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Selection for high seed oil content in soybean families derived from plants regenerated from protoplasts and tissue cultures

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Abstract Recurrent selection for high seed oil content was carried out with 2,008 progeny of 28 plants regenerated via embryogenesis, 95 via organogenesis and 25 from protoplasts via organogenesis from five different soybean cultivars. Two lines derived from plants regenerated from the cultivar Jack with small increases in seed oil content emerged after three selection cycles in the field but in both cases the protein content was decreased and the seed yield of one of the lines was also decreased. Apparently somaclonal variation for seed oil content can arise, but on the basis of the decreases in protein and yield found in this study, this small change is not useful for soybean improvement.

Keywords Seed oil content · *Glycine max* · Seed protein content · Somaclonal variation

Introduction

Plants regenerated from tissue cultures often carry mutations that arise due to the tissue culture process. These mutations have been defined as somaclonal variation by Larkin and Scowcroft (1981) and can be caused by the activation of transposable elements, single base changes, mitotic recombination, chromosome rearrangements and changes in gene copy number (Lee and Phillips 1988). Recently, activation of retrotransposons and insertion into gene coding regions has been documented by Hirochika et al. (1996). While the frequency of somaclonal variation is relatively low in soybean in comparison to some crop species, a number of reports have described a large number of variants, including maternally inherited wrinkled leaf, chlorophyll-deficiency, dwarf, sterility, maturity, height, leaf shape, variegation and isozymes (summarized by Widholm 1996). As with conventional mutagenesis most are detrimental, but it is possible that agronomically useful traits could be produced.

That all plants regenerated from tissue cultures do not carry deleterious genetic changes was demonstrated by Stephens et al. (1991) who evaluated 86 normal-appearing lines derived from soybean plants regenerated through organogenesis. There was no significant variation in seed weight, quality or yield, but there were small beneficial changes in maturity, height, lodging and seed protein and oil content in comparison with seed-derived controls. These changes were considered to be too small to be of use for breeding purposes, however.

We report here the selection for increased seed oil content in progeny from 148 families from a unique set of soybean plants of several genotypes regenerated via embryogenesis, organogenesis and from protoplasts. The families used here were normal in appearance, but somaclonal variants have been identified in the progeny of related regenerants. These include a maternally inherited wrinkled leaf mutant found in progeny of plants regenerated from protoplasts of cv. Clark 63 (Nguyen and Widholm 1997).

Materials and methods

Soybean [*Glycine max* L. (Merrill)] plants were regenerated from immature embryo axes via organogenesis as described by Barwale et al. (1986), from immature cotyledon protoplasts via organogenesis as described by Dhir et al. (1991) and from embryogenic suspension cultures initiated from immature cotyledons as described by Finer and Nagasawa (1988). The soybean cultivars used were Jack (Nickell et al. 1990a), Clark 63 (Williams and Bernard 1964), Chamberlain (Nickell et al. 1987), Burlison (Nickell et al. 1990b) and A2396 (Asgrow Seed Company). The number of families and plant regeneration method used are listed in Table 1.

In 1993, 1994 and 1995, 45 seeds of each of the selected lines were grown in 3.65-m long rows with 0.76 m between rows at the University of Illinois, Crop Sciences Research and Education Center in Urbana. In 1993, R1 seeds from plants regenerated from tissue cultures were grown (Table 1); subsequently, about 15 plants from each row were harvested individually and the seed threshed and analyzed for oil content. Seeds from individual plants with the 28 highest and 15 lowest oil contents were grown in 1994, and

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Table 1 Oil content of soybean seed produced by individual R1 plants grown in the field in 1993

Genotype	Origin	Number of rows (families)[a]	Number of plants tested	Range of oil contents (%)
Jack	Control		20	19.8–21.8
Jack	Embryogenesis	28	277	20.1–23.9
Jack	Organogenesis	51	759	18.9–26.0
Clark 63	Control		14	21.0–21.9
Clark 63	Protoplast	10	134	20.5–23.0
Chamberlain	Control		20	19.3–21.0
Chamberlain	Protoplast	11	142	20.0–22.6
Chamberlain	Organogenesis	38	551	15.1–22.7
Burlison[b]	Organogenesis	6	90	19.5–21.6
A2396[b]	Protoplast	4	55	20.3–22.1
Totals without controls		148	2008	

[a] Usually seed from 15 individual plants per row (family) were analyzed
[b] No control plants were grown for these cultivars

again seed from about 15 plants in each row were analyzed for oil content. In 1995, seed from the 15 rows grown from seed of single plants from the 18 families with the highest mean oil content in 1994 were bulked and analyzed for oil content. The oil concentration was measured in seed grown in 1993, 1994 and 1995 using the nondestructive nuclear magnetic resonance method (Alexander et al. 1967) with 25-g samples dried for 10 day at 43°C.

