Theor Appl Genet (1993) 87:49–53

Genetic variation for quantitative traits in soybean lines derived from tissue culture

M. S. Hawbaker [1], W. R. Fehr [2], L. M. Mansur [3], R. C. Shoemaker [4], R. G. Palmer [4]

[1] Department of Crop Science, North Carolina State University, Raleigh, NC 27695-7620, USA
[2] Department of Agronomy, Iowa State University, Ames, IA 50011, USA
[3] Department of Biology, University of Utah, Salt Lake City, UT 84110, USA
[4] Department of Agronomy, Zoology, and Genetics, USDA-ARS-FCR, Iowa State University, Ames, IA 50011, USA

Received: 31 October 1992 / Accepted: 1 March 1993

Abstract. Tissue culture may generate useful genetic variation for quantitative traits. The objective of this study was to analyze genetic variation for ten quantitative traits of soybean [*Glycine max* (L.) Merr.] among lines derived from the tissue culture of three cultivars. The three cultivars used to obtain R0 plants from tissue culture were "BSR 101", "Hodgson 78", and "Jilin 3". A total of 63 R0-derived lines of BSR 101, eight of Hodgson 78, and 42 of Jilin 3 was planted with the untreated controls in row plots in a randomized complete-block design with three replications at two locations for each of 2 years. The traits evaluated were days to beginning bloom (R1), beginning seed (R5), beginning maturity (R7), full maturity (R8), height, lodging, seed yield, seed weight, protein content, and oil content. Significant ($P < 0.05$) variation was observed among lines for each of the ten quantitative traits. There was 57.1% of the BSR 101 lines, 87.5% of the Hodgson 78 lines, and 76.2% of the Jilin 3 lines that were significantly different from the controls for at least one trait. The percentages of lines that were significantly different from the control for an individual trait ranged from 2.7% for oil content to 25.7% for R7. The magnitude of the changes was relatively small. Although this genetic variation may be useful for cultivar development, greater variability at less expense would be expected with conventional artificial hybridization.

Key words: Tissue culture – Somaclonal variation – Embryogenesis – *Glycine max* (L.) Merr.

Communicated by A. R. Hallauer
Journal Paper No. J-14958 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IOWA, USA. Project No. 2475.
Correspondence to: M. S. Hawbaker

Introduction

Tissue culture has been used for the clonal propagation of desirable genotypes (Scowcroft 1985). One potential disadvantage of tissue propagation is that genetic modifications may occur during regeneration. Although this genetic variation is a disadvantage for clonal propagation, it may be a method of creating useful genetic variation for plant improvement programs. The heritable genetic variability generated during tissue culture was termed somaclonal variation by Larkin and Scowcroft (1981). Somaclonal variation has been found at the agronomic, karyotypic, biochemical, and molecular levels in many crop species (Scowcroft 1985).

The nomenclature system introduced by Orton (1983) will be used to identify tissue culture regenerates and their progeny. An R0 plant is the regenerate obtained directly from tissue culture. An R1 plant is the selfed progeny of an R0 plant, and an R2 plant is the selfed progeny of an R1 plant.

Several factors affect the amount of somaclonal variation, including the type of tissue cultured, the media type used, the time the tissue is in culture, and the genotype of the explant (Scowcroft 1985). Single-gene mutations for qualitative traits have been documented for many crop species (Barwale and Widholm 1987; Zehr et al. 1987; Lal and Lal 1990). Identification of genetic alteration of quantitative traits by tissue culture generally involves replicated testing of regenerates to detect relatively small changes. There have been few studies that have evaluated a large number of lines derived from tissue culture over several environments (Dunwell et al. 1986; Graybosch et al. 1987; Lee et al. 1988; Dahleen et al. 1991; Stephens et al. 1991).

Tissue-culture-induced variation for specific qualitative traits in soybean [*Glycine max* (L.) Merr] has been

reported by Barwale and Widholm (1987), Freytag et al. (1989), and Amberger et al. (1992a, b). The single-gene mutations that they collectively reported in soybean include lethal sectoral albinos, twin seeds, multiple shoots, dwarfs, abnormal leaf morphology, abnormal leaflet number, wrinkled leaves, chlorophyll deficiency, partial sterility, complete sterility, lanceolate leaves, leaf variegation (chimeral variegated plants), pod variegation on otherwise normal plants, change in growth habit from indeterminate to determinate, yellow edges on cotyledons, no unifoliolate leaves, yellow-green plants, aconitase null, and malate dehydrogenase null.

