# Relationship Between Seed Mass and Linolenic Acid in Progeny of Crosses Between Cultivated and Wild Soybean

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**ABSTRACT:** Soybean [*Glycine max* (L.) Merr.] oil from current commercial cultivars typically contains *ca.* 8% linolenic acid (18:3). Applications of plant biotechnology have enabled plant breeders to develop germplasm having as low as 2.0% 18:3. Oils that are naturally low in 18:3 exhibited improved flavor characteristics and greater oxidative stability in high-temperature frying applications compared to hydrogenated soybean oil. As an extension of that research, efforts are underway to characterize genes in soybean that govern expression of higher than normal 18:3 concentration. Such oils may be of interest to the oleochemicals industry for various nonfood applications. Relatively high 18:3 in seed oil is a characteristic trait of the ancestor of modern soybean cultivars, *Glycine soja* (Sieb. and Zucc.). Accessions of this species have rarely been utilized in soybean improvement, and thus represent a virtually untapped genetic resource for genes governing 18:3 synthesis. We have hybridized cultivated soybean with wild soybean plant introductions. $F_{3:4}$ seed from the resultant *G. max* × *G. soja* populations exhibited a wide segregation pattern for 18:3 and seed mass. A strong negative association was found between 18:3 concentration and seed mass. Oil concentration was positively correlated with seed mass. Evaluation of glycerolipid composition revealed that high 18:3 was not associated with an altered proportion of phospholipid and triacylglycerol among lines segregating for seed mass. Thus, smaller seed mass may be a convenient trait to distinguish future soybean cultivars with highly polyunsaturated oils from other cultivars in production.
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The oil of current soybean [*Glycine max* (L.) Merr.] cultivars typically contains 8% 18:3 (1,2). Oxidation of 18:3 during high-temperature frying applications leads to the formation of undesirable odors and flavors that effectively shorten the useful life of refined, bleached, deodorized (RBD) soybean oil (3,4). Although oxidative stability of soybean oil may be improved through catalytic hydrogenation, biotechnological approaches have been used to produce more stable, high-quality RBD soybean oils (5). The initial biotechnological research on development of soybean oils that contain naturally low 18:3 concentration demonstrated that low 18:3 could be achieved by different breeding methods. At Raleigh, NC, recurrent selection was used to develop the soybean germplasms, N78-2245, N87-2120-3 and N87-2122-4, which contained *ca.* 4.2% 18:3 (6,7). Chemical mutagenesis with ethyl methanesulfonate (EMS) was conducted by Hammond and Fehr (8) to develop A5, a germplasm that contained 3.3 to 4.2% 18:3. EMS also was used by Wilcox and Cavins (9) to develop C1640, a germplasm that exhibited *ca.* 3.5% 18:3. This germplasm played a fundamental role in demonstrating that the low 18:3 trait in soybean was determined by two independently segregating recessive alleles (1). After gene recombination, these homozygous recessive alleles were recovered in the soybean germplasms, N85-2176 and BARC-12, which contained 2.0 to 3.5% 18:3 (10,11).

While the development of commercial soybean cultivars with naturally low 18:3 concentration remains a high priority, knowledge of the genetic mechanisms that govern this trait led to the hypothesis that a different complement of genes may condition expression of high levels of 18:3 in the wild ancestor of soybean. Oil from *Glycine soja* Sieb and Zucc. accessions in the United States Department of Agriculture (USDA) Wild Soybean Collection may contain as much as 23% 18:3 plus 53% linoleic acid (18:2) (12). Thus, transfer of genes to cultivated *G. max* germplasm could establish the foundation for development of highly polyunsaturated soybean oils that have application in the manufacture of lubricants and drying oils. Rahman *et al.* (13) have suggested that high-18:3 soybean oils may also be nutritionally beneficial in animal feeds.

Hybridization between wild and cultivated soybean germplasm gave populations in which progeny exhibited transgressive segregation for 18:3 (14). This finding indicated that separate genes from each parent plant were recombined and acted, possibly in an additive genetic manner, to effect higher and lower 18:3 concentrations than either parent. Work

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with these populations has been continued to characterize the genetic basis for this inheritance phenomenon. In that regard, another trait, smaller seed size, appeared to be associated with higher 18:3 concentration. A negative correlation between soybean seed size and 18:3 concentration was recently reported by Liu *et al*. (15). Following that observation, we analyzed the glycerolipid composition of inbred *G. max* × *G. soja* lines that varied in seed size, to determine the utility of that trait in the development of suitable cultivars for commercial production of highly polyunsaturated soybean oils.

