an open reading frame in Tgm4 and Tgm5 and ORF1, an open frame from the first intron of the maize Enhancer (Suppressor-mutator) transposable element, suggesting that both elements encode a common function (possibly a transposase) that requires a high degree of protein conservation. We estimate that there are approximately 50 copies of Tgm sequences in the soybean genome. Our results show that this family of elements has transposed in the past as they are found in a number of genomic sequences in soybean. The molecular analysis of these elements has yielded probes with which we can investigate whether Tgm elements are involved in unstable mutations in the anthocyanin pathway in soybean.

AN UNSTABLE MUTATION AFFECTING SOYBEAN SEED COAT COLOR

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The R gene of soybean is involved in anthocyanin synthesis in the seed coat and its r-m allele conditions a variegated distribution of black spots and/or concentric rings of pigment superimposed an otherwise brown seed coat. An examination of this allele through several generations has revealed an instability of expression of the allele in both the somatic and germinal cell layers.

Somatic instability of the allele is revealed in those plants which produce mixtures of seed for seed coat color (ie, black + mottled or brown + mottled). Germinal instability is indicated by progeny plants which produce all black or all brown seed. The two plant types essentially breed true for their respective seed coat color or, in the case of the black seeded plants, also have representatives that segregate 3:1 for black:mottled seeds. Surprisingly, subsequent generations can produce progeny with mottled or black + mottled seeds. This suggests that some mechanism is operating which causes the r-m allele to go through reversible changes in activity such that the gene is either fully expressed (black seed) or not expressed (brown seed).

It has been observed that sublines with mottled seeds produce offtype progeny at different rates. Studies have indicated, however, that these different mutation frequencies are not heritable. Also, an estimate of the minimal germinal reversion rates of the allele varied considerably between different sublines (from 3.5 - 49.5%).

Mutability of the r-m allele, in one case derived from a different genetic background than above, has been induced after intercrossing various soybean genotypes. In each case, the instability arose in a single plant in the F_3 generation. This suggests that the genetic background may be important in the expression of the instability.

ANALYSIS OF SOMACLONAL VARIANTS IN SOYBEAN (GLYCINE MAX L. MERR.).

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Immature embryos (± 4mm) from greenhouse-grown soybean plants were used as the explant source for induction of somatic embryogenesis. Plants were regenerated from nine soybean cultivars. Regenerated plants were scored for qualitative characteristics in the R0, R1, R2, and R3 generations to determine: types of variation, in what generation variation occurs, and the heritability of the variant phenotypes. The progeny of each R0, R1, and R2 plants were assigned an individual identification code. Progeny from each of these plants, in each generation, were grown in sufficient number so that there was a 99% probability of recovering a homozygous recessive trait. Variation was observed in the R0, R1, R2, and R3 generations. The variant phenotypes which have been found include albinos (R0), flower color (R0), partial sterility (R0, R1, R2), complete sterility (R0), abnormal leaf morphology (R0, R2), chlorophyll chimeras (R2, R3), chlorophyll deficiencies (R2, R3), changes in maturity group (R2) and changes in isozyme patterns (R2). The seed from the R1 and R2 plants are being evaluated for variation in isozyme patterns. The variant plant types are being evaluated for inheritance patterns.

TISSUE CULTURE INDUCED VARIATION IN SOYBEANS (GLYCINE MAX L. MERR.).

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Soybean plant regeneration can be achieved from tissue cultures via embryogenesis and organogenesis. Efficiency of plant regeneration via organogenesis was elevated with the use of thidiazuron. Plants regenerated from nine soybean genotypes show extensive qualitative variation. Variants seen include sectorial albinos, multiple shoots, dwarf growth habit, wrinkled leaf phenotype and partial\complete sterility. All generations from R_1 (R_0 being the regenerated plant) to R_∞ show variation. No significant differences were seen in the frequencies of variants for embryogenic and organogenic culture derived plants. Sterility, wrinkled leaf phenotype and dwarf growth habit are controlled by single recessive nuclear genes. Wrinkled leaf phenotype was associated with some chimeric plants indicating possible involvement of transposable elements. Somaclonal variation for disease tolerance and chromosome number is also discussed. Presence of heritable somaclonal variation in tissue culture derived plants of soybeans is demonstrated.

