Association of Seed Size with Genotypic Variation in the Chemical Constituents of Soybeans[1]

KeShun Liu[a,*] Frank Orthoefer[b] and Edward A. Brown[a]

[a]Jacob Hartz Seed Co., Inc. and [b]Riceland Foods, Inc., Stuttgart, Arkansas 72160

ABSTRACT: Ten soybean genotypes grown in 1992 with seed size ranging from 7.6 to 30.3 g/100 seeds and maturity group V or VI were selected and tested for oil and protein content and for fatty acid composition. In these germplasm, protein varied from 39.5 to 50.2%, oil, 16.3 to 21.6%, and protein plus oil, 59.7 to 67.5%. Percentages of individual fatty acids relative to total fatty acids varied as follows: palmitic, 11.0 to 12.8; stearic, 3.2 to 4.7; oleic, 17.6 to 24.2; linoleic, 51.1 to 56.3 and linolenic, 6.9 to 10.0. Seed size showed no significant correlations with individual saturated fatty acids, protein or oil content. However, significant correlations were found between seed size and individual unsaturated fatty acids: positive with oleic, and negative with linoleic and linolenic. Oil and protein content were negatively correlated with each other. Among the major fatty acids, only the unsaturated were significantly correlated with each other: negative between oleic and linoleic or linolenic, and positive between linoleic and linolenic. A subsequent study with soybeans grown in 1993 generally confirmed these findings. Variation in relative percentages of unsaturated fatty acids and *r* values for most pairs of relationships were even higher than those obtained from the 1992 crop.
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KEY WORDS: Fatty acid composition, protein and oil, seed size, soybeans.

There are three major factors affecting soybean quality. These are: i) protein and oil content, ii) the chemical components of protein and oil, and iii) seed appearance. The protein and oil content is important because of its economical and nutritional values. The amino acid composition of protein and fatty acid profile of oil are important because of their effects on not only the physicochemical properties but also nutritive values of protein and oil. In terms of fatty acid composition, studies have shown that a high-linolenic content in a food system is responsible for the oxidative rancidity and loss of flavor stability commonly encountered during preparation and storage (1), whereas a high saturated fat intake is correlated to elevated serum cholesterol, the effect leading to coronary heart disease (2). Seed appearance, such as seed size and hilum color, is important because of its influence on consumer's acceptance and food-product application.

Developing soybean varieties with superior chemical composition to meet special food applications has become a high research priority. During selection of soybeans for a particular food application or a particular seed breeding program, it is important to know whether relationships exist among these quality attributes. However, there are limited reports on the relationship between soybean seed size and oil or protein content (3), and fewer reports on relationships among individual fatty acids (4,5). Virtually no information has been published on the relationship between soybean seed size and fatty acid composition of the seed. Thus, the objectives of this study were to define potential relationships between seed size and individual fatty acid concentrations, between seed size and oil or protein content, and among fatty acids in a diverse set of soybean genotypes.

MATERIALS AND METHODS

Materials. Ten soybean genotypes, with seed size varying from 7.6 to 30.3 g/100 seeds, and maturity group of V or VI, were selected. All the genotypes were grown at the Agricultural Research Center of Jacob Hartz Seed Co. (Stuttgart, AR). Seeds were harvested at full maturity when seed moisture was reduced to 14% or less. To eliminate potential genetic contamination, seeds of the 1992 crop were planted in 1993 for a replicate study.

Proximate analysis. Crude oil content was determined by a Soxhlet extraction by the Association of Official Analytical Chemists' (AOAC) method 14.066 (6). Protein content was analyzed by an automated Kjeldahl equipment (Kjeltec Auto 1030 Analyzer; Tecator, Hoganas, Sweden) according to the AOAC method 14.067 (6). Oil or protein content was expressed on a dry weight basis. A conversion factor of 6.25 was used for protein computation. The moisture content of

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*To whom correspondence should be addressed at Jacob Hartz Seed Co. Inc., 901 N. Park Ave., Stuttgart, AR 72160.

seed samples was also determined by the AOAC method 14.004 (6). Duplicate analyses of chemical attributes were performed for each seed sample.

Analysis of fatty acid composition. The procedure described by Dahmer *et al.* (7) was adopted with some modifications. Seeds (11.5% moisture) were ground with a coffee mill. Fatty acid methyl esters (FAMEs) were prepared by direct transmethylation without extraction of oil from seeds. A Hewlett-Packard 5890 gas–liquid chromatograph fitted with an automatic sampler 7673A, flame-ionization detector (FID) detector, and ChemStation HP 3365 was employed for FAME analysis (Palo Alto, CA). A Supelco 0.53 mm i.d. fused silica capillary column, SUPELCOWAX™ 10 (Supelco Inc., Bellefonte, PA), was used with helium as the carrier gas. The temperature was increased from 170 to 210°C at 12°C/min. It was then raised to 240°C at 4°C/min and held at 240°C for 5.2 min. The column head pressure was 50 psi. Air pressure was 60 psi, and hydrogen was 50 psi. The injection volume was 1 μL. The percentage of an individual fatty acid relative to total fatty acids in seed lipids was expressed as area percent of the total peak area in each sample.