## MATERIALS AND METHODS

Interspecific crosses were made between *G. max* genotypes (N87-2120-3 or N87-2122-4) and *G. soja* genotypes (PI-342434, PI-424031, or PI-468910). Both *G. max* genotypes exhibited alleles that determined expression of low 18:3 (1,7). *Glycine soja* plant introductions were chosen from the USDA wild soybean germplasm collection on the basis of high 18:3 concentration (16). Three populations of $F_{2:3}$ plants were formed from the hybridizations N87-2120-3 × PI-342434 (population I), N87-2122-4 × PI-424031 (population II), and N87-2122-4 × PI-468910 (population III). Within each population, genotypes segregated for both 18:3 concentration and seed mass. $F_{3:4}$ progeny was characterized by sorting seed into size classes where class means were two-standard deviations apart. The range of these classes was between 3.0 to 14 g dry mass per 100 seed. For this investigation, seed of one genotype was selected for analysis from each size class, when available, from each population. Populations I and II produced individuals with seed masses that represented four size classes, and population III produced individuals with seed masses representing five size classes. Individuals were chosen for this experiment by using the random procedure of SAS (17) with a new randomization initiation value for each class within a population, to ensure representative sampling. All statistical analyses were performed with the analysis of variance, correlation, and regression procedures of SAS (17).

Oil concentration in dried seed was determined by nuclear magnetic resonance (NMR) (1). Oil was extracted from crushed seed with a mixture of chloroform/hexane/methanol (8:5:2, vol/vol/vol). Phospholipid (PL), diacylglycerol (DAG), and triacylglycerol (TAG) were fractionated from total lipid extracts by thin-layer chromatography (TLC) in petroleum-ether/diethyl-ether/acetic-acid (80:20:1, vol/vol/vol). Fatty acid methyl esters were prepared from all glycerolipid fractions by transmethylation with sodium methoxide (1). Fatty acid composition was determined by gas chromatography with a Hewlett-Packard 5890-II equipped with a model 7673 auto sampler, dual flame-ionization detectors, and a 0.53 mm × 30 m AT-Silar capillary column (Alltech Associates, Inc., Deerfield, IL). Operating conditions were: carrier, He (3 mL/min); 25:1 split injection; injection temperature, 250°C; detector temperature, 275°C; column temperature, 190°C. All data were reported as means of three replications.

## RESULTS AND DISCUSSION

Fatty acid compositions of *G. max* and *G. soja* germplasm, used as parents of the interspecific populations, are shown in Table 1. These germplasms were distinguished by species-dependent differences in relative 18:1-desaturation (18:1-D) and 18:2-desaturation (18:2-D) values, which indicate the genetic basis for the observed 18:3 concentration in each genotype. In *G. max* lines, low 18:1-D and 18:2-D values previously have been associated with recessive alleles that encode the respective desaturases, whereas high values have been associated with dominant alleles (1). Parental *G. max* lines, N87-2120-3 and N87-2122-4, both exhibited low 18:3 phenotypes, resulting from the expression of the two different recessive desaturation genes (7). Although little is known about genetic regulation of 18:3 concentration in *G. soja* germplasm, the relative estimates of 18:1- and 18:2-desaturation in the plant introductions used in this study are greater that those of common soybean cultivars. In comparison to *G. max*, this suggests a different complement or perhaps additional copies of genes that determine 18:3 concentration. Thus, intermating *G. max* and *G. soja* ensured a wide range of segregation for 18:3 among the progeny in the populations used in this investigation.

As described previously (14), crosses among wild and cultivated soybean genotypes produced fertile progeny. Segregation for 18:3 concentration was apparent in the oil of $F_{3:4}$ seed from all three populations. Across populations, 18:3 concentration ranged between *ca.* 5 and 14% of the total lipid fatty acid composition. Successful transfer of genes between species also was evident in the segregation pattern for growth and morphological characteristics among

**TABLE 1**
**Fatty Acid Composition (% total lipid) of Parental Germplasm Used to Develop *Glycine max* × *Glycine soja* Populations**

| Species | Line | 16:0 | 18:0 | 18:1 | 18:2 | 18:3 | 18:1-D[a] | 18:2-D[a] |
|---|---|---|---|---|---|---|---|---|
| *G. max* | N87-2120-3 | 6.6 | 4.0 | 29.7 | 56.2 | 3.5 | 66.8 | 5.9 |
| *G. max* | N87-2122-4 | 5.6 | 3.6 | 45.8 | 40.6 | 4.4 | 49.6 | 9.8 |
| *G. soja* | PI-342434 | 11.6 | 3.6 | 19.1 | 54.7 | 11.0 | 77.5 | 16.8 |
| *G. soja* | PI-424031 | 14.8 | 3.3 | 12.0 | 55.2 | 14.7 | 85.3 | 21.1 |
| *G. soja* | PI-468910 | 12.0 | 3.9 | 25.1 | 48.6 | 10.4 | 70.1 | 17.6 |
| | $LSD_{0.05}$ | 3.1 | 0.3 | 9.1 | 4.5 | 3.9 | 9.6 | 5.2 |

