that of the control but that of the oil degummed with 500 mg/L was higher than the control.

In conclusion, the oxidative stability of degummed oil decreased as the metal concentration increased. The development of oxidation was greater for $FeCl_2$ concentrations than for NaCl and $CaCO_3$-$MgCO_3$ concentrations under the same conditions.

The authors recommend that when degumming soybean oil, hard water with levels of $CaCO_3$ and $MgCO_3$ higher than 150 mg/L should not be used because of the undesirable effect of Ca and Mg on the effectiveness of the degumming process. In spite of the ability of high concentrations of $FeCl_2$ to remove more phosphorus during the degumming process, water with more than 100 μg/L $FeCl_2$ should not be used because of its deleterious effect on the oxidative stability of the degummed oil. Finally, deionized distilled water or softened water with 300 mg/L NaCl should be used for degumming, depending on availability and cost.

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Genetic Alteration of Soybean Oil Composition by a Chemical Mutagen[1]

J.R. WILCOX, J.F. CAVINS and **N.C. NIELSEN,** Department of Agronomy, Life Science Building, Purdue University, West Lafayette, IN 47907

ABSTRACT

Soybean (*Glycine max* [L.] Merr. cv. Century) seeds were treated with ethylmethanesulfonate (EMS) and the M_2 progeny were evaluated for fatty acid composition of the oil. Treatment with EMS significantly increased the variability in content of each of the fatty acids in comparison with those of the Century control. There was a strong inverse relationship between oleic and linoleic acids among seeds from M_2 plants. This supports the hypothesis of sequential desaturation as the method of formation of unsaturated fatty acids in soybean oil. A genetically stable mutant with 3.4% linolenic acid was identified that was similar to the cultivar Century in days to maturity, plant height and resistance to lodging.

INTRODUCTION

Soybean oil is the major edible vegetable oil produced and consumed in the USA. The high linolenic acid content of the oil, 7-9%, has been associated with objectionable flavors and poor stability (1,2). Industrial processes have been developed to hydrogenate and deodorize soybean oil to improve flavor and stability, but the processes are expensive and result in formation of *cis* and *trans* positional monoenes and *cis-trans* 9-12 dienes. The latter unnatural fatty acids have questionable nutritive value (3-5).

Efforts to identify soybean strains with low linolenic acid contents, 3.5% or less, have been only partially successful (6,7). The US soybean germplasm collection contains strains with minimum linolenic acid contents of ca. 4.2% (7-9). Related species in the genus *Glycine* have higher linolenic acid contents than the cultivated soybean (10).

Breeding programs have been initiated to develop soybean strains with inherently low levels of linolenic acid. White et al. (11) intercrossed low linolenic acid lines of soybeans and from their progeny identified an F_2 plant with 3.35% linolenic acid. However, this work was terminated because in succeeding generations the low linolenic acid value of the line was not maintained and environmental effects markedly influenced the linolenic acid content of the line.

Hammond and Fehr (6) used combinations of recurrent selection and both X-rays and chemical mutagens to produce soybean strains with low linolenic acid contents. By crossing strains with the lowest linolenic acid content available, they were able to produce strains with amounts of linolenic acid 1-1.5% lower than the best parental strain. Treatment of one low linolenic acid breeding line with ethylmethanesulfonate (EMS) resulted in a line, designated A5, with linolenic acid contents of 2.9-4.1%, depending on the environment where the line was grown. In that research, selection was only for low linolenic acid content and not for agronomic characteristics. Selected strains would have to be crossed with lines that possess good agronomic characteristics to develop low linolenic acid cultivars that would be competitive with currently grown cultivars.

Wilson et al. (12,13) used recurrent selection for high

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oleic acid and for high [18:1/(18:2 + 18:3)] ratios to alter fatty acid composition of soybean oil. Five cycles of selection resulted in an increase of 18:1 concentration from 31.2 to 51.0%, a decrease in 18:2 concentration from 48.7 to 31.9%, and a decrease in 18:3 concentration from 7.0 to 4.2%. However, these strains also need to be crossed with lines that have good agronomic characteristics to develop low 18:3 lines competitive with cultivars grown presently.

