Low-Palmitic, Low-Linolenic Soybean Development

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ABSTRACT: Plant breeding research efforts are currently focused on developing breeding procedures to decrease the saturated FA palmitic acid (16:0) and the PUFA linolenic acid (18:3) in U.S. soybean cultivars. Soybean oil with lower 16:0 may provide cardiovascular benefits to health-conscious consumers, and lower 18:3 could contribute to better flavor and stability of the oil. The purpose of this study was to determine genetic parameters that indicate the potential for breeding success and to characterize the correlated effect of the incorporation of the modified oil traits on the agronomic and seed quality traits of a soybean breeding population formed from a cross between the soybean cultivar Anand (normal) and germplasm N97-3708-13 (low 16:0, low 18:3). Although lines with only one modified oil quality trait (low 16:0 or 18:3) are useful as parents, commercial utilization requires productive cultivars with the combination of both oil traits. This paper shows the ease with which they may be combined with seed yield and other traits. Measurements were obtained from 179 F_2 single plants grown in 1999 and 121 $F_{2:4}$ lines grown in replicated plots in 2000. Modified FA lines were developed with *ca.* 4% 16:0 and 18:3, respectively. Very weak positive correlations were found between 16:0 concentration and seed yield (r = 0.12) and between 16:0 and seed oil concentration (r = 0.13). No correlation was found between 18:3 levels and seed yield, or between 18:3 levels and seed oil concentration. These results indicate that breeding for reduced 16:0 and 18:3 should not have a negative impact on seed yield or oil concentration. 16:0 and 18:3 had moderately high heritabilities of 0.65 and 0.73, respectively. This indicates that breeders using low 16:0, low 18:3 germplasm in crosses with normal, elite lines can expect to recover low 16:0 and low 18:3 in pure line progenies *via* selection and generation advancement of F_2 individuals that express low levels of these FA.

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Soybean [*Glycine max* (L.) Merr.] accounts for about 30% of the world's market for vegetable oil (1). Soybean seed is composed of approximately 20% oil (2). In the United States 84% of the fat in most margarines and 65% of the fat in most shortenings is soybean oil (3). The average FA composition of refined, bleached, and deodorized commercial soybean oil is 11% palmitic acid (16:0), 4% stearic acid (18:0), 24% oleic acid (18:1), 54% linoleic acid (18:2), and 7% linolenic acid (18:3) (2).

Health-conscious consumers currently favor a lower saturated fat diet. Nevertheless, saturated fat is an important component in the oxidative stability and nutritional quality of lipid-containing foods (4). This creates a challenge for the food processing industry to find efficient ways to reduce saturated fats in food products. 18:3 may be an essential unsaturated FA, but it is responsible for the development of an off-flavor problem known as flavor reversion (5). This is caused by autoxidation, a catalytic process leading to free radical chain reactions resulting in polymerization, undesirable odors and flavors, and oxidative products such as hydroperoxides and cyclic peroxides (6,7).

Hydrogenation is a standard technique used to enhance the functional properties and oxidative stability of oil (3). Although this process enables the food industry to convert liquid oils into semisolid fats (used for shortenings and margarines) and improves the oxidative stability, there is some concern about possible health effects from the formation of *trans*-FA by-products. During hydrogenation or partial hydrogenation, some of the acyl double bonds may be relocated and/or transformed from the usual *cis* to the *trans* configuration. These *trans*-FA may constitute 20 to 40% of some margarines and shortenings (5).

Recently, breeders and geneticists have developed soybeans with low 16:0 and low 18:3 FA. This combination of traits has not previously existed. The first soybean cultivar in which both low 16:0 and low 18:3 were combined was Satelite (8). The low 16:0 trait promotes less saturated oil, and the low 18:3 trait improves the oxidative stability of the oil, enabling the production of a lower *trans*-FA vegetable oil (1). Breeders using germplasm or cultivars such as Satelite need information on the genetic transmission (heritability) of the FA levels and their association with agronomic traits to plan modified FA development programs.