GENOMIC RELATIONSHIPS AMONG NINE OF THE TWELVE WILD PERENNIAL SPECIES (2N=40) OF THE SUBGENUS GLYCINE

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This study furnishes information about the current status of knowledge concerning the genomic relationships among nine of the twelve wild perennial species (2n = 40) of the subgenus Glycine. Crossability rate, hybrid inviability and meiotic pairing in intra- and interspecific F_1 hybrids revealed that genomically similar species, though morphologically distinct, crossed readily to produce hybrid progeny that were vigorous, fertile and normal in meiotic pairing (20 II at MI). However, a chromatin bridge and acentric fragment were recorded in certain hybrid combinations, suggesting that the evolutionary divergence in genomically similar species occurred due to paracentric inversions. In contrast, crosses between genomically dissimilar species set pods that often aborted, showed hybrid weakness, seedling and vegetative lethality, seed inviability and complete sterility. The sterility was attributed to disturbed meiotic pairing. It is obvious from this study that A-genome species - [G. canescens = AA; G. clandestina = A_1A_1; G. argyrea = A_2A_2] and B-genome species - [G. microphylla = BB; G. latifolia = B_1B_1; G. tabacina = B_2B_2] predominate in the subgenus Glycine. Glycine cyrtoloba (CC) showed stronger genome homology to B-genome species than to A-genome species. Likewise, G. tomentella (DD) appeared to be more closely associated with A-genome species than to B-genome species. Although, 38 and 40- chromosome tomentellas are indistinguishable morphologically they differed genomically, therefore, genome symbol EE, was assigned to 38- chromosome G. tomentella. Glycine falcata (FF) was found to be the most unusual species because it showed negligible chromosome homology with A- and B-genome species and did not set pods when cross-pollinated by C, D and E-genome species.

THE GENOMIC RELATIONSHIP BETWEEN GLYCINE MAX (L.) MERR. AND G. SOJA SIEB. AND ZUCC. REVEALED BY PACHYTENE CHROMOSOME ANALYSIS

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This study was conducted with the objective to determine the genomic relationship between cultivated soybean (Glycine max) and wild soybean (G. soja) of the subgenus Soja, genus Glycine. Observations on crossability rate, hybrid viability, meiotic chromosome pairing and pollen fertility in F_1 hybrids of G. max x G. soja and reciprocals elucidated that both species hybridized readily and set mature putative hybrid pods, generated vigorous F_1 plants, a majority of sporocytes showed 18II + 1IV chromosome association at diakinesis and metaphase I, and pollen fertility ranged from 49.2% to 53.3%. A quadrivalent was often associated with the nucleolus, suggesting that one of the chromosomes involved in the interchange is a satellited chromosome. Thus, G. max and G. soja genetic stocks used in this study have been differentiated by a reciprocal translocation. Pachytene analysis of F_1 hybrids helped construct chromosome maps based on chromosome length and euchromatin and heterochromatin distribution. Chromosomes were numbered in descending order of 1 to 20. The total length of chromosome 1 was 39.8 μ while chromosome 20 measured 10.6 μ. Pachytene chromosomes in soybean showed heterochromatin distribution on either side of the centromeres. Pachytene analysis revealed small structural differences for chromosomes 6 and 11 which were not detected at diakinesis and metaphase I. This study suggests that G. max and G. soja carry similar genomes and validates the previously assigned genome symbol GG.