[a]18:1-D = [(18:2 + 18:3)/(18:1 + 18:2 + 18:3) · 100], relative estimate of 18:1-desaturation; 18:2-D = [(18:3)/(18:2 + 18:3) · 100, relative estimate of 18:2-desaturation.

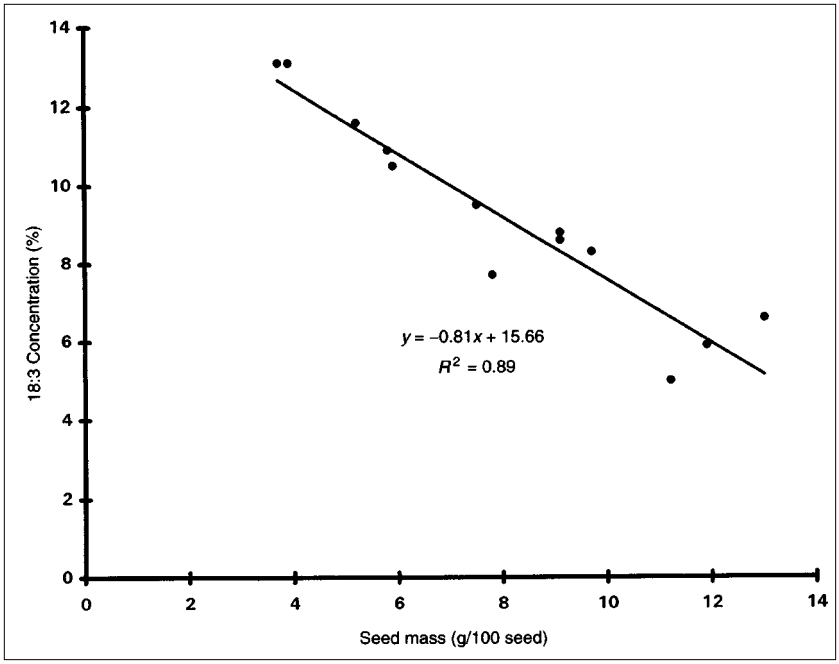


**FIG. 1.** Relationship between seed mass and 18:3 concentration in $F_{3:4}$ seed from random progeny of three *Glycine max* × *Glycine soja* populations.

progeny. In particular, seed mass ranged between 3 and 14 g per 100 seed.

Typically, there is no strong correlation between 18:3 concentration and seed mass in soybean (15). However, in our experiment, where wild soybean was crossed to cultivated soybean, the association was striking. Over all three populations, there was a strong negative correlation ($R^2 = 0.89$) between 18:3 concentration and seed mass (Fig. 1). This negative association remained strong when examined separately by population ($R^2 = 0.97$, 0.97, and 0.78 for populations I, II, and III, respectively). Fatty acid compositions of the genotypes studied in each population are shown in Table 2. These data indicated a possible transgressive segregate for high 18:3 concentration in population I. Line I-116b exhibited 13.1% 18:3, which was greater than the high 18:3 parent of that population (PI-342434). There also was evidence (based on relative estimates of 18:1-D and 18:2-D) for expression of recessive alleles that govern low 18:3 concentration among progeny. Low 18:3 in the line I-14b appeared to be determined by a recessive allele for the 18:2-desaturase. In line III-82a, the low 18:3 trait appeared to be due to recessive allele governing the 18:1-desaturase. Although present in the respective populations, germplasm exhibiting homozygous recessive alleles for both desaturases was not selected in the random sam-