Statistical treatment. Correlation coefficients (r) were calculated between each of two variables tested. The population was modeled as a bivariate normal distribution, and the Student's *t*-test was performed for each *r* value, with degrees of freedom = n-2.

RESULTS

Analytical data for soybeans grown in 1992 are shown in Table 1. The correlation coefficients between tested chemical parameters are presented in Table 2.

Among the ten genotypes selected, protein content ranged from 39.5 to 50.2%, and oil content from 16.3 to 21.6%. Protein plus oil content varied from 59.7 to 67.5%. There were no significant correlations between seed size and oil content and between protein content. There was a positive correlation between seed size and the total content of protein and oil with $r = 0.375$ but it was not significant at $P < 0.05$ level.

The relative percentage of individual fatty acids changed from 11.0 to 12.8 for palmitic acid (C16:0), 3.2 to 4.7 for stearic acid (C18:0), 17.6 to 24.2 for oleic acid (C18:1), 51.1 to 56.3 for linoleic acid (C18:2) and 6.9 to 10.0 for linolenic acid (C18:3). Within the ten genotypes selected, the seed size showed no significant correlation with each of two saturated fatty acids (C16:0 and C18:0), but it had significant, positive correlation with C18:1 ($r = 0.808$) and negative correlations with both C18:2 ($r = -0.796$) and C18:3 ($r = -0.603$). The larger the seed size, the higher the relative percentage of oleic acid in seed oil, and the lower the relative percentage of the linoleic and linolenic acids. When the total percentage of unsaturated fatty acids was considered, no significant correlation with seed size was found.

The correlation coefficient between oil and protein content in soybeans of the 92 crop was −0.421. This indicates that the two parameters were negatively correlated with each other but not significant at $P < 0.05$ within the ten soybean genotypes selected.

Among the five major fatty acids in soybeans, there were ten pairs of relationships. The majority showed little significant correlation. However, within the individual unsaturated fatty acids, significant correlations existed. More specifically, oleic acid correlated negatively with both linoleic and linolenic acids, with *r* values of −0.806 and −0.815, respectively, whereas linoleic acid correlated positively with linolenic acid, with an *r* value of 0.407.

The repetition study with the same soybean genotypes grown in 1993 (Tables 3 and 4) generally agreed with the findings from the initial study with the 1992 crop. However, the subsequent study exhibited a greater variation in relative percentages of unsaturated fatty acids, with oleic ranging from 16.4 to 27.3, linoleic, 49.5 to 57.1, and linolenic, 5.7 to 9.4. Furthermore, the correlations between seed size and each of the unsaturated fatty acids became stronger as indicated by

TABLE 1
Seed Size, Protein and Oil Content and Relative Percentages of Major Fatty Acids in Ten Soybean Genotypes Grown in 1992[a]

Genotype line	Maturity group	Seed size (g/100 sd)	Protein (%)	Oil (%)	Protein and oil	Major fatty acids (relative %) C16:0	C18:0	C18:1	C18:2	C18:3	Total unsaturated
H91-27343	VI	7.6	42.2	17.6	59.8	11.9	4.3	18.1	55.1	10.0	83.2
HX608	VI	8.6	42.0	17.7	59.7	12.2	4.3	17.6	56.3	8.8	82.7
HX5403	V	8.8	41.4	18.6	60.0	12.0	4.7	19.2	55.3	8.0	82.5
HX307	VI	9.3	39.5	20.4	59.9	11.8	4.0	22.5	53.1	7.8	83.4
H79-17006	VI	16.8	42.1	19.1	61.2	12.2	3.9	20.7	53.6	8.8	83.1
HX4501	VI	17.5	50.2	17.3	67.5	11.5	4.3	22.1	54.2	7.1	83.4
HX5149	V	19.2	42.4	21.6	64.0	11.6	3.2	22.8	54.8	6.9	84.5
HX6397	VI	21.1	43.7	19.2	62.9	12.8	4.0	21.6	52.6	8.1	82.3
HX1039	VI	23.0	44.1	19.4	63.5	11.0	4.2	24.2	52.8	7.1	84.1
H90-149	VI	30.3	43.8	16.3	60.1	12.4	4.3	24.1	51.1	7.4	82.6
Minimum		7.6	39.5	16.3	59.7	11.0	3.2	17.6	51.1	6.9	82.3
Maximum		30.3	50.2	21.6	67.5	12.8	4.7	24.2	56.3	10.0	84.5
Average		16.2	43.1	18.7	61.9	11.9	4.1	21.3	53.9	8.0	83.2
SD		7.2	2.7	1.5	2.5	0.5	0.4	2.2	1.5	0.9	0.7

[a]Means of duplicate measurements.