TRANSFORMATION OF PERENNIAL *GLYCINE* SPECIES BY *AGROBACTERIUM RHIZOGENES*

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Transformed roots were induced by infecting cotyledons and hypocotyls of 6 to 12 days old seedlings of *G. argyrea* (accession G1626), *G. canescens* (G1114, G1171, G1240, G1249, G1340, G1699) and *G. clandestina* (G1001, G1145, G1231) with *Agrobacterium rhizogenes* strain LBA 9402 containing pRi 1855. Hypocotyls were the most responsive with 70% of the seedlings of *G. canescens* G1240 and G1340 producing roots. Transformed roots were plagiotropic and negatively geotropic in culture on hormone-free medium. Cultured roots of *G. canescens* G1171 regenerated shoots on a B5 based medium containing 0.05 mg/l IBA and 10.0 mg/l BAP; those of *G. argyrea* produced shoots on a medium with 0.005 mg/l IBA and 0.2 mg/l BAP. Agropine and mannopine were detected in cultured roots and regenerated shoots. Southern blot analysis confirmed the genomic integration of pRi TL- and TR-DNA in a clonally propagated, transformed plant of *G. canescens*[1].

Additional studies with *A. rhizogenes* strain A4R 160-1 containing a kanamycin resistance gene co-integrated into Hind III fragment 21 of the TL-DNA of pRiA4 (E.W. Nester, Seattle, USA) have resulted in plants of *G. canescens* G1171 resistant to 100 µg/ml of kanamycin. In comparison, LBA 9402-derived transgenic plants were only resistant to 25 µg/ml of the antibiotic, as were non-transformed plants.

The ability to produce transgenic plants of *G. argyrea* and *G. canescens* will facilitate future studies on gene expression both in these species and in other members of the genus *Glycine*, including soybean.

[1]E.L. Rech, T.J. Golds, N. Hammatt, B.J. Mulligan and M.R. Davey (1988) J. Exp. Bot. (in press).

EXOGENOUS DNA TRANSFER INTO SOYBEAN CELLS AND TISSUE

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Four methods were compared for directly introducing exogenous DNA into Glycine max (L.) Merr. and Glycine canescens F. J. Herm. cells: electroporation, macroinjection, microinjection, and microprojectile bombardment. Two different genes were used to monitor the transformation: the hygromycin phosphotransferase (Hyg) gene and the reporter gene, beta-glucuronidase (GUS). All constructs contained the cauliflower mosaic virus (CaMV) 35S promoter and were terminated by the nopaline synthase 3′ (Nos 3′) polyadenylation sequences. Various conformational isomers of the vectors, i.e., supercoiled, relaxed circular and linear DNA, were employed in an attempt to improve the efficiency of transformation. The recipient protoplasts and seedling hypocotyl. Alternatively, zygotic embryos, excised five to seven days after fertilization, also were tested as transformation targets. Depending on the vector construct, concentration and conformation, and the method of introduction, we obtained; (1), transformation of protoplasts with the subsequent development of hygromycin resistant calli and (2), transient expression of exogenous DNA in intact cultured soybean cells. Investigations as to the possible transformation of embryonic cells are continuing.

PROGRESS IN THE DEVELOPMENT OF GENE TRANSFER SYSTEMS FOR SOYBEANS

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Our work has focused on the coupling of *Agrobacterium* - mediated transformation to efficient somatic embryogenesis in soybeans. Improvements in the efficiency of somatic embryogenesis from immature cotyledons have been achieved by adjustment of the NAA/sucrose ratio, reduction of the duration of exposure to moderately high auxin levels, use of genotypes with cv. 'Manchu' in their pedigree, and desiccation of matured embryos to enhance germination. The time taken to produce soil-ready plantlets from explant tissues has been reduced from 9 months to 2-3 months. Recovery of transformed somatic embryos from explants inoculated with *Agrobacterium* has proved difficult. Two transformed regenerant plants were recovered from ca. 10,000 inoculated explant cotyledons, but neither plant gave rise to transformed progeny. It appears that these plants were chimeric and the germ-line cells were not transformed. Histological analysis of somatic embryos transformed with the beta-glucuronidase (GUS) reporter gene indicated layers of transformed and untransformed cells. We are currently working on improved targeting of gene transfer to morphogenetically competent cells, and on altering regeneration protocols to improve the efficiency of whole-plant transformation.

