# Environmental Effects on Fatty Acid Levels in Soybean Seed Oil

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**ABSTRACT:** FA composition determines the quality of vegetable oil. Soybean breeders have generated and used mutations in FA genes to develop altered FA profiles in the seed. However, the expression of the alleles and the relative activity of the gene products are often dependent on the environment, and these facts have hampered the breeding efforts. To investigate the environmental effect on FA composition of soybean seed oil in specific mutant material developed at the University of Guelph, a recombinant inbred line (RIL) population was developed from a cross between a low palmitate (16:0) line and a high-stearate (18:0) parent. The RIL population was field-tested across three environments over 2 yr. A combined ANOVA for FA composition was conducted to determine the year and location effects on the expression of FA alleles in this material. The results indicated that linolenic (18:3) level was most vulnerable to the environmental changes. Year effects accounted for a greater amount of variance than location effects for 16:0, 18:0, and 18:1, whereas location effects were more important than year effects for the relative amounts of 18:2 and 18:3. Genotype × environment (year, location) interaction effects were significant for the relative amounts of all five FA according to the combined ANOVA. Our results indicated that the extreme minimum daily temperatures during September seed fill period, rather than the means or the maximum temperature, may be responsible for the ratio of saturated vs. unsaturated FA in soybean oil.

Paper no. J11248 in *JAOCS 83*, 759–763 (September 2006).

**KEY WORDS:** Environmental effect, fatty acid composition, mutation, oil quality, soybean breeding.

An improved understanding of environmental effects on FA composition of soybean seed oil could assist breeders and agronomists to interpret data, predict performance, and select proper cultivation strategies for altered FA cultivars. The effects of many environmental factors on the FA composition of soybean seed oil have been investigated, including temperature, year, planting date, planting location, seed-fill duration, photoperiod, light intensity and quality, drought stress, irrigation, total rainfall, mean solar radiation, soil type, and nutrition (1–7). Temperature has been consistently shown to be a predominant environmental factor affecting the FA composition, especially for the unsaturated FA. Elevated levels of oleate (18:1) and reduced linoleate (18:2) and linolenate (18:3) have been reported as a result of increased temperatures during seed development in many studies (3,5–9). However, a more precise estimate of the effects that various temperature profiles have on the final FA composition, during different periods in the growing season is still warranted, especially regarding saturated FA.

In soybean breeding, the segregating progeny is often advanced to homozygous recombinant inbred lines (RIL) for evaluation and selection under multiple environments. For soybean oil quality improvement, lines with differently altered FA levels are often chosen as parents in order to combine the genes for desirable FA composition (10). In most of the previous studies regarding the environmental effect on the FA composition of soybean seed oil, soybean cultivars and mutant lines with normal or altered FA profiles have been used as experimental materials. In this study, an RIL population derived from a cross between a low-16:0 line and a high-18:0 line, developed at the University of Guelph, was field-tested to evaluate environmental effects on FA composition and expression of the mutant alleles under multiple environments. The population was developed to combine these traits, because 16:0 has been shown to increase blood cholesterol levels by increasing the undesirable LDL (low-density lipoprotein) (11), which in turn increases the risk of heart disease (12). On the other hand, an elevated 18:0 level is desirable for the production of certain baking fats such as margarine and shortenings (13–15). The objective of the current study was to measure and compare the location, year, and genotype × environment (location, year) effects on FA composition using this unique, previously uncharacterized RIL population.

## MATERIALS AND METHODS

*Field experiment.* Indoor hybridization was made between the low-16:0 soybean line RG2 and the high-18:0 line RG7. The progeny was advanced to $F_5$ using single seed descent. The harvested $F_5$ seeds from each $F_4$ plant were planted at Ridgetown [Ontario (ON), Canada] in June 2000 in two-row plots, which were 2 m long with between-row spacing of 43 cm. A type two modified augmented design was used (16). In total, 115 RIL were harvested at maturity for FA analysis. The harvested $F_6$ seeds were planted in 2001 at two locations, Ridgetown (ON, Canada) and Talbotville (ON, Canada), using a lattice design ($11 \times 11 = 121$ entries, 2 replications). The plots in Ridgetown were three-row plots with row length of 2.69 m and row width

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of 47 cm. The plots in Talbotville were two-row plots with row length of 5.3m and row width of 35 cm. FA were analyzed for both replications at each location.