Studies of genetic variability for quantitative traits resulting from tissue culture of soybean have given contrasting results. Graybosch et al. (1987) studied lines derived by tissue culture and found significant ($P<0.05$) variability for seed yield and height, but not for lodging and days to maturity. In contrast, Stephens et al. (1991) observed significant ($P<0.05$) variation among soybean lines derived from tissue culture for time of maturity, lodging, height, protein content, and oil content, but not for seed yield, seed weight, or seed quality. Because studies of somaclonal variation for quantitative traits in soybean have been limited and the results have been contradictory, our study was conducted to further examine the extent of variation that could be generated by tissue culture.

Materials and methods

The 113 lines used for this study originated from R0 plants that had been regenerated by the induction of direct somatic embryogenesis on immature embryo cotyledons (Amberger et al. 1992a). Three soybean cultivars, 'BSR 101', 'Hodgson 78', and 'Jilin 3', were used as explant sources for tissue culture. These cultivars were chosen for this study because they had different genetic backgrounds and acceptable maturity for central Iowa. BSR 101 is a high-yielding cultivar of Maturity Group I developed at Iowa State University that has PI 84946-2 in its parentage (Tachibana et al. 1987), a source of resistance to brown stem rot [caused by *Phialophora gregata* (Allington and Chamberlain) W. Gams], Hodgson 78 is a high-yielding cultivar of Maturity Group I developed by the University of Minnesota (Lambert and Kennedy 1979). Jilin 3 (PI 427.099) is a cultivar of Maturity Group I from Jilin Province of The People's Republic of China, and exhibits a similar yielding-ability to BSR 101 when grown in central Iowa.

Tissue-culture regeneration consisted of initiation, maturation, and conversion phases. During the initation phase, immature embryo cotyledons (4 mm) were excised from immature seed and placed on induction media containing 20–40 μg ml^{-1} 2,4-Dichlorophenoxy acetic acid (2,4-D) to initiate somatic embryogenesis, as described by Ranch et al. (1985). Somatic embryos were transferred to a Murashige and Skoog (MS) medium containing 3% sucrose and 0.5% activated charcoal and cultured for 20 days. During the maturation phase, embryos were cultured for 45 days on semisolid MS media with 3% sucrose and 0.2% Gelrite® (Ranch et al. 1985). For the conversion phase, embryos were germinated on MS media with 1.5% sucrose and 0.2% Gelrite® to produce R0 plants that were transplanted into a greenhouse.

For this study, R0-derived lines were evaluated in the R3 generation ($R_{0:3}$ lines) during 1990 and the R4 generation ($R_{0:4}$ lines) during 1991. R0 plants were grown in a greenhouse to obtain R1 seeds. The R1 seeds from each R0 plant were planted in a progeny row, and the plants were evaluated visually for obvious phenotypic changes in the field at Ames, Iowa, during the summer of 1988. All the lines used in this study had no obvious phenotypic changes from the controls. The R1 plants from each line were threshed individually. In 1989, 17 R2 seeds from each R1 plant were planted in the field at Ames in progeny rows. Three pods (approximately nine R3 seeds) were harvested in bulk from each row. To obtain enough seed for a replicated field trial in 1990, all R3 seeds tracing to the same R0 plant were bulked. The $R_{0:4}$ lines evaluated in 1991 resulted from bulking equal amounts of seed from each replication of the $R_{0:3}$ lines evaluated in 1990. In addition, self-pollinated seed from each parental line not derived from tissue culture was planted each season to serve as control populations. Any residual heterozygosity of the original parent lines would be apparent in these entries. Comparisons of the early generation somaclonal lines with the parental cultivars were accomplished by planting at least 50 seed from each of the three parental lines during phenotypic evaluation of R1 somaclonal lines and 50 seed from each parental line for every 100 R2 somaclonal lines (Amberger 1990). During each evaluation, no variability was observed within each parental line. Seed within each parental line was then bulked for use as controls during evaluation of the $R_{0:3}$ and $R_{0:4}$ somaclonal lines.

In 1990 and 1991, field tests were conducted with three replications using a randomized complete-block design at two locations in central Iowa. The test consisted of 120 entries: 63 BSR 101 lines, eight Hodgson 78 lines, 42 Jilin 3 lines, three control entries of BSR 101, and two control entries each of Hodgson 78 and Jilin 3.

The experimental unit in 1990 was a two-row unbordered plot 76 cm long with 69 cm between rows of the same plot and 102 cm between rows of adjacent plots. The seeding rate was 23 seed m^{-1} of row. The experimental unit in 1991 was a two-row unbordered plot 152 cm long with 69 cm between rows of the same plot and 102 cm between rows of adjacent plots. The seeding rate was 30 seed m^{-1} of row.