SOYBEAN REGENERATION AND INVESTIGATIONS OF *AGROBACTERIUM TUMEFACIENS*-MEDIATED TRANSFORMATION.

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Transformation of soybean plants (*G. max*) should be possible provided that the soybean genotype can be regenerated from tissue culture efficiently and is susceptible to the transformation vector, *Agrobacterium tumefaciens.* The objectives of this study were to: 1) evaluate Minnesota genotypes for plant regeneration from tissue culture, 2) determine the combination of soybean genotype and *Agrobacterium* strain that give rise to the most tumors, and 3) use the most responsive strain and genotype to attempt regeneration from transformed tissue. Ten Minnesota soybean genotypes were evaluated for tissue culture initiation and whole plant regeneration using an *in vitro* organogenic system (Wright, 1980). Fourteen days after seed germination on 1/2 MS media containing 5 μM BAP, cotyledonary node explants were excised and placed on fresh medium. Shoots were removed every 2-4 weeks and placed on hormone-free media to allow shoot elongation and root formation. The genotypes producing the most shoots that developed into whole plants during the 30 weeks after culture initiation were: HHP, PI 445799, Hodgson 78, and Corsoy 79.

The same ten genotypes and Peking (highly susceptible cultivar) were evaluated for susceptibility to *Agrobacterium tumefaciens* strains with various Ti (tumor inducing) plasmids. Cotyledons from *axenic* 1 day seedlings were excised, wounded, and inoculated with *Agrobacterium*. After 21 days of culture on water agar media containing 200 mg/L carbenicillin and 200 mg/L vancomycin, the relative frequency of tumor formation was scored. The *Agrobacterium* strains, A208 and C58, were the most virulent and the soybean genotypes, Peking, PI 180529, and Hodgson 78, were highly susceptible, especially to these strains.

The *Agrobacterium* strain, C58, containing a disarmed Ti plasmid, was used as a vector for a binary plasmid with T-DNA (transfer DNA). The T-DNA included genes coding for neomycin phosphotranferase (NPTII) and β-glucuronidase. Plant cells transformed with these genes should be selectable in the presence of kanamycin or G418 and also test positive for β-glucuronidase activity. Cotyledonary nodes of Hodgson 78 and Peking were wounded and inoculated with the disarmed vector containing the binary plasmid 6-8 days after germination. Cotyledonary nodes were cultured in the presence and absence of kanamycin or G418. Shoots were removed at 2-4 week intervals for 6 months and assayed for β-glucuronidase activity. Transformed shoots have not been positively identified to date, but experiments are still in progress. This research was supported by the Minnesota Soybean Research and Promotion Council.

GENETICALLY ENGINEERED SOYBEAN

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Through use of an electric discharge apparatus, we have devised a method to introduce the kan gene into soybean and have maintained and expressed that gene in subsequent generations. The electric discharge propels DNA-coated metallic beads into regenerable tissue. The discharge is finely tunable to maximize entry of the beads, while minimizing damage to the tissue.

EVALUATION OF CYTOPLASMICALLY INHERITED CHLOROPHYLL-DEFICIENT SOYBEAN MUTANTS

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Several cytoplasmically inherited chlorophyll-deficient soybean mutants have been evaluated at the levels of transcription and translation. Mung bean chloroplast DNA probes have been used in Northern blots to compare RNA populations between chlorophyll-deficient mutants and their normal green siblings. SDS-PAGE gel electrophoresis is also being used concurrently to compare protein constituents of yellow and green leaves. Because normal and mutant plants are derived from the same chimeric parent any RNA or protein differences observed should be attributable to the mutation causing the chlorophyll deficiency. To date, no qualitative differences have been observed in RNA or protein constituents between yellow or green leaves. Chlorophyll deficiencies of the mutants studied may be due to quantitative differences or binding-site alterations. The mode of action of each of the mutations is being investigated.