Two related objectives were addressed in the study described below. The first was to evaluate the effectiveness of EMS to induce mutants with altered seed fatty acid content. This was done by comparing the distributions of stearate, oleate, linoleate and linolenate content in seeds of M_2 progeny derived from treated and untreated seeds. The second objective was to isolate an experimental line that had a low level of linolenic acid which might be useful for the development of soybean varieties whose oil products have improved stability. The features of a low linolenate line, C1640, and the population of soybean plants from which it was derived are described.

EXPERIMENTAL PROCEDURE

Ca. 3,000 seeds of Century soybean, a high yielding maturity group II cultivar (14), were soaked in an aerated 2,500 ppm solution of EMS for 24 hr. Seeds were rinsed exhaustively with distilled water and sown in a greenhouse sandbench. Use of the sandbench was necessary because EMS-treated seeds were slow to germinate and produced weak seedlings, which drastically reduced survival when planted in the field. Seedlings were obtained from ca. 40% of the seeds planted, and were transplanted to 0.25-L styrofoam containers filled with soil. When unifoliolate leaves were fully expanded, the seedlings were transplanted to the field 10 cm apart in rows spaced 75 cm apart. At maturity, these M_1 plants were harvested and threshed individually. The following year, 25 seeds from each M_1 plant were sown in rows 1 m in length spaced 75 cm apart. At maturity, five M_2 plants were pulled from each row, threshed, and the identity of seed from each M_2 plant and each M_1 progeny row was maintained. Ca. 20 seeds from each plant were analyzed for fatty acid composition of the oil, and the remnant seed was retained to maintain the line of any M_2 plants with altered fatty acid composition.

For fatty acid analyses, seed samples were ground in an electric food grinder (Varco model MX-228). Ca. 200 mg of ground seed were placed in a 30-mL glass vial and 5 mL of sodium methoxide solution (1 g sodium dissolved in 100 g methanol) was added. After 30 min, 1 mL of 10% acetic acid solution was added, followed by 10 mL heptane. The material was mixed thoroughly and then allowed to stand until the organic and aqueous phases separated. The organic phase was removed and 5-μL samples were analyzed in a Varian 3700 gas chromatograph equipped with autoinjectors and flame ionization detectors. Glass columns (2 m × 2 mm) packed with 100/200 mesh Gas-Chrom Q coated with 5% LAC-2R-446 were used for separation. Isothermal analyses were made at 180 C with the injector at 230 C and the detector at 240 C.

Ca. 500 seeds of Century soybean, as checks, were treated as described above, except that they were soaked for 24 hr in distilled water instead of the EMS solution. Progeny from these seeds were examined for their fatty acid composition and the variability measured was compared with that in progeny of the EMS-treated seeds.

Ten remnant seeds from an M_2 plant low in linolenate were sown, one seed per 1-L clay pot of soil, in the greenhouse in February, 1982. At maturity, 10 seeds from each

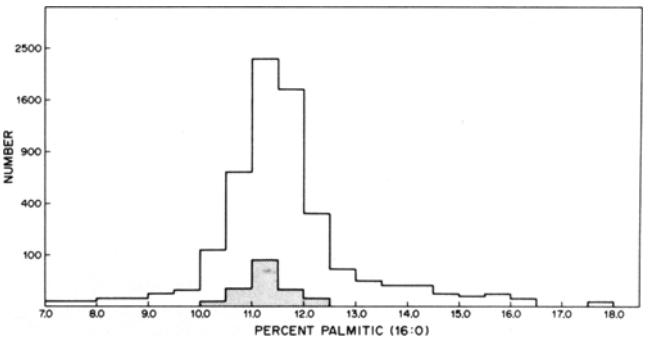

FIG. 1. Distribution of M_2 plants and of Century plants (shaded) for palmitic acid content of the oil.