In 1996 the seed from the two families (No. 9, 14 lines and No. 25, 15 lines) with the highest oil contents was grown at four locations: Dekalb, Dwight, Urbana-1 and Urbana-2, all in Illinois. Entries were planted in 4-row plots, each row 4.5 m long, with 0.76 m between rows. The experimental design was a randomized complete block with two replicates per location.

The agronomic traits evaluated were (1) yield (g plot^{-1}–adjusted to 130 g kg^{-1} moisture); (2) maturity (date when at least 95% of the pods had a mature color); (3) lodging (scored on the basis of 1=all plants erect to 5=all plants lying flat on the soil surface); (4) plant height at harvest (cm); (5) seed quality, based on the amount of wrinkled and discolored seed (on a scale of 1=excellent to 5=poor); (6) seed weight (cg); (7) seed protein (%); (8) seed oil (%).

Seed protein and oil were measured in 1996 and 1997 on a 25-g seed sample combined from the two replications of each entry. Protein and oil concentrations were determined using infrared reflectance (Rinne et al. 1975) at the National Center for Agricultural Utilization Research, Peoria, Illinois and reported on a dry weight basis.

Results and discussion

When the oil content was determined in seeds produced by a total of 2,008 individual R1 plants that were progeny of 148 R0 plants regenerated via embryogenesis, organogenesis or from protoplasts, the highest levels were found in 28 cv. Jack lines regenerated via embryogenesis or organogenesis (Table 1). The oil concentrations in these 28 lines were above 23%, while the highest Jack control plant was 21.8%. When seeds from these 28 highest oil plants were planted in 1994 in individual rows and the seed from 15 individual plants in each row analyzed for oil content, the mean oil concentration was higher than the control for 18 of the rows (Table 2).

In 1994 we also grew 15 rows from seed of individual plants that had the lowest oil contents in 1993 (Table 1). However, the seed produced by 15 plants in each row showed no differences in oil concentration from the control, indicating that the low oil concentrations found in 1993 was due to some physiological factor like immaturity and had no genetic basis.

The seeds from 15 plants from each of the 18 rows with the highest oil content in 1994 were grown in individual rows in 1995. The mean oil content of only 2 of these families, consisting of all the plants in the row, was higher than the Jack control grown in an adjacent row (Table 2). Row 9 (denoted 9) contained 22.5% oil versus 21.2% for Jack, and row 25 (denoted 25) contained 22.1% versus 21.0% for the control.

Seed from the highest 14 of the number 9 rows and 15 of the number 25 rows produced in 1995 were grown in 1996 at four locations. The combined results from the four locations showed that the mean oil concentration was 20.5% in Jack, 20.9% in No. 25 and 21.3% in No. 9, while the protein was 41.9%, 41.2% and 39.2%, respectively (Table 3). This shows that No. 9 family oil level had increased by 0.8% and No. 25 by 0.4% but that the protein content had decreased by about twice the oil percentage increase in both cases. The mean yield for No. 25 of 4,065 kg/ha was close to that of Jack, 4,207 ka/ha, but No. 9 yielded only 3,441 ka/ha. Both No. 9 and 25 were 2 days earlier in maturity and were shorter, which correlated with a lower lodging score. Seed quality was not different, while No. 25 seed were somewhat larger than that of cv. Jack, and No. 9 was smaller.

Seed from the 4 rows of family No. 9 that showed the highest oil concentrations in 1996 were grown again in 1997 at three locations (same as in 1996 except only one site at Urbana). In this case the oil content of the No. 9 line was 20.7% versus 20.1% for the cv. Jack control, and the respective protein contents were 37.9% versus 41.6%. The yield of the No. 9 rows averaged 3,480 kg/ha compared to 4,038 ka/ha for the control.