ORGANIZATION AND EXPRESSION OF THE SOYBEAN MITOCHONDRIAL GENOME

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Plant mitochondrial genomes are very large and variable in size, ranging from 200-2400 kb, with estimates of around 400 kb for the soybean mitochondrial DNA. Plant mitochondrial DNA can be organized as a single master chromosome or as a series of multipartite, subgenomic circles that may undergo recombination at a number of repeated sequences. A definitive role for rearrangements in the mitochondrial DNA has not been elucidated but rearrangements appear to be quite frequent, involving coding sequences as well as intergenic regions. A number of specific rearrangements that are related to the phenotype of cytoplasmic male sterility have been examined in considerable detail in several plants including maize and petunia.

Plant mitochondrial DNA encodes a number of subunits for the enzyme complexes of the inner mitochondrial membrane as well as ribosomal RNAs and tRNAs. Genes for subunits I, II and III of the cytochrome oxidase and subunits 1, 6 and 9 of the ATPase have been isolated from a soybean mitochondrial cosmid library and are under investigation. The genes for the cytochrome oxidase subunits appear to be present as single copies within the soybean mitochondrial DNA whereas the ATPase genes are repeated. The structure of the two copies of the ATPase subunit 6 gene (atp6) are of particular interest because they exist as chimeric sequences, very likely the result of some type of rearrangements. Both copies of the atp6 gene contain sequences at their 5' ends that are homologous to other known genes and which contain in-frame methionine codons contiguous with the remainder of the coding region (1).

Attempts at studying cytoplasmic diversity in soybean by comparing mitochondrial restriction patterns yielded very few differences (2) and did not address the nature of those changes in the mitochondrial DNA. We have initiated studies on the comparison of a number of soybean cultivars and have demonstrated a restriction length polymorphism in a number of cultivars of the Mandarin cytoplasm that differs from other cultivars tested. Southern analysis has shown that the difference could result from a deletion in the Mandarin mitochondrial DNA but the molecular analysis of this region awaits further characterization.

(1) Grabau et al. (1988). Curr Genet 13, 83-89.
(2) Sissons et al. (1978). Crop Sci 18, 991-996.

CHLOROPLAST DNA RESTRICTION SITE POLYMORPHISM IN GLYCINE SUBGENUS SOJA

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Restriction fragment length polymorphisms (RFLPs) have been used to detect intragenic sequence diversity in soybean chloroplast DNA. The distribution of these RFLPs allow soybean accessions to be grouped according to cytoplasmic relatedness. DNA clones from mung bean chloroplast DNA were used to locate RFLPs to specific regions of the chloroplast genome. Several previously undetected RFLPs were also identified. The changes appear to include both insertion/deletion events as well as loss or gain of restriction sites. At least seven molecular changes were detected. The distribution of these changes within the chloroplast chromosome is non-random.

Probes detecting polymorphisms in representative genotypes were used to screen additional cultivars and Plant Introductions. The distribution of RFLP patterns in these accessions were consistent with the patterns of the previously described cytoplasmic groupings with the exception of one accession which formed a new plastome group.