The following traits were evaluated for each plot in 1990 and 1991, except where indicated:

Beginning bloom (R1) – recorded as days after planting, when 50% of the plants in a plot had an open flower at any node on the main stem (Fehr and Caviness 1981).

Beginning seed (R5) – recorded as days after planting, when 50% of the plants had a seed 3 mm long in a pod at one of the four uppermost nodes on the main stem with a fully developed leaf (Fehr and Caviness 1981).

Beginning maturity (R7) – recorded as days after planting, when one normal pod on the main stem had reached its mature pod color (Fehr and Caviness 1981).

Full maturity (R8) – recorded as days after planting, when 95% of the normal pods on the main stem had reached their mature pod color (Fehr and Caviness 1981).

Lodging – scored at R8 on a scale from 1 (all plants erect) to 5 (all plants prostrate).

Plant height – measured in cm at R8 as the distance from the soil surface to the terminal node.

Seed weight – measured in mg sd^{-1} as the average weight of 200 random whole seeds for each plot, except for one replication at one location in 1990.

Seed yield – measured as g m^{-2} for each plot, except for one replication at one location in 1990.

Protein content – measured as g kg^{-1} on a moisture-free basis for each plot, except for one replication at one location in 1990. Oil content – measured as g kg^{-1} on a moisture-free basis for each plot, except for one replication at one location in 1990.

Protein and oil analyses were made with a near-infrared analyzer by the USDA Northern Regional Research Center, Peoria. Illinois.

A separate analysis of variance was computed for each trait and cultivar for individual environments and combined across environments. Entries and environments were considered as random effects. Comparisons between tissue-culture-derived lines and the control entries were made for each trait, and significant differences were determined by use of the L.S.D.

Results

There were significant ($P<0.05$) differences among R0-derived lines for each of the ten quantitative traits in the three cultivars, except days to R1 and R5 for lines derived from Hodgson 78 (Table 1). For BSR 101, the trait with the greatest number of lines significantly different from the control was seed yield (Table 1). There were 20 lines (31.7%) that had significantly lower yields than the BSR 101 control, with a maximum reduction of 56 g m^{-2} (18.6%). There were five (62.5%) of the Hodgson 78 lines that had significantly lower yields than the control, with the greatest reduction being 54 g m^{-2} (21.0%). There were two (4.8%) Jilin 3 lines that had significantly higher yields than the control, with increases of 24 g m^{-2} (10.0%) and 27 g m^{-2} (11.5%). These two lines were also significantly different from the control for earlier R1 maturity, increased height, and greater lodging.

The variation observed for days to R1 differed among the three cultivars (Table 1). The only significantly different BSR 101 line (1.6%) was 1 day later than the control. None of the Hodgson 78 lines was significantly different from the control. There were 14 (33.3%) of the Jilin 3 lines significantly earlier than the control by up to 2.2 days. Similar results were observed for R5 maturity (Table 1). One (1.6%) BSR 101 line was significantly later (1.5 days) than the control. No significant differences from the control were observed for the Hodgson 78 lines. There were nine (21.4%) of the 14 Jilin 3 lines significantly earlier for R1 maturity that also were significantly earlier for R5 by up to 2.4 days.

For R7 maturity, five (7.9%) BSR 101 lines were significantly later than the control, with a maximum differences of 1.5 days (Table 1). One (12.5%) Hodgson 78 line was significantly earlier by 1.6 days, and one line was significantly later by 1.7 days. There were 22 (52.4%) Jilin 3 lines significantly earlier than the control, with the greatest difference being 4.8 days.

The three cultivars had about the same percentage of variants for R8 maturity (Table 1). There were nine (14.3%) BSR 101 lines significantly different from the control for later R8 maturity, with a maximum difference of 1.5 days. There was one (12.5%) Hodgson 78 line significantly earlier by 2.3 days, and seven (16.7%) Jilin 3 lines significantly earlier by up to 2.1 days.

There were significant differences among lines for lodging in each cultivar (Table 1). There was one (1.6%) BSR 101 line that lodged significantly more than the control by a score of 0.5, five (62.5%) Hodgson 78 lines lodged significantly less than the control by up to a score of 0.8, and three (7.1%) Jilin 3 lines lodged significantly more than the control by up to a score of 1.1.