The results of this study indicate that it is possible to select for small increases in oil concentration in progeny of soybean plants regenerated from tissue cultures. Line No. 9 was derived from progeny of a plant regenerated from an embryogenic suspension culture of the cv. Jack, and line No. 25 was from a plant regenerated by organogenesis from an immature embryo axis of cv. Jack. This

Table 2 Seed oil concentration of selected plants in 1993, 1994 and 1995. Families nos. 1–10 originated from plants regenerated via embryogenesis and nos. 11–28 via organogenesis

Plant no.	1993 Oil concentration (%)	1994 Oil concentration (%)		1995 Oil concentration (%)		
		Mean	Range	Mean	Range	Jack adjacent Row
Jack	19.8–21.8	22.7	21.6–23.6			
1	23.9	23.2	21.6–24.0	21.5	20.6–22.2	21.3
2	23.5	22.4	20.1–23.0	21.6	20.1–22.4	21.2
3	23.6	22.8	21.1–23.4			
4	23.3	22.2	21.0–23.3			
5	23.2	22.2	20.6–23.9			
6	23.5	23.4	20.8–23.9	21.4	20.4–22.2	20.7
7	23.3	23.9	21.2–24.8	21.5	20.9–22.4	21.4
8	23.1	23.7	21.4–24.0	21.8	21.3–22.3	21.0
9	23.1	23.0	21.7–23.7	22.5	21.1–23.3	21.2
10	23.3	21.8	21.1–23.3			
11	23.2	23.1	21.6–24.3	21.5	20.5–22.5	21.3
12	23.8	24.1	21.8–24.3	21.4	20.1–22.0	21.1
13	23.1	21.6	21.1–22.3			
14	23.2	21.5	20.7–22.2			
15	23.2	22.3	20.9–23.1			
16	23.1	22.8	22.7–23.4	21.4	20.8–22.2	21.1
17	26.0	22.5	21.7–23.5			
18	23.1	22.5	22.1–23.1			
19	23.0	23.4	22.9–24.2	21.0	20.5–21.7	21.5
20	23.3	22.7	22.5–23.1	21.3	19.2–22.6	20.3
21	23.0	22.7	22.2–23.5	21.4	21.1–21.7	21.8
22	23.1	22.4	21.9–22.9			
23	23.6	22.8	22.2–23.4	21.6	20.7–22.4	22.3
24	23.0	22.8	22.1–23.4	21.8	20.5–22.5	21.9
25	25.2	23.0	21.4–24.0	22.1	21.5–22.7	21.0
26	23.0	23.0	22.5–23.7	21.9	21.3–22.6	21.6
27	23.0	23.8	23.2–24.6	21.8	21.3–22.5	21.6
28	23.2	23.4	22.9–24.2	22.2	21.5–22.7	22.1

Table 3 Averaged performance of soybean cv. Jack and 2 regenerated soybean lines at Urbana-1, Urbana-2, Dekalb and Dwight IL in 1996

Entry name	Yield (kg/ha)	Lodging score (1–5)	Height (cm)	Seed				
				Quality score	Weight (cg)	Protein (%)	Oil (%)	Maturity date
Jack	4,207	2.8	104	1.5	15.1	41.9	20.5	9/28
No. 9	3,441	1.6	84	1.4	12.3	39.2	21.3	9/26
No. 25	4,065	2.4	99	1.5	15.7	41.0	20.9	9/26
Average	58.1	2.3	97	1.5	14.3	40.7	20.9	9/26.4
LSD (0.05)	7.6	0.6	9	n.s.	0.8	1.5	0.7	2.1
CV %	7.6	15.3	5.7	7.4	3.4	2.1	1.9	0.1

effect seems to be under genetic control, since the oil levels are relatively stable in related lines over succeeding generations. The increased oil content is associated with a decrease in protein content and in line No. 9 with a decrease in yield.

Hawbaker et al. (1993) found that three of the progeny lines produced from 113 soybean plants regenerated by direct embryogenesis on immature cotyledons of three cultivars were significantly ($P<0.05$) different from the control with respect to oil content. Only one of these lines showed an increase in oil content (0.3%). The seed that was analyzed was of the R3 and R4 generations grown from bulked seed progeny of the individual R0 plants. Thus, segregation in progeny lines would not have been seen.

Breeders have also found that there generally is an inverse correlation between soybean seed protein and oil contents (Wilcox 1998) so that increasing one without lowering the other has been difficult.

This study does provide information about the utility of somaclonal variation found in a unique set of germplasm produced from plants regenerated by three different methods from five different cultivars It would appear that the somaclonal variation for oil content present in

this material was both small and associated with detrimental effects like decreased protein and yield so cannot contribute to soybean improvement. Previous studies using conventional breeding methods have also often shown some of these undesirable correlations. It is possible, however, that evaluation of much larger numbers of families derived from more regenerated soybean plants could produce better results.

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