This study determined F_2 parent–offspring heritability estimates for 16:0 and 18:3 and the correlations between these two FA and agronomic and seed-quality traits. This information will provide breeders with useful knowledge to combine desirable traits in their breeding programs and meet anticipated processor goals for soybeans.

MATERIALS AND METHODS

Field experiment. A soybean population was created in 1998 from a cross between N97-3708-13, a low-16:0, low-18:3

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*To whom correspondence should be addressed at Dept. of Plant Sciences, 2431 Center Dr., University of Tennessee, Knoxville, TN 37996-4561. E-mail: vpantalo@utk.edu germplasm, and the cultivar Anand, which is a high-yielding cultivar with a normal oil composition. N97-3708-13 was selected from the cross Soyola × [Brim (2) × N88-431 (2) × (N90-2013 × C1726)]. Soyola contributed the low-18:3 trait, Brim and N88-431 contributed the high yield, and N90-2013 and C1726 contributed the low-16:0 trait.

F_1 seeds of N97-3708-13 × Anand were grown at a winter nursery in Isabela, Puerto Rico, during the winter of 1998–1999. The F_2 seeds were grown at the Knoxville Experiment Station in Knoxville, Tennessee, during the summer of 1999. One hundred seventy-nine F_2 single plants were grown in a honeycomb design (seeds were planted in rows spaced approximately 76 cm apart, with 88 cm between each plant within a row, and every other row was offset by 44 cm). This design allowed equidistant plant spacing in every direction. Three seeds were sown per hill, and after 10 to 14 d of growth the plots were thinned to one seedling per hill. As the seedlings developed into mature F_2 plants, the following traits were measured or recorded for each hill plot: maturity date, mature plant height, 100-seed weight, seed protein and oil concentration, and FA composition. Maturity was defined as the age when 95% of the pods reached their mature color. Plant height was measured from the soil surface to the apex of the main stem. In the fall of 1999, each F_2 plant was harvested individually using a single-plant thresher. Seed subsamples were taken for protein, oil, and FA analyses. Approximately 25 g of the $F_{2:3}$ seeds were packaged and sent to the USDA National Center for Agricultural Utilization Research (Peoria, IL) for near-IR transmission analysis of protein and oil, using a Model 1255 Infratec Feed and Food Analyzer.

The remainder of the $F_{2:3}$ seeds were sent to Costa Rica to increase seed production during the winter of 1999–2000 so that sufficient seed would be available for replicated field tests in 2000. In the spring of 2000, each of the $F_{2:3}$ rows was harvested separately in Costa Rica and the $F_{2:4}$ seeds from each of the individual rows were shipped to Knoxville, Tennessee.

In June 2000, a randomized complete block design of three replicates of $F_{2:4}$ plots was planted on Sequatchie silt loam soil at the Knoxville Experiment Station in Knoxville, Tennessee. The plot entries included 121 random $F_{2:4}$ genotypes of the N97-3708-13 × Anand population. Seeds were sown at *ca.* 8 seeds per 0.30 m in two-row plots, planted 6.1 m in length with 76 cm between rows. In addition to all of the traits measured for the individual F_2 plants, we also measured seed yield and lodging for the $F_{2:4}$ plots. Lodging was given a score ranging from 1 (all plants erect) to 5 (all plants prostrate). Before harvesting, the rows were end-trimmed to 4.9 m to normalize their lengths. Plot moisture and yield were then measured after passing the two-row plots through a combine.

FA concentration. Soybean seeds from single F_2 plants (1999) and from each of the replicated $F_{2:4}$ rows (2000) were sampled for FA analyses. Each sample of 5 crushed seeds was transferred to a test tube with 1.0 mL of chloroform/hexane/methanol (8:5:2, by vol). After 4 h, 100 µL of the oil sample was transferred to a 1.5-mL autosampler vial. Then 75 µL of methylating reagent (a mixture of 50 mL sodium methoxide, 20 mL petroleum ether, 10 mL ethyl ether, and 1 mL hexane) was added to the vial before capping. FAME were analyzed using a Hewlett-Packard 6890 series gas chromatograph equipped with a model 7683 autosampler, FID, and a 30 m × 0.53 mm i.d. J&W 125-2332 capillary column. Operating conditions were as follows: carrier: helium (20 mL/min); split injection: 20:1 (vol/vol); injection temperature: 250°C; detector temperature: 275°C; and column temperature: 230°C. The RM-1 standard (Supelco Co., Bellefonte, PA) was used to calculate the relative FA concentrations of the experimental samples.