**TABLE 2**
**Analytical Traits of Random Genotypes from an Array of Seed Size Classes of Three *G. max* × *G. soja* Populations**

| Population[a] | Line | 100-seed mass (g) | Oil (%) | 16:0 (%) | 18:0 (%) | 18:1 (%) | 18:2 (%) | 18:3 (%) |
|---|---|---|---|---|---|---|---|---|
| I | 116-b | 3.7 | 13.3 | 12.1 | 3.4 | 14.4 | 57.0 | 13.1 |
| I | 123-a | 5.8 | 16.8 | 7.2 | 3.0 | 19.2 | 59.7 | 10.9 |
| I | 204-a | 9.1 | 18.1 | 7.3 | 3.2 | 24.6 | 56.3 | 8.6 |
| I | 14-b | 11.2 | 18.1 | 10.5 | 4.1 | 21.2 | 59.1 | 5.0 |
| II | 215-c | 3.9 | 12.8 | 13.1 | 3.6 | 16.3 | 54.0 | 13.1 |
| II | 105-a | 5.2 | 17.0 | 12.6 | 3.6 | 14.4 | 57.9 | 11.6 |
| II | 68-b | 7.5 | 16.5 | 7.5 | 3.4 | 24.9 | 54.7 | 9.5 |
| II | 12-b | 9.1 | 18.2 | 7.0 | 3.2 | 23.7 | 57.3 | 8.8 |
| III | 191-a | 5.9 | 16.4 | 8.9 | 3.6 | 22.7 | 54.5 | 10.5 |
| III | 174-b | 7.8 | 21.1 | 7.3 | 4.0 | 23.5 | 57.5 | 7.7 |
| III | 20-b | 9.7 | 19.5 | 8.5 | 3.9 | 19.9 | 59.4 | 8.3 |
| III | 82-a | 11.9 | 18.8 | 5.9 | 3.0 | 46.0 | 39.3 | 5.9 |
| III | 235-a | 13.0 | 18.4 | 6.8 | 3.4 | 41.2 | 42.0 | 6.6 |

[a]Populations I, II, III = N87-2120-2 × PI342434, N87-2122-4 × PI424031, N87-2122-4 × PI468910, respectively.

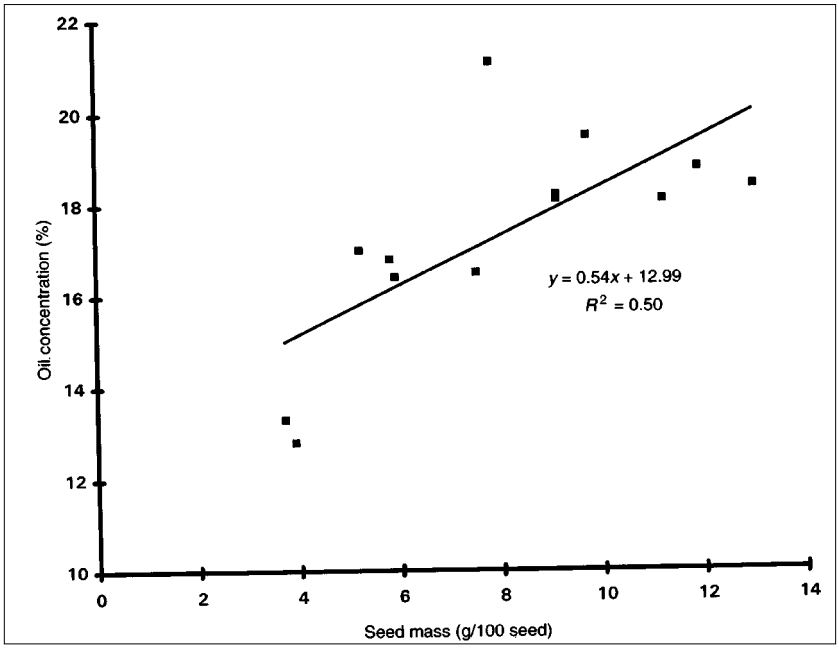


**FIG. 2.** Relationship between seed mass and oil concentration in $F_{3:4}$ seed from random progeny of three *Glycine max* × *Glycine soja* populations.

ples used in this investigation. No high-18:3 phenotype was found in the largest seed class among all progeny of these populations, and no low-18:3 phenotype was found in the smallest seed class. Therefore, the observed negative correlation between 18:3 concentration and seed mass appeared to be valid.

The data suggested the possibility of a genetic linkage between 18:3 concentration and seed mass in *G. max* × *G. soja* populations. There also could be a physiological explanation for this finding, given the impact of environmental conditions upon expression of these traits. Seed mass and 18:3 concentration in soybean usually are correlated negatively with growth temperature during plant development (18–20). However, a combined analysis of variance over environments for these populations revealed that genotypes were consistent in their relative performance for fatty acid traits (21). In addition, no significant correlation was found between seed size and plant maturity in the populations examined.