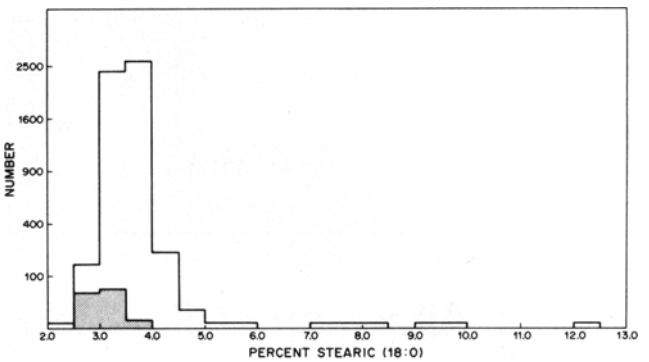

FIG. 2. Distribution of M_2 plants and of Century plants (shaded) for stearic acid content of the oil.

M_3 plant were analyzed for fatty acid composition. The remaining 25-30 seeds from each plant were sown in the field in 10 rows 3 m in length and 1 m apart. At maturity, a sample of seed from fully fertile plants in each row were analyzed for oil composition. Three Century plants grown in the greenhouse and 5 Century plant rows grown in the field along with the low linolenate plants provided seed used as controls when comparing the M_3 and M_4 generations.

RESULTS AND DISCUSSION

Treatment with EMS significantly increased the variability in fatty acid composition of M_2 plants. There was about a 2-fold increase in variability of stearic and linolenic acid and a 3- to 4-fold increase in the variability of palmitic, oleic and linoleic acid content in oil from M_2 plants compared with oil from Century controls.

The frequency distributions of 5355 M_2 lines compared with 106 Century controls are shown in Figures 1-5. The range in palmitic acid concentration (Fig. 1) of the M_2 population was from 7.1 to 17.6%, compared to the range for Century of 10.5-12.5%. Variability appeared to be equally distributed about the mean of both the M_2 and the Century control plants. Stearic acid content (Fig. 2) of the M_2 population varied from 2.3 to 12.1%, compared with 2.6 to 3.7% for Century. The low level of stearic acid in Century soybeans was not altered appreciably by mutagenic treatment, although there was as much as a 4-fold increase of this fatty acid in one M_2 plant. Oleic acid content (Fig. 3) of the M_2 plants varied from 14.9 to 33.8%, compared with a range in the Century controls of 17.2-24.7%. The distributions of both the M_2 plants and the Century controls were skewed toward higher oleic acid values and

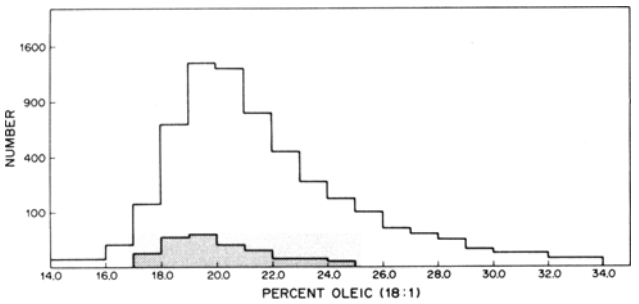

FIG. 3. Distribution of M_2 plants and of Century plants (shaded) for oleic acid content of the oil.

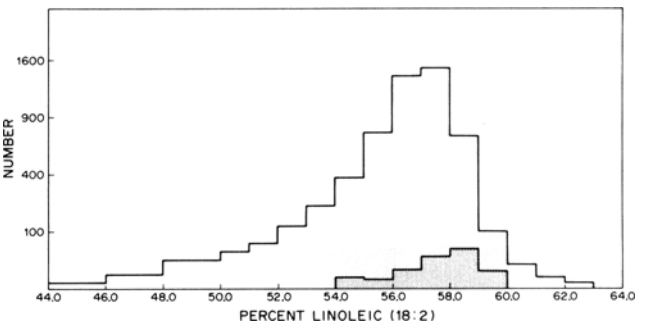

FIG. 4. Distribution of M_2 plants and of Century plants (shaded) for linoleic acid content of the oil.

there was a small but statistically significant shift in the mean oleic acid content of seeds from the M_2 population toward higher 18:1 content. Linoleic acid contents (Fig. 4) varied from 44.7 to 62.2% in the M_2 population and from 54.3 to 59.7% among the Century control plants. In contrast to the values for oleic acid, the linoleic acid contents of seeds in the M_2 plants were skewed toward the lower values and there was a small, statistically significant shift to a lower average 18:2 content. The linolenic acid content of seeds in the M_2 populations varied from 3.4 to 11.1% and for the Century controls from 6.6 to 9.4% (Fig. 5). Variability appeared to be distributed normally about the mean of the M_2 population, although there was a small statistically significant shift to a lower mean 18:3 content compared to Century.

