Fatty Acid Composition of Oil from Soybean Seeds Grown at Extreme Temperatures

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Temperature during seed development is known to influence the level of the various fatty acids in soybean [*Glycine max* (L.) Merr.] oil. In order to determine the range of values that can be obtained for each fatty acid, five lines (A5, C1640, N78-2245, PI 123440 and PI 361088B) known to possess low linolenic acid (18:3) levels, one line (A6) known to possess a high stearic acid (18:0) level, and two cultivars (Century and Maple Arrow) were grown at 40/30, 28/22, and 15/12°C day/night. At 40/30°C, high oleic acid (18:1), low linoleic acid (18:2), and low linolenic acid levels were obtained that were beyond the range of levels reported for the soybean germplasm. The linolenic acid levels for A5, C1640 and N78-2245 grown at 40/30°C were below 2.0%, and are the lowest values reported for soybean oil. A6 displayed a high level of stearic acid at 28/22 and 40/30°C but displayed a relatively low level at 15/12°C. This indicates that temperature may affect the expression of the *fas*^{a} allele, which is responsible for high stearic acid levels in A6. The linolenic acid levels of PI 361088B and C1640, both possessing the *fan* allele, were the lowest for all lines grown at 15/12°C. Therefore, the *fan* allele is an appropriate source for the development of low linolenic acid lines adapted to cool areas.

Oil from the seeds of field-grown soybean cultivars is characterized by linolenic acid levels that range from below 6% to almost 14% (1). These levels of linolenic acid have been associated with poor flavor stability in the extracted oil (2). In order to increase flavor stability, several research groups have developed, or isolated, soybean lines with reduced levels of linolenic acid. The low linolenic acid lines A5 (3) and C1640 (4) were produced by chemical mutagenesis; PI 123440 and PI 361088B are naturally occurring lines with low linolenic acid levels; N78-2245 was produced by recurrent selection for high oleic acid levels (5). The genetic basis for low linolenic acid levels in these lines differ: in C1640 (4) and PI 361088B (6) a single allele at the *Fan* locus is involved; in A5 (7) and N78-2245 (5) alleles at many loci are involved; the basis for low levels in PI 123440 has not yet been established. Although the linolenic acid levels in these lines are controlled by a diverse set of factors, each line is characterized by linolenic acid levels that range from 2.5-4.6%, depending upon growing conditions (3-9). Researchers have not been able to develop a line with a linolenic acid level below 2.5%, and this has caused some researchers to consider that this may be the minimum level of linolenic acid that can be achieved.

The relative levels of the fatty acids in a soybean seed can be influenced substantially by the temperatures to which the developing seeds are exposed. Cool temperatures are associated with: lower levels of palmitic acid (10), and oleic acid (10); and higher levels of linoleic acid (10,11) and linolenic acid (10,11). The range of levels within a single line exposed to a variety of different temperatures (11) may be as great as the range in diverse lines grown at a single temperature (1). Therefore, growing at both a very high and a very low temperature, lines that possess a wide range of levels for various fatty acids should generate maximum diversity of fatty acid levels.

This research was conducted to determine if an extremely high temperature would generate unusually low linolenic acid levels, and if so, what effect would this have on the levels of other fatty acids. In addition, low linolenic acid lines were exposed to an extremely low temperature to determine if there was a differential response of these lines to cool conditions.

EXPERIMENTAL PROCEDURES

Seed from five soybean lines (A5, C1640, N78-2245, PI 123440 and PI 361088B) with low linolenic acid levels, one line (A6) with a high stearic acid level, and two commercial cultivars (Century and Maple Arrow) was used. One seed of each of these lines was planted in each of three 20-cm plastic pots filled with pre-mixed soil (Metromix 245, W.R. Grace & Co., Ajax, Ontario) in a controlled environment cabinet or growthroom facility. Commercial granular inoculant (Nitragin Co., Milwaukee, WI) was added to each pot to ensure adequate nodulation.

Three experiments were run, each at a different temperature during seed development and maturation: 40/30, 28/22 or 15/12°C day/night. The 40/30°C experiment, with 12 hr daylength, was applied throughout the growth of the plants. The 15/12°C experiment was applied postflowering and continued throughout seed development and maturation. For this experiment, the pots were placed in the cabinet at 30/25°C, 12 hr daylength, until the oldest four pods on each plant had reached a length of one cm. At that time, the temperature was reduced 5°C per day until a temperature of 15/12°C was obtained. A third experiment at 28/22°C, with 16 hr of daylength, was included as a reference, as these are the standard conditions used in our indoor research program.