An atypical relation was also found between oil concentration and seed mass among progeny lines (Fig. 2). Oil concentration among all populations ranged from *ca.* 12.8%, in the smallest seed size class, to *ca.* 18.8% in the largest seed size class. Generally, there is no association between these traits in soybean (15), but, in this case the correlation ($R^2$ = 0.50) was positive. This meant that plants from these populations with small seed mass generally also had low oil concentration.

Although these data support a genetic basis for the association of high 18:3 concentration with small seed mass in *G. max* × *G. soja* populations, low oil concentration could be a function of lower TAG content in relation to polar (membrane) lipids. Because membrane lipids often are rich in polyunsaturated fatty acids (22), such a condition arbitrarily could give rise to higher 18:3 concentration in seed oil. In this case, however, TAG accounted for approximately 90% or more of total glycerolipids in all genotypes, regardless of seed mass or oil concentration (Table 3). The distribution of 18:3 among glycerolipid classes also confirmed that the 18:3 concentration of TAG was representative of that in crude oil extracts. Nearly all 18:3 was found in the TAG fraction, resulting in a strong positive correlation ($r$ = 0.95) between 18:3 concentration in oil of crushed seed and 18:3 concentration in the TAG fraction of that oil.

In conclusion, the strong negative association between seed mass and 18:3 concentration in these interspecific lines has practical ramifications to soybean breeding. Seed size is a highly heritable trait. Heritability estimates for this trait often exceed 90% (23). This condition effectively reduces the number of generations required to select and develop inbred lines. Other research at Raleigh, NC, has shown that small-seeded soybean cultivars may be developed with high yield potential. The application of that technology is evidenced by the release of a new small-seeded soybean cultivar, Pearl, which is used in the manufacture of soy food products such as "natto" (24). Pearl has a characteristic seed mass of 8.4 g per 100 seed. Unique genes for high 18:3 from *G. soja* plant introductions could be backcrossed to Pearl or other small-seeded soybean cultivars. This approach could expedite development of commercial soybean cultivars that produce oils with more than 70% polyunsaturated fatty acid concentration.

**TABLE 3**
**Relative Proportion and Concentration of 18:3 in Phospholipid, Diacylglycerol (DAG), and Triacylglycerol (TAG) Oil Fractions[a]**

| | | | 18:3 Relative proportion (%) | | | 18:3 Concentration (%) | | |
|---|---|---|---|---|---|---|---|---|
| Population[b] | Line | 100-seed mass (g) | PL | DAG | TAG | PL | DAG | TAG |
| I | 116-b | 3.7 | 5.2 | 0.6 | 93.7 | 10.1 | 10.7 | 12.4 |
| I | 123-a | 5.8 | 4.8 | 0.6 | 93.0 | 9.6 | 10.1 | 11.6 |
| I | 204-a | 9.1 | 4.6 | 0.6 | 95.3 | 6.6 | 6.8 | 8.5 |
| I | 14-b | 11.2 | 4.9 | 0.8 | 93.8 | 4.9 | 5.6 | 4.7 |
| II | 215-c | 3.9 | 8.0 | 1.4 | 88.8 | 9.4 | 10.7 | 13.6 |
| II | 105-a | 5.2 | 7.2 | 1.0 | 90.0 | 15.2 | 14.5 | 12.0 |
| II | 68-b | 7.5 | 7.0 | 2.0 | 91.0 | 7.1 | 9.5 | 9.8 |
| II | 12-b | 9.1 | 3.8 | 1.6 | 92.5 | 7.0 | 9.0 | 9.2 |
| III | 191-a | 5.9 | 5.3 | 2.0 | 92.1 | 8.4 | 15.6 | 11.3 |
| III | 174-b | 7.8 | 3.8 | 2.1 | 94.1 | 11.9 | 12.7 | 9.0 |
| III | 20-b | 9.7 | 6.0 | 1.5 | 92.4 | 6.4 | 9.1 | 8.3 |
| III | 82-a | 11.9 | 6.2 | 1.2 | 91.5 | 7.6 | 6.1 | 6.3 |
| III | 235-a | 13.0 | 5.8 | 1.5 | 92.7 | 7.3 | 10.9 | 6.9 |

[a]From random genotypes from an array of seed size classes of three *G. max* × *G. soja* populations.
[b]Population I, II, III = N87-2120-3 × PI342434, N87-2122-4 × PI424031, N87-2122-4 × PI468910, respectively.

Such oils should be of interest to the oleochemicals industry, and the associated small seed trait would provide a convenient means to preserve the identity of these specialized soybeans throughout production and processing.

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