TABLE 2
Correlation Coefficients Between Quality Factors in Ten Soybean Genotypes Grown in 1992

Factors	Protein	Oil	Protein + oil	C16:0	C18:0	C18:1	C18:2	C18:3	Total unsaturated
Seed size	0.421	-0.130	0.375	0.044	-0.032	0.808[a]	-0.796[a]	-0.603[b]	0.105
Oil	-0.421								
C16:0				0 095	-0.239	-0.158	0.404		
C18:0					-0.362	0.077	0.293		
C18:1						-0.806[a]	0.815[a]		
C18:2							0 407		

[a]These numbers correspond correlations which are significant at $P < 0.01$.
[b]These numbers correspond correlations which are significant at $P < 0.05$.

TABLE 3
Seed Size, Protein and Oil Content and Relative Percentages of Major Fatty Acids in Ten Soybean Genotypes Grown in 1993[a]

Genotype line	Maturity group	Seed size (g/100 sd)	Protein (%)	Oil (%)	Protein and oil	Major fatty acids (relative %) C16:0	C18:0	C18:1	C18:2	C18:3	Total unsaturated
H91-27343	VI	7.6	43.7	17.6	61.3	12.0	4.4	16.4	57.1	9.3	82.8
HX608	VI	8.6	42.8	16.4	59.2	11.8	4.3	18.6	55.1	9.4	83.1
HX5403	V	8.8	42.1	18.0	60.1	12.7	4.7	18.8	55.0	8.0	81.8
HX307	VI	9.3	40.0	19.9	59.9	12.3	4.5	18.7	55.5	8.3	82.5
H79-17006	VI	16.8	41.2	19.9	61.1	12.3	3.8	22.3	53.2	7.6	83.1
HX4501	VI	17.5	50.5	17.0	67.5	11.8	4.5	24.7	53.1	5.8	83.6
HX5149	V	19.2	42.5	20.7	63.2	12.7	3.9	25.0	51.8	5.7	82.5
HX6397	VI	21.1	42.9	19.5	62.4	12.8	4.2	23.5	52.6	6.6	82.7
HX1039	VI	23.0	45.3	17.8	63.1	11.6	4.9	27.3	49.5	6.3	83.1
H90-149	VI	30.3	43.2	19.9	63.1	12.5	4.7	25.5	50.1	6.8	82.4
Minimum		7.6	40.0	16.4	59.2	11.6	3.8	16.4	49.5	5.7	81.8
Maximum		30.3	50.5	20.7	67.5	12.8	4.9	27.3	57.1	9.4	83.6
Average		16.2	43.4	18.7	62.1	12.3	4.4	22.1	53.3	7.4	82.8
SD		7.2	2.7	1.4	2.3	0.4	0.3	3.5	2.3	1.3	0.5

[a]Means of duplicate measurements.

TABLE 4
Correlation Coefficients Between Quality Factors in Ten Soybean Genotypes Grown in 1993

Factors	Protein	Oil	Protein + oil	C16:0	C18:0	C18:1	C18:2	C18:3	Total unsaturated
Seed size	0.243	0.427	0.558	0.55	0.118	0.898[a]	–0.930[a]	–0.751[a]	0.100
Oil	–0.554								
C16:0					–0.349	–0.010	–0.010	–0.184	
C18:0						0.089	–0.179	0.010	
C18:1							–0.962[a]	–0.907[a]	
C18:2								0.791[a]	

[a]Numbers correspond correlations which are significant at $P < 0.01$.

higher r values of the 1993 crop. The r values for relationships among three unsaturated fatty acids, including the correlation between C18:2 and C18:3, were also higher, all being significant at the 99% confidence interval. In addition, the negative relationship between protein and oil and the positive relationship between seed size and the total content of protein and oil was more distinctive in the study with the 1993 crop than with the 1992 crop.

DISCUSSION

The relationships between seed size and individual fatty acids have been studied with other plant species. Mozingo *et al.* (8) reported that an increase in the seed size of five selected peanut cultivars resulted in a significant increase in the percentage of stearic, oleic, and arachidic acids and a significant decrease in the percentage of palmitic, linoleic, eicosenoic, behenic and lignoceric acids. Izzo *et al.* (9) studied the variation of the oil composition of ten rapeseed cultivars in relation to seed size and found that oleic acid was highest in seeds of diameters of 1.5–2.0 mm, linoleic, palmitic and stearic acids were always highest in seeds of diameters less than 1.5 mm, and linolenic, eicosenoic and erucic acids were highest in the seeds larger than 2.0 mm. A negative correlation between size and stearic acid and positive correlation between linolenic acid were also reported in linseeds (10). In this study with soybeans, however, we found different relationships between seed size and individual fatty acids. Regarding the relationships among individual unsaturated fatty acids, our finding agrees with that of previous studies (4,5).