The three experiments were each in a randomized complete block design with the eight different genotypes as treatments. There were three blocks, each containing one plant of all genotypes for each treatment. The blocks consisted of three equal divisions of the growth cabinet, and the genotypes were randomized within each block. Five seeds were analyzed for each plant, and the mean of the five seeds was used as the value for each plant.

The seeds were harvested at maturity and were dried at 65°C for 24 hr prior to being ground to a fine powder with a mortar and pestle. The oil was extracted from these tissues, methyl esters were prepared, and the fatty acid compositions were determined by means of gas chromatography (GC) as described by Rennie *et al.* (6). The standards used for identification of the fatty acid peaks

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were D102 and D103 from Serdary Research Laboratories Inc. (London, Ontario).

RESULTS AND DISCUSSION

At all temperatures, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) were present in the oil from all lines at a level greater than 1.0%. In addition, the level of arachidic acid (20:0) in A6 at 40/30 and 28/22°C was greater than 1.0%.

At 28/22°C (Table 1), the fatty acid composition of each of the lines was generally consistent with the range of levels reported for each line in the literature (3-9,12,13). This is the first report of all five low linolenic acid lines grown under a standard set of conditions to allow comparisons. Although there were numeric differences, the linolenic acid levels of A5, C1640, N78-2245, PI 123440 and PI 361088B were not significantly different.

TABLE 1

Fatty Acid Composition by Relative Area Percent of the Seeds of Eight Soybean Lines Grown at 28/22°C

		Fatty acid composition by GC, %					
Type	Line	16:0[a]	18:0	18:1	18:2	18:3	20:0
Low 18:3	A5	11.0	3.3	28.6	52.1	3.7	0.2
	C1640	12.4	3.4	19.7	60.4	3.5	0.3
	N78-2245	10.6	3.9	35.1	44.1	4.5	0.4
	PI 123440	10.8	2.9	22.1	59.7	3.6	0.2
	PI 361088B	12.8	3.5	16.8	61.3	4.2	0.3
Cultivars	Century	10.7	3.3	18.6	58.4	7.7	0.2
	Maple Arrow	11.2	3.2	23.7	53.4	7.3	0.3
High 18:0	A6	8.0	22.3	17.9	42.7	6.6	1.4
	L.S.D.	0.7	1.1	2.3	2.5	1.0	0.1

[a]Palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), and arachidic acid (20:0).

At 40/30°C (Table 2) the linolenic acid levels of A5, C1640, and N78-2245 were below 2.0% These are the lowest levels of linolenic acid so far reported for soybean seeds. The low levels of linolenic acid were accompanied by low levels of linoleic acid and were compensated by high levels of oleic acid. The linoleic acid levels of A5, Maple Arrow and N78-2245 were lower than any previously reported levels (1) of field-grown soybeans. The oleic acid levels of Maple Arrow and N78-2245 were higher than the highest level previously reported for any line grown in the field (14). The stearic acid and palmitic acid levels were minimally different from those obtained at 28/22°C for field-grown soybean lines (1).

At 15/12°C (Table 3) the levels of linolenic acid were much higher than the levels observed at 28/22°C. There were significant differences between the linolenic acid levels of the five low linolenic acid lines. The mean linolenic acid level of N78-2245 was higher than that of the cultivar Century. The linolenic acid level of A5 and PI 123440 were significantly less than the levels of the cultivars, but significantly higher than the level of C1640 or

TABLE 2

Fatty Acid Composition by Relative Area Percent of the Seeds of Eight Soybean Lines Grown at 40/30°C

		Fatty acid composition by GC, %					
Type	Line	16:0	18:0	18:1	18:2	18:3	20:0
Low 18:3	A5	13.4	3.2	58.5	20.1	1.9	0.4
	C1640	13.5	2.7	46.8	33.3	1.9	0.4
	N78-2245	11.7	3.3	70.3	8.5	1.9	0.6
	PI 123440	11.0	2.7	46.2	34.9	2.4	0.3
	PI 361088B	13.0	3.8	46.9	30.4	2.2	0.5
Cultivars	Century	12.6	3.0	40.8	41.8	3.6	0.3
	Maple Arrow	13.0	2.0	66.2	16.1	2.3	0.4
High 18:0	A6	8.4	18.2	38.7	30.3	2.8	1.6
	L.S.D.	1.0	0.9	3.1	3.0	0.6	0.1