*FA analysis.* Ten random seeds from each line were ground together into powder and samples of them were placed into labeled glass tubes. After adding 0.6 mL of 0.25 M KOH solution (7.01 g KOH in 250 mL methanol and 250 mL ethylether mixture), each tube was vortexed for 15 s and heated in a hot water bath (60°C) for 1 min. After the samples had cooled to room temperature, 2.0 mL of saturated NaCl solution and 1.5 mL of iso-octane were added. Each tube was vortexed for 30 s, and the phases were allowed to separate for 45 min. From each tube, 0.85 mL of supernatant was transferred to a small labeled autosampling vial for FA analysis using the Hewlett-Packard 6890 Series gas chromatograph (GC). The GC was equipped with a capillary column that was 15 m in length and 0.25 mm in diameter (J & W Scientific, Folsom, CA) and an FID. Helium was used as a carrier gas. The temperatures of the oven, injector, and detector were set at 180, 290, and 330°C, respectively. The hydrogen flow rate was 45 mL/min, and the air flow rate was 450 mL/min. The FA composition of each sample was determined by integrating the recorded peaks using Hewlett-Packard ChemStation Software.

*Statistical analysis.* Combined ANOVA for FA composition were conducted in three ways: over three environments (Ridgetown 2000, Ridgetown 2001, and Talbotville 2001), over two years (2000 and 2001) at the Ridgetown location, and over two locations (Ridgetown and Talbotville) in 2001. The expected mean squares for each source of variation were obtained using PROC GLM in SAS ver8.2 (SAS Institute Inc., Cary, NC, 1999). All the effects were considered random. The appropriate error term for testing the significance of each effect was defined based on the expected mean squares from the output of PROC GLM. In the combined analysis over three environments, the significance of genotype × environment interaction effects and block (environment) effects were tested against the residual, genotype effects were tested against the genotype × environment interaction effects, and environmental effects were tested by performing a quasi *F*-test on the ratio of $(MS_{environment} + MS_{residual})/[(MS_{blocks(environment)} + MS_{genotype\times environment})]$ (17). The same principles were applied to the other two combined ANOVA. The estimates of variance components were obtained from PROC MIXED in SAS version 8.2 (SAS Institute Inc., Cary, NC, 1999). Climatic data (including monthly maximum, minimum and mean temperatures; monthly precipitation) at three environments (Ridgetown 2000, Ridgetown 2001, and Talbotville 2001) were obtained from Environment Canada on-line data (http://www.climate.weatheroffice.ec.gc.ca/).

## RESULTS AND DISCUSSION

The means of 16:0 and 18:3 relative amounts in the RIL population differed by more than 2% across the three environments. For the relative amounts of 18:0, 18:1, and 18:2, the means differed by more than 5% across environments (Table 1). The means for 18:0 and 18:1 relative amounts under the three environments were in the following order: Ridgetown 2000 < Ridgetown 2001 < Talbotville 2001, whereas for 16:0, 18:2, and 18:3 relative amounts, the means from low to high were in the exact opposite order. The differences of means between years (Ridgetown 2000 and Ridgetown 2001) were greater than between locations (Ridgetown 2001 and Talbotville 2001) for 16:0, 18:0, and 18:1 relative amounts, but smaller for 18:2 and 18:3 relative amounts (Table 1). The effect and contribution of each source of variation to FA relative amounts were investigated through the combined ANOVA over three environments (Ridgetown 2000, Ridgetown 2001, and Talbotville 2001), in one location over 2 yr (Ridgetown in 2000 and 2001), and over two locations (Ridgetown and Talbotville) in 1 yr (2001) (Table 1).

**TABLE 1**
**Means and Ranges of FA Relative Amounts in a Recombinant Inbred Line (RIL) Population Derived from the Cross of RG2 × RG7 of Soybean, Grown at Ridgetown, Ontario (ON), Canada, in 2000 (RT00) and 2001 (RT01), and Talbotville, ON, in 2001 (TV01)**