As shown in Figure 1, the fatty acids in soybeans and some other plant species are synthesized in plastids up to oleoyl-CoA. Oleoyl-CoA is then transported out of plastids, undergoing a variety of fates, such as further desaturation, elongation, etc. (11). In other words, oleoyl-CoA is an intermediate and the polyunsaturated fatty acids, C18:2 and C18:3, are produced by the consecutive desaturation of C18:1. Based on observations of the present and previous studies (4,5), it is possible that the biological steps affected by natural selection be primarily at the level of linoleic and linolenic biosynthesis. It is also possible that genes encoding enzymes responsible for desaturation of oleoyl–phosphatidylcholine (PC) to linoleyl–PC and further to linoenoyl-PC be related not only to each other but also to genes controlling seed size. We believe that knowing these relationships is of importance for an efficient soybean breeding program, as in selecting for one trait, other traits may also be changed. Understanding these relationships is also important to food scientists and nutritionists who may select beans with a unique seed size for a particular food application.

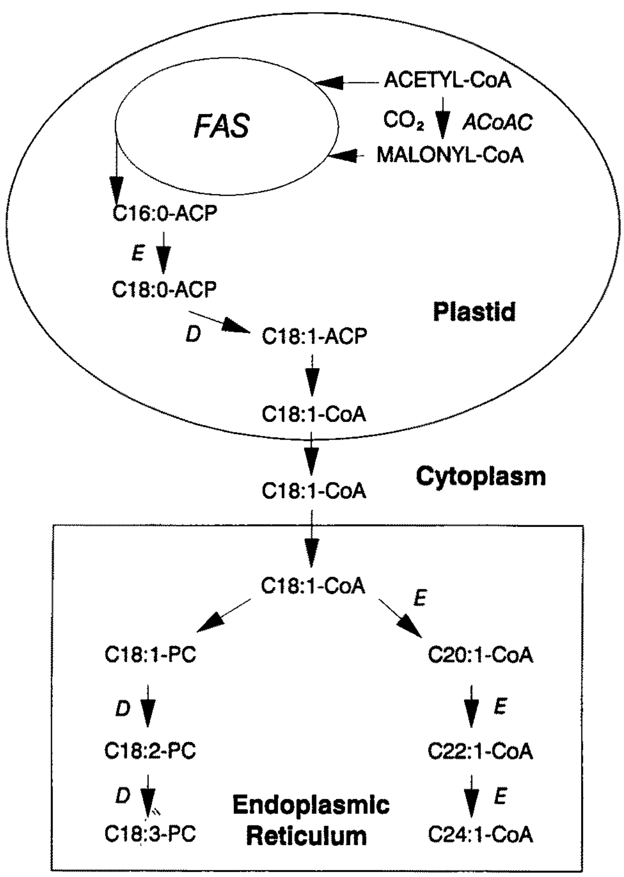

FIG. 1. Biosynthesis of fatty acids in plants. FAS, fatty acid synthetase; ACP, acyl carrier protein; PC, phosphatidylcholine; D, desaturase; E, elongase; and ACoAC, acetyl-CoA carboxylase.

Burton (12), in an excellent review article, pointed out that with few exceptions, correlations between protein and oil in breeding populations of soybeans have been negative with absolute r values greater than 0.50. Our data with the 1992 and 1993 crops showed r values close to this figure, although a slight variation existed between the two year studies. In terms of the relationship between seed size and oil or protein content, a general presumption has been that the larger the seeds, the higher the oil and protein content. Although this may be true with cottonseed, based on a study by Abbel-Rahman and Youssef (13), it is not true with soybean seeds, as the present study showed that there was no correlation between seed size and oil or protein content.

In summary, we conclude from this study with ten selected soybean genotypes grown in two years: i) changes in seed size were related to changes in individual unsaturated fatty acids; the larger the seeds, the lower the relative percentage of oleic acid in seed oil, and the higher the relative percentages of linoleic and linolenic acids, ii) seed size had no significant correlation with the percentage of saturated fatty acids and with protein or oil content, and iii) among the five major fatty acids, the unsaturated fatty acids correlated significantly with each other; the oleic acid correlated negatively with each of two polyunsaturated fatty acids and linoleic acid correlated positively with linolenic acid.

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