RESULTS AND DISCUSSION

A chi-square analysis of the 16:0 data from the seeds of the F_2 plants fit a two-gene model ($x^2 = 0.75$). Fourteen plants, representing approximately 8% of the population, were observed to have a double-recessive phenotype, with a mean of 4.4% 16:0. The other 165 plants had a mean 16:0 content of 8.3%. A chi-square analysis of the 18:3 data from the seeds of the F_2 plants fit a one-gene model ($x^2 = 0.31$). Forty-eight individual plants had a recessive phenotype, with a mean of 4.9% for 18:3. The remaining 131 individuals had a mean of 7.3% for 18:3. Our conclusion, i.e., that two recessive genes govern low-16:0 and a single recessive gene governs low-18:3, is consistent with the pedigree of the modified FA parents. This suggests that approximately 1.5% of the recombinant F_2 progeny from a cross with normal, elite lines carries both traits. Hence, breeders would need to keep population sizes adequately large to find the low frequency of individuals with the desired traits.

Tables 1 and 2 show the mean, SE, and minimum and maximum ranges for FA concentrations of the N97-3708-13 × Anand F_2 and $F_{2:4}$ populations, respectively. The lowest levels of 16:0 and 18:3 were *ca.* 4%, representing a greater than 50% reduction in FA amounts relative to their levels in the normal parent Anand (Table 3) and in other typical soybeans. The consistency of the F_2 vs. $F_{2:4}$ FA values suggests that early-generation selection for these traits is possible.

It is noteworthy that the low-16:0, low-18:3 parent, N97-3708-13, yielded 2482 kg ha^{-1}, in the replicated yield test of $F_{2:4}$ lines (data not shown), which is statistically equivalent to that of the parent cultivar, Anand (2473 kg ha^{-1}); Anand is a high-yielding cultivar that was recently released. Ross *et al.*

TABLE 1
Mean, SE, and Minimum and Maximum Ranges for Palmitic and Linolenic FA Concentrations from a Population of 121 F_2 Individuals of N97-3708-13 × Anand Soybeans

	% Total lipid		Range	
Trait	Mean	SE	Minimum	Maximum
Palmitic acid (16:0)	8.0	±0.15	4.1	12.9
Linolenic acid (18:3)	6.6	±0.11	4.1	11.3

TABLE 2
Mean, SE, and Minimum and Maximum Ranges for Palmitic and Linolenic FA Concentrations from a Population of 121 $F_{2:4}$ Lines of N97-3708-13 × Anand Soybeans

	% Total lipid		Range	
Trait	Mean	SE	Minimum	Maximum
Palmitic acid (16:0)	8.7	± 0.16	3.9	12.6
Linolenic acid (18:3)	6.9	± 0.13	4.2	10.6

TABLE 3
Mean and SE for Parents (Anand and N97-3708-13) for Palmitic, Stearic, Oleic, Linoleic, and Linolenic FA Concentrations Grown with Replicated $F_{2:4}$ Lines of the N97-3708-13 × Anand Soybean Population

	% Total lipids				
Parent	16:0	18:0	18:1	18:2	18:3
Anand	12.2 ± 1.4	3.5 ± 0.1	20.1 ± 1.0	55.5 ± 1.3	8.6 ± 0.7
N97-3708-13	4.9 ± 1.4	3.9 ± 0.1	21.4 ± 1.0	64.3 ± 1.3	5.5 ± 0.7

(9) also reported few major agronomic differences between very low 18:3 lines and check cultivars. The population of random $F_{2:4}$ lines from N97-3708-13 × Anand in our study had a mean yield of 2734 kg ha^{-1} (data not shown), which exceeded that of either parent, indicating that characteristics favorable to a productive yield capacity were combined among many of the recombinant lines. This population may have value in the development of pure lines with low 16:0, low 18:3, and acceptable yield performance.

