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Agronomic evaluation of tissue-culture-derived soybean plants

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Summary. Genetic alterations of regenerated plants based on the tissue culture process (somaclonal variation) have become common for many plant species including soybean [*Glycine max* (L.) Merr.]. The objective of this study was to test for the presence of tissue-culture-derived genetic variation in eight agronomic traits in homozygous progeny regenerated by organogenesis using the commercially important cultivar Asgrow 'A3127.' A total of 86 lines derived by repeated self-pollination of nine regenerated plants was grown in two locations for 2 years. When compared to the unregenerated parent, statistically significant variation ($P < 0.05$) was found for maturity, lodging, height, seed protein and oil, but not for seed quality, seed weight, or seed yield. All of the variation noted was beneficial and did not involve decreased yield. Since the differences were not large, the results indicate that the tissue culture process is not necessarily detrimental to plant performance, which is an important consideration since tissue culture techniques are used in many genetic engineering methods.

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

Introduction

Soybean plant regeneration from tissue culture has been difficult and only recently has the process become routine. There are two routes for plant regeneration from culture: somatic embryogenesis and shoot organogenesis. Embryogenesis is the production of embryos from single or multiple cells, while organogenesis is the regeneration of plantlets usually from preexisting structures (e.g., cotyledon, meristem, or embryo axis). Christianson et al. (1983) published the first report on successful soybean regeneration in which several somatic embryoids were produced. Reports of more efficient embryogenic regeneration systems soon followed (Ranch et al. 1985; Lazzeri et al. 1985; Barwale et al. 1986), as well as organogenic regeneration systems (Barwale et al. 1986; Wright et al. 1986).

Plants regenerated from tissue culture have exhibited various morphological and biochemical variation due to mutations which Larkin and Scowcroft (1981) termed somaclonal variation. Chromosomal aberrations, ranging from changes in ploidy to whole chromosome loss, have been demonstrated for tissue-culture-derived plants. Mutations that are single gene, multigene, and cytoplasmic have also been described (Lee and Phillips 1988; Dahleen et al. 1991).

In some regenerated soybean progeny, Ranch and Palmer (1987) found 80 chromosomes and Barwale and Widholm (1989) reported mixaploidy with chromosome counts ranging from 10 to 60. Freytag et al. (1989) described changes in leaf morphology and growth habit from indeterminate to determinate in organogenically regenerated soybean plants. In another study by Graybosch et al. (1987), three soybean genotypes regenerated with the organogenic system of Wright et al. (1986) were found to show significant variability for plant height and yield, while differences in lodging and maturity were not significant. The significant yield differences were lower than the control. Barwale and Widholm (1987) studied the morphology of 212 SC_3 families and 789 SC_4 families from nine soybean genotypes regenerated through both the embryogenic and organogenic systems. The variant phenotypes included chlorophyll deficiency, sterility, abnormal leaf morphology, abnormal leaf number, and dwarf growth habit. One variant phenotpye, a wrinkled-leaf trait was found to be maternally inherited (Stephens

Table 1. Means and ranges of somaclonal (SC) lines compared to A3127 pooled from two locations and 2 years

Character		Parent	SC lines		LSD (0.05)[a]
		Mean	Mean	Range	
Yield	kg ha^{-}	3,365.0	3,297.0	2,701.0–3,709.0	NS
Maturity	date[b]	19 Sept.	18 Sept.	16 Sept.–20 Sept.	1.4 d
Lodging	score[c]	1.9	1.6	1.3– 2.2	0.4
Height	cm	88.0	86.0	81.0– 90.0	4.0
Seed quality	score[d]	2.0	1.9	1.8– 2.1	NS
100-seed weight	g	14.2	13.8	13.3– 14.5	NS
Protein	g kg^{-1}	368.0	370.0	362.0–376.0	5.0
Oil	g kg^{-1}	216.0	216.0	212.0–223.0	4.0

[a] Comparison between parent mean and somaclone (SC) entry mean
[b] Maturity date: when 95% of pods have mature pod color
[c] Score: 1 = all plants erect, 5 = all plants prostrate
[d] Score: 1 = excellent, 5 = poor

et al. 1991). However, the regenerants were not extensively evaluated for agronomic traits.

The objective of this sutdy was to test for the presence of tissue-culture-derived genetic variation in agronomic traits under field conditions in the progeny of plants regenerated by organogenesis of a commercially important cultivar. The plants used did not show obvious visual alterations.