| | | | | Difference of means between | | |
|---|---|---|---|---|---|---|
| FA | Environment | Mean | Range | RT00/RT01 | RT01/TV01 | RT00/TV01 |
| Palmitate (%) | RT00 | 8.55 | 4.03–13.87 | 2.02 | 0.22 | 2.24 |
| | RT01 | 6.53 | 3.72–11.04 | | | |
| | TV01 | 6.31 | 3.58–10.51 | | | |
| Stearate (%) | RT00 | 6.60 | 2.60–13.27 | 3.10 | 2.09 | 5.19 |
| | RT01 | 9.70 | 3.29–20.89 | | | |
| | TV01 | 11.79 | 4.61–23.71 | | | |
| Oleate (%) | RT00 | 17.54 | 13.75–23.95 | 2.85 | 2.54 | 5.39 |
| | RT01 | 20.39 | 14.81–29.43 | | | |
| | TV01 | 22.93 | 16.02–31.97 | | | |
| Linoleate (%) | RT00 | 55.95 | 49.39–61.65 | 2.67 | 2.97 | 5.64 |
| | RT01 | 53.28 | 46.20–60.02 | | | |
| | TV01 | 50.31 | 44.57–55.01 | | | |
| Linolenate (%) | RT00 | 11.36 | 9.35–13.73 | 1.25 | 1.45 | 2.70 |
| | RT01 | 10.11 | 8.56–11.66 | | | |
| | TV01 | 8.66 | 7.38–10.14 | | | |

**TABLE 2**
**Combined ANOVA and Estimates of Variance Components (VC) for FA Composition in a Soybean RIL Population Derived from the Cross of RG2 × RG7, Grown at Ridgetown, ON, in 2000 (RT00) and 2001 (RT01) and at Talbotville, ON, in 2001[a] (TV01)**

| | Combined ANOVA over three environments | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Palmitate | | | | Stearate | | | Oleate | | | Linoleate | | | Linolenate | | |
| Source | DF | MS[b] | VC | %[c] | MS | VC | % | MS | VC | % | MS | VC | % | MS | MS | % |
| Genotype (G) | 115 | 16.91[a] | 3.51 | 63 | 101.96[a] | 22.51 | 66 | 58.54[a] | 12.70 | 53 | 23.21[a] | 4.64 | 31 | 1.92[a] | 0.35 | 15 |
| Environment (E) | 2 | 206.19[b] | 1.43 | 26 | 989.82[b] | 6.43 | 19 | 1235.31[a] | 7.67 | 32 | 1374.40[a] | 8.34 | 55 | 301.56[a] | 1.78 | 74 |
| G × E | 230 | 0.79[a] | 0.35 | 6 | 6.63[a] | 3.00 | 9 | 4.37[a] | 1.95 | 8 | 2.70[a] | 1.07 | 7 | 0.36[a] | 0.15 | 6 |
| Block (E) | 2 | 6.16[a] | 0.05 | 1 | 6.85[b] | 0.04 | <1 | 2.53 | 0.01 | <1 | 5.61[a] | 0.04 | <1 | 1.10[a] | 0.01 | <1 |
| Residual | 230 | | 0.22 | 4 | | 2.18 | 6 | | 1.41 | 6 | | 1.01 | 7 | | 0.11 | 5 |
| | Combined ANOVA over RT00 and RT01 | | | | | | | | | | | | | | | |
| | Palmitate | | | | Stearate | | | Oleate | | | Linoleate | | | Linolenate | | |
| Source | DF | MS | VC | % | MS | VC | % | MS | VC | %[a] | MS | VC | % | MS | MS | % |
| Genotype (G) | 115 | 11.35[a] | 4.01 | 62 | 47.47[a] | 16.73 | 65 | 27.48[a] | 9.65 | 59 | 15.86[a] | 5.12 | 46 | 1.23[a] | 0.37 | 29 |
| Year (Y) | 1 | 331.09[b] | 2.11 | 33 | 769.21[b] | 4.87 | 19 | 650.30[b] | 4.16 | 25 | 610.03[b] | 3.87 | 34 | 104.19[a] | 0.67 | 53 |
| G × Y | 115 | 0.40[a] | 0.21 | 3 | 4.94[a] | 2.92 | 11 | 2.89[a] | 1.56 | 10 | 2.53[a] | 1.20 | 11 | 0.24[a] | 0.09 | 7 |
| Block (Y) | 1 | 4.16[a] | 0.03 | <1 | 12.76[a] | 0.10 | <1 | 4.74[b] | 0.03 | <1 | 9.61[a] | 0.07 | 1 | 0.77[b] | 0.01 | 1 |
| Residual | 115 | | 0.11 | 2 | | 1.29 | 5 | | 0.94 | 6 | | 0.99 | 9 | | 0.12 | 10 |
| | Combined ANOVA over RT01 and TV01 | | | | | | | | | | | | | | | |
| | Palmitate | | | | Stearate | | | Oleate | | | Linoleate | | | Linolenate | | |
| Source | DF | MS | VC | % | MS | VC | % | MS | VC | % | MS | VC | % | MS | VC | % |
| Genotype (G) | 115 | 12.67[a] | 2.94 | 83 | 129.03[b] | 30.76 | 84 | 71.33[a] | 16.74 | 72 | 22.95[a] | 4.99 | 44 | 1.87[a] | 0.36 | 20 |
| Location (L) | 1 | 0.64 | 0 | 0 | 417.68[b] | 1.75 | 5 | 814.37[a] | 3.49 | 15 | 1032.60[a] | 4.42 | 39 | 263.19[a] | 1.13 | 64 |
| G × L | 115 | 0.90[a] | 0.34 | 10 | 6.00[a] | 1.90 | 5 | 4.39[a] | 1.48 | 6 | 2.98[a] | 0.98 | 9 | 0.43[a] | 0.16 | 9 |
| Block (L) | 2 | 6.16[a] | 0.04 | 1 | 6.85[b] | 0.04 | <1 | 2.53 | 0.01 | <1 | 5.61[a] | 0.04 | <1 | 1.10[a] | 0.01 | <1 |
| Residual | 230 | | 0.22 | 6 | | 2.19 | 6 | | 1.42 | 6 | | 1.01 | 9 | | 0.11 | 6 |