MAPPING THE SOYBEAN (G. MAX) GENOME USING NEAR-ISOGENIC LINES

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Near-isogenic lines (NILs), because of their unique characteristics, could provide a genetic resource in the search for linkages between molecular marker loci and conventional loci. NILs have been developed in a number of crop species via the backcross breeding method, and in soybean (Glycine max (L.) Merr.), there exists a relatively large stock of NILs. These NILs possess remnant donor parent (DP) DNA, most of which is present in the chromosomal segment linked to and surrounding the introgressed marker. If this DP-derived DNA contains molecular marker loci for which a recurrent parent (RP) and DP possessed allelic contrasts, then DP-derived molecular alleles may still be present in an NIL and could be detected posteriori by molecular genetic analysis of specific trio sets of RP/NIL/DP lines. Given this proposition, we have examined the theoretical basis for, and have examined the practical use of the "near-isogenic gene mapping technique". Using equations derived by previous workers, we calculated that about four of 100 randomly chosen DP-derived molecular markers would be expected to be retained in a BC_5S_1 NIL constructed in a hypothetical species that possesses 20 chromosomes of equivalent 50 cM genetic map length. Of these four markers, two or three would be expected to be located on the introgressed marker chromosome. This suggests that the "near-isogenic gene mapping technique" would provide a means for subsetting molecular and introgressed markers into presumptive linkage groups. Such a priori information would make traditional methods of integrating both marker types into a single linkage map a much more efficient undertaking. The utility of the technique was assessed in an initial screening of the soybean NILs and corresponding DP and RPs for just eight isozymes (aconitase, acid phosphatase, endopeptidase, isocitrate dehydrogenase, malate dehydrogenase, mannose-6-phosphate isomerase, phosphoglucomutase, and phoshohexose isomerase). Three presumptive linkages were found; the endopeptidase locus (ENP) and the narrow leaflet locus (ln), the mannose-6-phosphate isomerase locus (MPI) and the semi-determinate growth habit locus (Dt_2), and the aconitase-4 locus (ACO-4) and the semi-determinate growth habit locus (Dt_2). F_2 segregation data for the cosegregation of the endopeptidase locus and narrow leaflet locus revealed a 8.81% recombination frequency between the two markers. Segregation data for the other two presumptive linkages will be obtained in the near future. The utility of the technique relative to RFLP loci will also be examined using eleven probes of the soybean genome obtained from other researchers.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) IN TWO (G. MAX) CULTIVARS AND CELL CULTURES PREPARED FROM THESE CULTIVARS

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DNA preparations extracted from leaves of the cultivar Minsoy (PI 27890) and Noir 1 (PI 290136) or from cell suspension prepared from these same cultivars, was analyzed for changes in Restriction Fragment Lengths (RFLP). Previous work (Apuya et al., Theor. Appl. Genet. in Press, 1988) had shown that about one fragment in five was polymorphic and that usually only two alleles were found in different cultivars of G. max and G. soja (Keim et al., Agron. Soc. Mtg., 1987, Abstract); Apuya et al., 1988). We have now examined more than 50 such markers for linkage. Linked markers are not distributed at random, but are clustered. This situation, reminiscent of Tomato, could mean that probes are not selected at random from the DNA pool, that large chromosomes exist on which recombination is inhibited, and/or that polymorphisms are clustered in localized regions of DNA.

DNA from cell cultures prepared from stem, leaves or cotyledon appeared to have an array of restriction fragment length alleles identical to the array in the intact plant from which each culture was prepared.

However, DNA extracted from cell cultures prepared from secondary roots contained a high proportion of fragments not observed in the plant of their origin. Each of these changes corresponded to the acquisition of the other allele already characterized in this dimorphic system. This observation suggests the working hypothesis, that soybean, an inbred plant, can generate its genetic variation internally, possibly in response to stress, such as that which may occur when tissues are cultured.

MEASURING GENETIC DIVERSITY IN THE SOYBEANS USING RFLP MARKERS

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Cultivars and accessions from Glycine soja, G. gracilis, and G. max were screened for allelic differences at ca. 20 RFLP loci. The allele frequencies varied from a very even distribution (50:50), to cases where an allele was present in only one accession. In only one case were more than two alleles observed. The most molecular diversity was seen among accessions of G. soja. Accessions from G. max were less diverse and elite cultivated lines from G. max exhibited the least genetic diversity. The uniformity in elite lines may be due to "genetic bottlenecks" which occurred during elite line development. Though principal components analysis did not reveal a discrete separation between G. soja and G. max genotypes, the maximum genetic distance observed was between individual G. max and G. soja accessions. Therefore, a cultivated G. max line and a wild accession of G. soja were chosen for our studies. These lines are being screened with random probes to identify a large number of RFLP markers which can be used to construct a soybean genetic linkage map and to identify quantitative trait loci.