Materials and methods

A total of 86 lines from nine SC_1 plants of the cultivar Asgrow 'A3127' ('Williams' × 'Essex') was grown in the experiment. The regenerated plant is termed the SC_1 generation, and subsequent generations of selfed progeny, the SC_2, SC_3, etc. (Larkin et al. 1984). Two groups of 36 SC_6 entries that traced back to two SC_1 plants made up the bulk of the lines evaluated. The two SC_1 plants had given rise to several of the variant families reported by Barwale and Widholm (1987). A third group consisted of 14 SC_5 entries, two from each of seven SC_1 plants. The cultivars A3127, 'Williams 82' (Bernard and Cremeens 1988), 'Burlison' (Nickell et al. 1990), and 'Resnik' (A3127[4] × Williams 82) were included as standards for a total of 90 entries. Entries were planted in four-row plots in a randomized complete block design at two locations (Agronomy-Plant Pathology Farm and Cruse Farm) for 2 years 1988–1989). Each location consisted of two blocks, with each entry entered once per block. Plots were 3 m long with a 76-cm spacing between rows. The two center rows were harvested and seed yields was adjusted for 13% moisture. The eight traits studied were: (i) yield (kg ha^{-1}), (ii) plant height at harvest (cm), (iii) maturity (date when at least 95% of the plants had mature pod color), (iv) lodging (scored on the basis of 1 = all plants erect to 5 = all plants prostrate), (v) 100-seed weight (g), (vi) seed quality (on a scale of 1 = good to 5 = poor), (vii) seed protein (g kg^{-1} dry wt), and (viii) seed oil (g kg^{-1} dry wt). Protein and oil percentage were determined using a Dickey-John near infrared reflectance analyzer after the samples had been processed in a flour mill (Magic Mill Inc., Salt Lake City/UT).

Data from the experiment were subjected to analysis of variance (ANOVA). Years and locations were considered random, while genotypes were considered a fixed effect. Statistical signficance was determined at the 0.05 probability level and means were separated using the LSD (0.05), calculated with a pooled error term since no interactions were detected.

Table 2. Number of regenerated entries significantly different from parent (A3127), based on comparison using LSD ($P<0.05$)

	Asgrow A3127	
	Greater no.	Less no.
Yield	0	0
Maturity	3	11 (earlier)
Lodging	0	17 (better standability)
Height	0	8 (shorter)
Seed quality	0	0
100-seed weight	0	0
Protein	3	1
Oil	2	0

Results and discussion

Comparison of the 86 somaclonal (SC) lines with the parental cultivar A3127 showed significant variation ($P<0.05$) for maturity, lodging, height, protein, and oil but not for seed yield, seed quality, or seed weight. Although the differences were significant ($P<0.05$), they were not large since the range for the regenerated lines was reasonably close to the means for the parent (Table 1). Regenerated SC_6 entries that were significantly earlier than the parent in maturity also had significantly decreased height and lodging, but not decreased yield. Significant differences for protein and oil were an effect of maturity, since entries higher in protein were earlier in maturity while entries higher in oil were later in maturity, as has been noted before in soybean population (Miller and Fehr 1979).

Results of this 2-year study show that statistically significant somaclonal variation can be generated for a number of agronomic characters in soybean (Table 2). Although the entries were randomly selected from advanced lines, most of the significant variation came from two SC_1 (36 entries each) lines. These two lines were

originally noted in the SC_4 as showing segregation for chlorophyll and partial sterility mutation. The normal appearance and small differences in the variants suggest that several genes or a minor gene may have been altered. If chromosome aberrations were present or if genes responsible for qualitative traits had been altered, we would expect to see abnormal plants and greater variation.

With the advent of transformation techniques using organogenic regeneration systems (McCabe et al. 1988; Hinchee et al. 1988; Zhou and Atherly 1990), researchers need to be concerned about the possible somaclonal variation-induced mutations in the transformed product. The results presented here show that soybean plants cultured through an organogenic regeneration procedure would not necessarily have detrimental mutations resulting from the tissue culturing process and would retain the yield potential of the parental cultivar, which is in contrast to the results of Graybosch et al. (1987) who showed a decrease in yield for some of the regenerated soybean lines. The results show that somaclonal variation did cause significant beneficial variation for maturity, lodging, height, seed protein, and oil. However, due to the narrow degree of variation, it would not benefit a soybean breeder if used directly to generate genetic variation among regenerated progeny.

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