[a]DF, degrees of freedom MS, mean square. For other abbreviations see Table 1.
[b]Superscript roman letter a significant at $P = 0.05$; superscript roman letter b significant at $P = 0.01$. Values without superscript letters are not significantly different.
[c]Percentage of the total variation in the model.

Genotype (RIL) effects were significant for FA relative amounts in all three of the combined ANOVA (Table 2). For 16:0, 18:0, and 18:1 relative amounts, genotype effect was the most important source of variation and accounted for over 50% of the variance for the three FA relative amounts. Environmental effect was the most prominent source of variation for 18:2 relative amount in the combined ANOVA over all three environments, but the genotype effect was greater than year and location effects when a combined ANOVA was conducted over 2 yr in one location and over two locations in one year. For the 18:3 relative amount, environmental effect including year and location effect accounted for the largest amount of its variance. This indicated that the 18:3 relative amount was more vulnerable to the environmental changes than other FA, which is consistent with previous reports.

Year effect accounted for a larger amount of variance for 16:0, 18:0, and 18:1 relative amounts than location effect (Table 2), which agreed with the larger difference of means for these traits between years than between locations (Table 1), whereas for 18:2 and 18:3 relative amounts, location effect accounted for a larger amount of variation than year effects, which was also in agreement with the larger difference of means for 18:2 and 18:3 relative amounts between locations than between years. Genotype × environment interaction, genotype × year interaction, and genotype × location interaction effects were significant for all of the five FA relative amounts in the three combined ANOVA over three environments, over 2 yr, and over two locations (Table 2).

Previous studies have consistently reported the association between high temperature during seed development and low 18:2 and 18:3 and high 18:1 relative amounts (3,5–9). Relatively little attention has been paid to what happens with the saturated FA, which was the main goal of our experiment. The means and relative amounts of 16:0 from high to low were in the order of Ridgetown 2000, Ridgetown 2001, and Talbotville 2001; while the mean relative amounts of 18:0 in the three environments were in the exact opposite order (Table 1). The unsaturated FA—18:1, 18:2 and 18:3—followed the trend of 16:0, which again was opposite of 18:0. Since 18:0 is the precursor of all 18-carbon unsaturated FA in soybean oil, the higher activity of the desaturase enzymes at lower temperatures would lead to an increase in the desaturates at the expense of 18:0, as our data confirmed.

The months of August and September were the most important periods for soybean seed development across the three environments, since they coincided with seed fill and maturation.