SOYBEAN SOMATIC EMBRYOGENESIS AS AN AVENUE FOR TRANSFORMATION VIA PARTICLE BOMBARDMENT

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The application of the Biolistics particle delivery system for the transformation of soybean was evaluated in two different regeneration systems. The first regeneration system was multiple shoot proliferation from shoot tips obtained from immature zygotic embryos, and the second regeneration system was somatic embryogenesis from a long term proliferative suspension culture. Bombardment of soybean shoot tips with tungsten particles coated with precipitated DNA containing the gene for ß-glucurondiase (GUS) produced a relatively high (10% of explants) number of transformed sectors in the subsequently regenerated shoots. The shoots were chimeric for GUS expression. However, none of these transformed sectors were in indeterminate cells since shoots which were grown into plants did not continue to produce GUS positive tissue. However, similar bombardments of embryogenic suspension cultures did produce GUS positive globular somatic embryos which were capable of proliferating other GUS positive embryos and plants. The different transformation results obtained with these two systems is directly related to differences in the cell types which are responsible for regeneration. The shoots obtained from the shoot tip regeneration system are derived primarily from multiple subepidermal cells, while the somatic embyros are derived from single epidermal cells on the surface of pre-existing somatic embryos. The tungsten particles bombarded from the Biolistics gun penetrate primarily only epidermal cell layers, and therefore target transformation to regeneration competent cells best in the somatic embryogenesis system.

TEMPORAL AND SPATIAL EXPRESSION OF *CAB* GENES DURING SOYBEAN DEVELOPMENT

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The soybean genome has approximately 10 genes (*Cab*1- *Cab*10) that encode the chlorophyll a/b binding proteins of light-harvesting complex II. We have characterized the structure and expression of eight members of this gene family (*Cab*1- *Cab*8). Using quantitative S1 nuclease reconstruction experiments, we have determined the contribution of 5 *Cab* genes to the *Cab* mRNA populations during soybean embryogenesis in cotyledons after germination, in leaves, stems, and roots. *Cab*1 and *Cab*2 are pseudogenes, and their mRNAs do not accumulate during soybean development. *Cab*3, *Cab*4, and *Cab*5 mRNAs accumulate to different levels in each stage in soybean development. *In situ* hybridizations, using [^{3}H]-dGTP-labeled *Cab* antisense RNAs, indicate that *Cab* RNAs accumulate in a cell-specific manner in cotyledons after germination. Collectively, these data suggest that developmental signals, as well as light signals, modulate the accumulation of *Cab* mRNAs. *In vitro* cotyledon culture experiments have identified one of the embryonic developmental regulators as the plant hormone, abscisic acid. Five μM and 50 μM ABA dramatically decrease *Cab* mRNA levels.

PRODUCTION OF TRANSGENIC SOYBEAN PLANTS USING AGROBACTERIUM-MEDIATED DNA TRANSFER

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ABSTRACT

Transgenic soybean plants have been produced using an *Agrobacterium* -mediated gene transfer system. This procedure relied on a regeneration protocol in which shoot organogenesis was induced on cotyledons of soybean genotypes selected for susceptibility to *Agrobacterium*. Cotyledon explants were inoculated with *Agrobacterium tumefaciens* pTiT37-SE harboring pMON9749 (conferring kanamycin resistance and ß-glucuronidase "GUS" activity) or pTiT37-SE :: pMON894 (conferring kanamycin resistance and glyphosate tolerance) and cultured on shoot induction medium containing kanamycin. Plantlets were tested for gene insertion 3 - 4 months post-inoculation. Approximately 6% of the shoots (8 plants to date) produced on the kanamycin-selected cotyledons were transgenic based on assays for GUS expression, kanamycin resistance or glyphosate tolerance. Progeny from two of these plants demonstrated co-segregation of kanamycin resistance and either GUS expression or glyphosate tolerance in a 3:1 ratio indicating a single insert inherited in a Mendelian fashion.