The three cultivars had about the same number of variants for height (Table 1). There were three (4.8%) BSR 101 lines significantly shorter than the control by up to 6 cm, one (12.5%) Hodgson 78 line significantly short-

Table 1. Number of R0-derived lines significantly ($P<0.05$) different from the controls and the range of the deviations

Trait	Cultivar					
	BSR 101[a]		Hodgson 78[b]		Jilin 3[c]	
	No. of lines	Range	No. of lines	Range	No. of lines	Range
Yield (g m^{-2})	20	−29 to −56	5	−33 to −54	2	+24 to +27
Beginning bloom (days)	1	+1.0	0	0	14	−1.3 to −2.2
Beginning seed (days)	1	+1.5	0	0	9	−1.6 to −2.4
Beginning maturity (days)	5	+1.3 to +1.5	2	−1.6 to + 1.7	22	−1.3 to −4.8
Full maturity (days)	9	+0.8 to +1.5	1	−2.3	7	−1.1 to −2.1
Lodging (score)	1	+0.5	5	−0.4 to −0.8	3	+0.5 to +1.1
Plant height (cm)	3	−5 to −6	1	−8	2	+5 to +6
Seed weight (mg sd^{-1})	15	−4 to −13	4	+9 to +16	6	−7 to −15
Protein content (g kg^{-1})	6	+4 to +6	1	+8	2	+4 to +6
Oil content (g kg^{-1})	1	−3	1	+3	1	−6

[a] 63 R0-derived lines from BSR 101 were evaluated
[b] 8 R0-derived lines from Hodgson 78 were evaluated
[c] 42 R0-derived lines from Jilin 3 were evaluated

er than the control by 8 cm, and two (4.8%) Jilin 3 lines significantly taller than the control by up to 6 cm.

There were significant differences among lines for seed weight in each cultivar (Table 1). There were 15 (23.8%) BSR 101 lines significantly smaller than the control by up to 13 mg sd^{-1}, four (50.0%) Hodgson 78 lines significantly larger than the control by up to 16 mg sd^{-1}, and six (14.3%) Jilin 3 lines significantly smaller, with a maximum difference of 15 mg sd^{-1}.

The three cultivars had about the same percentage of variants for protein content (Table 1). There were six (9.5%) BSR 101 lines with a significantly greater protein content than the control by up to 6 g kg^{-1}, one (12.5%) Hodgson 78 line significantly greater by 8 g kg^{-1}, and two (4.8%) Jilin 3 lines significantly greater in protein content by up to 6 g kg^{-1}.

The three cultivars had the same number of variants for oil content (Table 1). One of the same BSR 101 lines that had a significantly greater protein content also had a significantly lower (3 g kg^{-1}) oil content than the control. There was one (12.5%) Hodgson 78 line that had a significantly greater (3 g kg^{-1}) oil content. One of the same Jilin 3 lines that had a significantly greater protein content was also significantly lower (6 g kg^{-1}) in oil content than the control.

There were 38 (60.3%) BSR 101 lines that had at least one trait significantly different from the control and 25 (39.7%) lines not significantly different for any trait. There were 22 (34.9%) lines that had only one trait significantly different, 11 (17.5%) lines were different for two traits, two (3.2%) lines were different for three traits, and three (4.8%) lines were different for four traits.

There were seven (87.5%) Hodgson 78 lines that had at least one trait significantly different from the control and one (12.5%) line not significantly different for any trait. There was one (12.5%) line that had only one trait significantly different, two (25.0%) lines were different for two traits, two (25.0%) lines were different for three traits, one (12.5%) line was different for four traits, and one (12.5%) line was different for five traits.

There were 32 (76.2%) Jilin 3 lines that differed for at least one trait. There were ten (23.8%) lines that had no traits different from the control. There were 13 (31.0%) lines that had only one trait significantly different, ten (23.8%) lines were different for two traits, four (9.5%) lines were different for three traits, three (7.1%) lines were different for four traits, one (2.4%) line was different for five traits, and one (2.4%) line was different for six traits.

Discussion

Bidirectional variation was detected for all the quantitative traits measured in the R0-derived lines from the three cultivars. The traits evaluated included all those studied by Graybosch et al. (1987) or Stephens et al. (1991), except seed quality. Graybosch et al. (1987) evaluated a total of 24 R0-derived lines from three cultivars in one environment. They found lines significantly ($P<0.05$) different from the control for yield and height, but not for lodging and days-to-maturity. Stephens et al. (1991) evaluated progeny from nine R0 plants of one cultivar in four environments. They observed lines significantly ($P<0.05$) different from the control for maturity, lodging, height, protein content, and oil content, but not for yield, seed weight, or seed quality. Failure to detect genetic variation for some traits among lines derived from tissue culture in the previous studies may have been due to the limited number of R0-derived lines evaluated, the limited amount of replicated testing, or both.