**TABLE 3**
**The Means and Ranges of Monthly Maximum, Minimum and Mean Temperatures Recorded at Ridgetown, ON, in 2000 (RT00) and 2001 (RT01) and at Talbotville, ON, in 2001[a] (TV01)**

| | | Temperatures (°C) | | |
|---|---|---|---|---|
| Month | Environment | Maximum (range) | Minimum (range) | Mean (range) |
| June | RT00 | 25 (14.8–32.6) | 14.9 (6.1–22.6) | 20 (12.9–26.2) |
| | RT01 | 25 (15.7–32.5) | 14.1 (5.6–21.4) | 19.6 (11.0–26.0) |
| | TV01 | 24.4 (14.0–31.0) | 13 (11.5–25.0) | 18.7 (6.5–19.5) |
| July | RT00 | 25 (20.9–28.4) | 14.2 (7.2–21.5) | 19.7 (15.8–23.8) |
| | RT01 | 27 (20.9–33.5) | 15.3 (4.3–23.9) | 21.2 (12.6–28.6) |
| | TV01 | 26.6 (19.5–33.5) | 14.7 (13.8–28.5) | 20.7 (6.5–23.5) |
| August | RT00 | 25 (16.5–29.0) | 14.6 (4.5–20.8) | 19.8 (12.7–24.5) |
| | RT01 | 28.1 (23.3–36.0) | 15.9 (7.8–23.0) | 22.1 (17.0–28.5) |
| | TV01 | 27.7 (23.0–34.5) | 15.4 (8.5–21.0) | 21.6 (17.8–27.0) |
| September | RT00 | 21.3 (12.0–29.3) | 11.3 (0.5–20.9) | 16.3 (7.5–24.5) |
| | RT01 | 21.7 (8.8–31.6) | 10.1 (1.7–22.0) | 15.9 (7.3–26.2) |
| | TV01 | 21.5 (11.0–29.5) | 10.1 (7.0–26.3) | 15.8 (3.0–23) |
| October | RT00 | 17.2 (8.3–25.1) | 6.3 (–4.5–16.9) | 11.8 (3.7–21.0) |
| | RT01 | 15.2 (6.0–25.5) | 7 (–3.1–15.1) | 11.1 (3.4–19.6) |
| | TV01 | 14.9 (7.0–23) | 6.4 (4.3–18.3) | 10.7 (–2.0–15.5) |

[a]For abbreviation see Table 1.

In most years, soybeans planted in Ontario do not enter R4 stage (18) until August. Thus, the temperature profiles during August and September were critical for the FA composition of soybean oil. When the mean temperature is evaluated, very little difference can be observed between the three environments in August and September (Table 3). However, a closer look at the ranges, especially for the minimum temperature, shows that the absolute minima recorded for the three environments in September were 0.5°C in Ridgetown 2000, 1.7°C in Ridgetown 2001, and 7°C in Talbotville 2001. This coincides with the greatest relative amount of stearic acid being recorded at Talbotville 2001 followed by Ridgetown 2001, followed by Ridgetown in 2000. Low temperature may have a stimulatory effect on desaturase gene expression. A previous study found that desaturase activities, especially for 18:1- and 18:2-desaturases, were increased at lower temperatures (19). Since the desaturase enzymes seem to have enhanced expression or activity at lower temperatures, the highest relative amounts of stearic acid were at locations with the lowest minimum temperature extremes (e.g., Talbotville 2001), which was accompanied by an increase in the relative amounts of 18:1, 18:2, and 18:3 (Table 1). It needs to be reiterated that the mean minimum temperature alone could mask the effects of a few days with lower temperatures (data for individual dates are available from Environment Canada; 20), especially when they occur during a key period of seed fill and development. Hence, the temperature ranges appear to be as important, if not more, as the mean minimum temperatures during the seed fill and oil accumulation period in determining the relative amounts of saturated compared to unsaturated FA soybean seed oil. Thus, it is important to consider the climatic conditions of the growing environment, especially minimum temperatures during seed fill, when trying to develop or grow lower-palmitic and higher-stearic soybean genotypes.

## ACKNOWLEDGMENTS

The technical assistance of Julia Zilka, Wade Montminy, Cal Klager, Yesenia Salazar, Dennis Fischer, Bryan Sterling, and Dr. Joe Omielan is gratefully acknowledged. The financial support for this research was generously provided by the Agricultural Adaptation Council of Canada (CanAdapt program), the Ontario Ministry of Agriculture, Food and Rural Affairs—Special Research Projects Program, and the Ontario Soybean Growers.

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[Received October 3, 2005; accepted June 7, 2006]