Table 4 shows correlation coefficients derived for agronomic and seed-quality traits. Very weak positive correlations were found between reduced 16:0 and seed yield ($r = 0.12$), and between reduced 16:0 and seed oil concentration ($r = 0.13$). This indicates that breeding for reduced 16:0 should not have a negative impact on seed yield or oil concentration. This point has been elaborated on by Rebetzke *et al.* (10), who reported that seed oil content was significantly greater among reduced palmitic lines. Rebetzke *et al.* also reported that there was no significant association between 16:0 and protein concentration, an observation that we confirmed in our $F_{2:4}$ population. Hence, it should be possible to improve seed protein concentration while pursuing the goal of reducing total saturates in soybean oil.

A very weak negative correlation was found between 18:3 and protein ($r = -0.10$): As 18:3 decreased there was little change in the amount of protein. Improvements in soybean protein concentration would stimulate the soyfood processing industry by reducing production costs. Genetic gains in protein would require breeders to transfer genes from high-protein germplasm. Given the weak correlation between the protein concentration and 18:3 levels observed in this study, it should be possible to increase protein while selecting for low 18:3 in soybean oil.

Parent–offspring regression of FA contents was analyzed using data from single F_2 plants and their derived $F_{2:4}$ rows for all traits. Estimates were determined from the slope of the line, i.e., how well F_2 data predicted their offspring $F_{2:4}$ lines. 16:0 had the second-highest heritability at 0.65, with $r^2 = 0.66$ (Fig. 1). This compares to the high narrow-sense heritabilities for 16:0 (0.98) reported by Rebetzke *et al.* (11) in their normal 16:0 populations. In our study, 18:3 had the highest heritability of all the traits measured, at 0.73 with $r^2 = 0.66$ (Fig. 2). We expand on the conclusion of Rebetzke *et al.* that selection for low 16:0 and/or low 18:3 (within the same or different populations) could be accomplished in early generations by identifying superior F_2 plants that expressed low levels of these FA.

To explore this idea further, we examined the FA compositions of seeds harvested from single F_4 plants within a sample of seven $F_{2:4}$ lines that were derived from F_2 plants with either a low-16:0 and/or a low-18:3 concentration. A high heritability was shown through linear regression among the F_2 single-plant population and the mean among F_4 single plants within the derived $F_{2:4}$ rows for both 16:0 and 18:3 ($r^2 = 0.78$ and 0.75, respectively; data not shown). This supports our findings of moderate to high heritabilities for 16:0 and 18:3 levels and indicates that recombinant plants carrying the traits of interest can be selected in the early breeding generations, allowing rapid genetic transfer of FA traits. More research is needed to develop optimal selection strategies to modify soybean FA composition and improve seed yield and

TABLE 4
Pearson Correlation Coefficients (r)[a] Comparing Maturity (Mat), Height (Ht), Lodging (Lod), Seed Yield (Yld), Seed Size (SS), Seed Protein Concentration (Pro), Seed Oil Concentration (Oil), and Palmitic (16:0) and Linolenic (18:3) Acid Concentrations from a Population of 121 $F_{2:4}$ Lines of N97-3708-13 × Anand Soybeans

	Ht	Lod	Yld	SS	Pro	Oil	16:0	18:3
Mat	0.20**	–0.04	0.20**	0.16**	–0.25**	–0.30**	0.06	–0.05
Ht		0.09	0.10	0.04	–0.12*	–0.05	0.20*	–0.08
Lod			0.12*	0.13*	0.12*	–0.14	–0.05	–0.05
Yld				0.19*	0.11*	–0.09	0.12*	–0.00
SS					0.17**	–0.09	–0.12*	0.01
Pro						–0.19*	0.02	–0.10*
Oil							0.13*	0.05
16:0								–0.09

[a] Approximately 363 observations for each trait. *Significance at $P < 0.05$; **significance at $P < 0.01$.