TABLE 3

Fatty Acid Composition by Relative Area Percent of the Seeds of Eight Soybean Lines Grown at 15/12°C

		Fatty acid composition by GC, %					
Type	Line	16:0	18:0	18:1	18:2	18:3	20:0
Low 18:3	A5	8.9	1.9	12.7	64.8	10.7	0.1
	C1640	10.4	2.3	13.6	65.2	7.8	0.2
	N78-2245	9.6	2.1	11.9	55.7	18.2	0.1
	PI 123440	10.0	2.1	13.0	63.6	11.1	0.1
	PI 361088B	12.0	2.6	15.3	62.8	7.4	0.2
Cultivars	Century	10.0	2.2	13.3	59.2	14.6	0.1
	Maple Arrow	9.1	2.2	13.7	53.7	20.5	0.1
High 18:0	A6	8.4	2.4	11.3	56.8	18.2	0.2
	L.S.D.	1.0	0.3	1.3	3.2	1.4	0.1

PI 361088B (Table 3). The high levels of linolenic acid were accompanied by high levels of linoleic acid and were compensated by low levels of oleic acid.

The low linolenic acid levels in both C1640 and PI 361088B are controlled by a recessive allele at the *Fan* locus (4,6). Thus, it would appear that these *fan* alleles may be responsible for a reduction in the temperature-sensitive response of linolenic acid levels. As a result, these lines should be the most useful for breeders attempting to develop low linolenic acid lines for cool, short-season areas.

A6 had a substantially higher level of stearic acid than all other lines at 28/22 and 40/30°C (Table 3), or in the field (12,13). However, A6 plants grown at 15/12°C had levels of stearic acid that were at or below the levels of the other lines. Thus it appears that exposure to cool temperatures may alter the stearic acid metabolism in A6. The *fas*[a] allele, which is responsible for the high level of stearic acid in A6 (12), has a secondary effect of increasing the level of arachidic acid (13). At 40/30 and 28/22°C, the levels of arachidic acid in A6 were four- or five-fold higher than levels in the other lines, but at 15/12°C the level in A6 was similar to other lines. This is consistent with an alteration in the expression of the *fas*[a] allele at 15/12°C. Further studies of the interactions between the *fas*[a] allele and low temperatures are presently underway.

The maturity of the eight lines used in this study range from Group 0 (A5) to Group IV (PI 123440). When grown at 16 hr daylength the time to flowering ranged from 43-116 days (unpublished data). Temperatures in the cabinet are known to fluctuate over time when operating at the limits of the temperature range for the cabinet. Therefore, it was necessary to conduct the extreme temperature studies at 12 hr daylength to enhance the synchronization of flowering and to ensure that the temperature in the cabinet during seed development was consistent for all lines under study.

Howell and Collins (11) noted that 12 hr daylength was associated with elevated levels of linolenic acid, across temperatures. However, a sample of seed of A6 grown at 15/12°C with 16 hr daylength had fatty acid levels that were not different from those observed with 12 hr daylength (unpublished data). In addition, several experimental low linolenic acid lines grown at 28/22°C had similar fatty acid levels for both 16 and 12 hr daylength regimes (unpublished data).

The temperatures used in the current study were lower and higher than those that can be reasonably expected in most soybean fields during seedfill. However, these temperatures are useful to evaluate the relative activity of enzymes responsible for extreme phenotypes such as high stearic acid (A6) or high oleic acid (N78-2245) levels. The use of extreme temperatures allowed us to produce fatty acid profiles that have not been previously observed for soybeans. The high oleic acid, low linoleic acid, and low linolenic acid levels observed at 40/30°C were beyond the range of values observed from the soybean germplasm in the field (1). It is apparent that soybean oil with less than 2.0% linolenic acid can be produced under the appropriate conditions. Therefore, it is reasonable to conclude that breeders may be able to select for soybean lines with linolenic acid levels below 2.5% under field conditions if the appropriate genetic combinations can be created.

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