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References

Burton JW (1985) Breeding soybeans for improved protein quantity and quality. In: R Shibles (ed) World soybean research conference III: Proceedings. Westview Press, Boulder CO, pp 361-367

Edwards MD, Stuber CW, Wendel JF (1987) Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics 116:113-125

Erickson LR, Voldeng HD, Beversdorf WD (1981) Early generation selection for protein in Glycine max x G. soja crosses. Can J Plant Sci 61:901-908

Ertl DS, Fehr WR (1985) Agronomic performance of soybean genotypes from Glycine max x Glycine soja crosses. Crop Sci 25:589-592

Fehr WR (1987) Principles of Cultivar Development. Macmillan Publishing Company, New York

Graef GL, Fehr WR, Cianzio SR (1989) Relation of isozyme genotypes to quantitative characters in soybean. Crop Sci 29:683-688

Graef GL, Fehr WR, Hammond EG (1985) Inheritance of three stearic acid mutants of soybean. Crop Sci. 25:1076-1079

Howell, RW, Brim CA, Rinne RW (1972) Plant geneticists' contribution toward changing lipid and amino acid composition of soybeans. J Am Oil Chem Soc 49:30-32

Hymowitz T, Singh RJ (1987) Taxonomy and speciation. In: JR Wilcox (ed) Soybean: Improvement, production and uses. 2nd ed. Agronomy 16:23-48

SOYBEAN TRANSFORMATION

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The regeneration of soybean plants from somatic cells has been achieved by culturing thin cotyledonary epidermal layers from immature cotyledons.

This cell system was used in transformation experiments mediated by *Agrobacterium* or by a "particle gun" with which DNA was shot into the cells. The marker genes GUS, NPT as well as a gene (bxn) coding for resistance to the herbicide Bromoxynil were used for transformation. The results obtained so far, including transient expression of these genes and stable transformation, are compared for both *Agrobacterium* and the particle gun.

Keim P, Shoemaker RC (1988) Construction of a random recombinant DNA library that is primarily single copy sequences. Soybean Genet. Newsl. 15:147-148

Keim P, Diers BW, Shoemaker RC (1990a) Genetic analysis of soybean hard seededness with molecular markers. Theor Appl Genet (In press)

Keim P, Shoemaker RC, Palmer RG (1989) Restriction fragment length polymorphism diversity in soybean. Theor Appl Genet 77:786-797

Keim P, Diers BW, Olson T, Shoemaker RC (1990b) RFLP mapping in soybean: Association between marker loci and variation in quantitative traits. Unpublished.

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181

Osborn TC, Alexander DC, Fobes JF (1987) Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. Theor Appl Genet 73:350-356

Palmer RG, Kilen TC (1987) Qualitative genetics and cytogenetics. In: JR Wilcox (ed.) Soybean: Improvement, production and uses. 2nd ed. Agronomy 16:23-48

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721-726

Smith KJ, Huyser W (1987) World distribution and significance of soybean. In: JR Wilcox (ed) Soybeans: Improvement, production, and uses. 2nd ed. Agronomy 16:23-48

Suarez JC (1989) Association between isozyme genotypes with quantitative traits in soybean. Ph.D. diss. Iowa State Univ., Ames, IA (Diss. Abstr. 89-20190)

Weber CR (1950) Inheritance and interrelation of some agronomic and chemical characters in an interspecific cross in soybeans, Glycine max x G. ussuriensis. Iowa Agric.Exp. Stn. Res. Bull. 374.

Wilcox JR (1985) Breeding soybeans for improved oil quantity and quality. In: R Shibles (ed) World soybean research conference III: Proceedings. Westview Press, Boulder CO, pp 380-386

Wilcox JR, Cavins JF (1985) Inheritance of low linolenic acid content of the seed oil of a mutant in Glycine max. Theor. Appl. Genet. 71:74-78.

Wilson RF (1987) Seed metabolism. In: JR Wilcox (ed) Soybeans: Improvement, production, and uses. 2nd ed. Agronomy 16:643-686

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