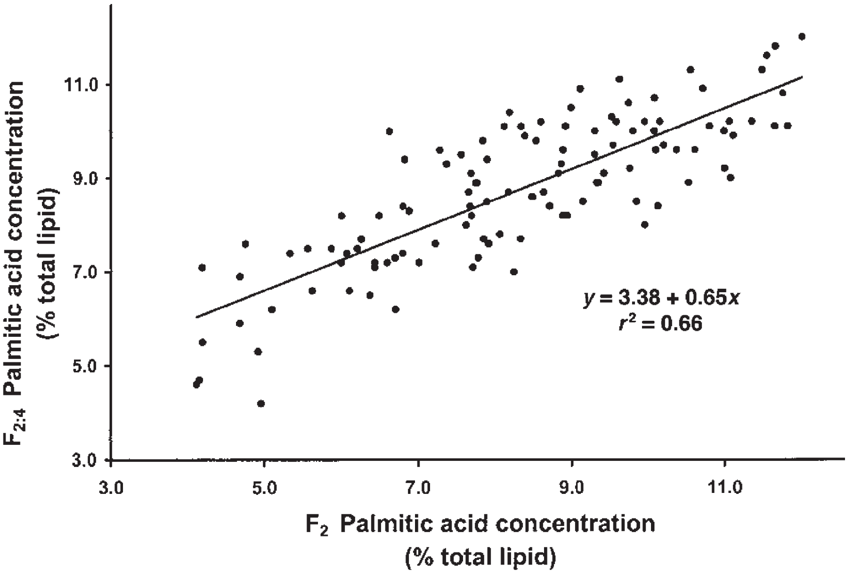

FIG. 1. Parent–offspring regression of F_2 single-plant palmitic acid (16:0) concentration vs. $F_{2:4}$ progeny row 16:0 concentration. Slope represents an estimate of heritability for the 16:0 concentration.

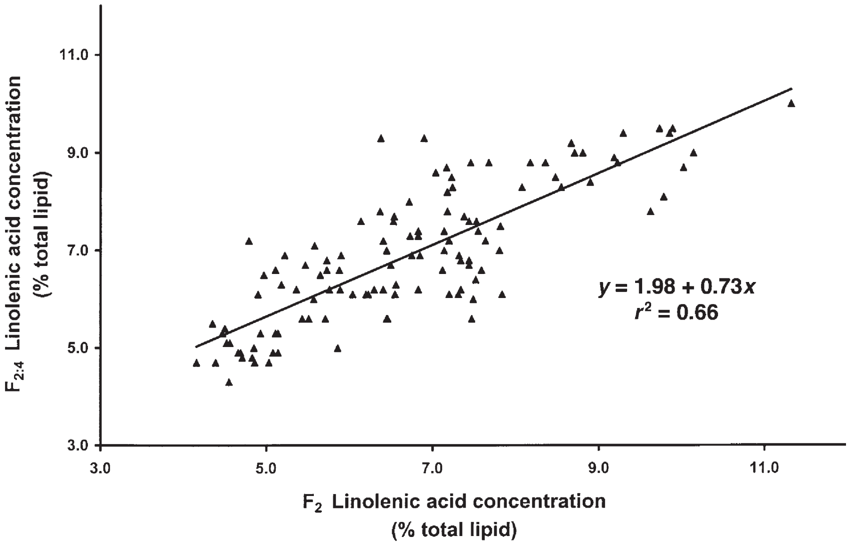

FIG. 2. Parent–offspring regression of F_2 single-plant linolenic acid (18:3) concentration vs. $F_{2:4}$ progeny row 18:3 concentration. Slope represents an estimate of heritability for the 18:3 concentration.

seed protein and oil concentrations. Newly created lines with stable, high-yielding capacity and suitability for food processing applications would benefit the oil processing industry.

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