The percentage of lines significantly ($P<0.05$) different from the controls was not the same for the three cultivars, indicating that genotypes may not respond the same to tissue-culture regeneration. Similar results were reported by Dahleen et al. (1991) in oat. They evaluated progeny from 56 R0 plants of two cultivars in four environments. Their results indicated that somaclonal variation was greater for one cultivar than for the other. In our study, more useful variation was found in lines derived from Jilin 3 than from BSR 101 and Hodgson 78. Jilin 3 was the only cultivar of the three used in this study in which improved yield was observed.

We agree with Dahleen et al. (1991), who indicated that the presence of genetic variability among lines derived from tissue culture should allow for only small genetic gain from selection for quantitative traits. Although our lines had bidirectional variation for all the quantitative traits evaluated, most of the variation observed did not show great enough change for economically important traits to justify the use of tissue culture to induce variation compared with conventional artificial hybridization. Also, each cultivar had a large percentage of lines that had more than one trait significantly different from the control. Because tissue culture may alter many quantitative traits, large numbers of lines would need to be evaluated in order to identify those with changes only in the desired trait.

Although it is theoretically possible that the observed somaclonal variation could be the result of some pre-existing variability present in the original parental lines, this is unlikely. Phenotypic evaluation of the three control cultivars for four seasons indicated homogeneity within each of the controls. Also, for none of the traits evaluated were control lines within a cultivar significantly different from each other. Thus, residual heterozygosity in the original parent lines was minimal and should not, therefore, be used to explain the variation observed in these R0-derived lines.

Acknowledgements. Research was supported in part by grants from the Iowa Soybean Promotion Board and The American

Soybean Association. The mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by Iowa State University or the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

References

Amberger LA (1990) Analyses of somaclonal variation in soybean. MS dissertation, Iowa State University, Ames

Amberger LA, Palmer RG, Shoemaker RC (1992a) Analyses of culture-induced variation in soybean. Crop Sci (in press)

Amberger LA, Shoemaker RC, Palmer RG (1992b) Inheritance of two independent isozyme variants in soybean plants derived from tissue culture. Theor Appl Genet (in press)

Barwale UB, Widholm JM (1987) Somaclonal variation in plants regenerated from cultures of soybean. Plant Cell Rep 6:365–368

Dahleen LS, Stuthman DD, Rines HW (1991) Agronomic trait variation in oat lines derived from tissue culture. Crop Sci 31:90–94

Dunwell JM, Cornish M, Powell W, Borrino EM (1986) An evaluation of the field performance of the progeny of plants regenerated from embryos of *Hordeum vulgare* cv Golden Promise. J Agri Sci 107:561–564

Fehr WR, Caviness CE (1981) Stages of soybean development. Iowa Coop Ext Serv, Iowa Agri Home Econ Exp Stn Spec Rep 80

Freytag AH, Rao-Arelli AP, Anand SC, Wrather JA, Owens LD (1989) Somaclonal variation in soybean plants regenerated from tissue culture. Plant Cell Rep 8:199–202

Graybosch RA, Edge ME, Delannay X (1987) Somaclonal variation in soybean plants regenerated from the cotyledonary node tissue culture system. Crop Sci 27:803–806

Lal R, Lal S (1990) Crop improvement utilizing biotechnology. CRC Press, Inc. Boca Raton, Florida, USA

Lambert JW, Kennedy BS (1979) Registration of 'Hodgson 78' soybean. Crop Sci 19:296

Larkin PJ, Scowcroft WR (1981) Somaclonal variation – a novel source of variability from cell culture for plant improvement. Theor Appl Genet 60:197–214

Lee M, Geadelmann JL, Phillips RL (1988) Agronomic evaluation of inbred lines derived from tissue cultures of maize. Theor Appl Genet 75:841–849

Orton TJ (1983) Experimental approaches to the study of somaclonal variation. Plant Mol Biol Rep 1:67–76

Ranch JP, Oglesby L, Zielinski AC (1985) Plant regeneration from embryo-derived tissue cultures of soybean. In Vitro 21:653–658

Scowcroft WR (1985) Somaclonal variation: The myth of clonal uniformity. In: Hohn B, Dennis B, Dennis ES (eds) Genetic flux. Springer-Verlag, Vienna pp 217–245

Stephens PA, Nickell CD, Widholm JM (1991) Agronomic evaluation of tissue culture-derived sobean plants. Theor Appl Genet 82:633–635

Tachibana H, Voss BK, Fehr WR (1987) Registration of 'BSR 101' soybean. Crop Sci 27:612

Zehr BE, Williams ME, Duncan DR, Widholm JM (1987) Somaclonal variation in the progeny of plants regenerated from callus cultures of seven inbred lines of maize. Can J Bot 65:491–499