The inverse relation between oleic and linolenic acids was examined in more detail by comparing the fatty acid content of the 10 highest and 10 lowest M_2 plants. The 10 with the highest mean oleic acid content ($\bar{x}$ = 32.4 ± 0.31%) contained 46.8 ± 0.27% linoleates. Conversely, the 10 plants with the highest mean linoleic acid content ($\bar{x}$ = 61.1 ± 0.17%) averaged 17.8 ± 0.48% oleate. Although the reasons underlying this inverse relation remain obscure, it is a common feature of plants in which the degree of fatty acid unsaturation has been altered. An inverse relationship between oleic acid and linoleic acid was reported by Hammond and Fehr (6,7) among selections from X-ray treated soybean seeds. The recurrent selection methodology used by Wilson et al. (12,13) to alter the fatty acid composition of soybean oil resulted in a large increase in 18:1 content and a decrease in 18:3. A similar phenomenon was observed in safflower by Knowles (15), where three alleles at one chromosome locus that govern the proportions of oleic and linoleic acid in the seed were described.

Linolenic acid is considered to be formed by a sequential desaturation pathway (see ref. 16 for a review), although there is uncertainty about the precise intermediates, the enzymes involved in the pathway, and the subcellular compartments within which the reactions occur (17,18). The skewed inverse relation between oleate and linoleate content in the population of M_2 plants studied suggests that the part of the desaturase pathway which connects these two intermediates is particularly sensitive to mutation. Alterations in this region which restrict the flow of metabolites through the pathway would be expected to increase oleate content and decrease linoleate and linolenate like the experimental soybean line N78-2245 (12). Such mutations would produce high positive correlation between changes in the level of linolenic and linoleic acid, as first observed by White et al. (11).

The M_2 plant with the lowest linolenic acid content was designated C1640 and studied further. The fatty acid composition of the oil from seeds of this plant was 16:0, 11.5%;

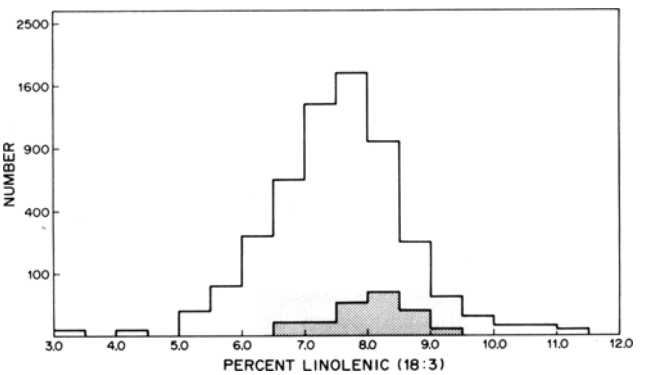

FIG. 5. Distribution of M_2 plants and of Century plants (shaded) for linolenic acid content of the oil.

18:0, 3.8%, 18:1, 21.5%; 18:2, 60.2%; and 18:3, 3.4%. Fatty acid analyses of oil extracted from seeds of M_3 and M_4 plants are shown in Table I. The M_3 plants were grown in the greenhouse, and the M_4 plants were grown in the field. Despite this difference, the 18:3 content of oil from lines derived from the M_2 plant was consistently low in the two generations succeeding the M_2, which indicates this was an inherent change in fatty acid composition of the oil. Percentages of 18:3 in seed from field-grown plants were higher than from greenhouse-grown plants or from the original M_2 plant. This is attributed to the late planting, June 11, of the strains in the field in 1982. Late planting and seed development under cool conditions results in higher 18:3 contents than early planting or seed development under high temperatures (19-22).

It is unclear where in the desaturase pathway the genetic lesion which produces C1640 is having its effect. The 2-fold decrease in linolenic acid level in this variant was not accompanied by large changes in either oleate or linolenate, although the small decrease in oleate content was statistically significant. This is in contrast to the situation in N78-2245, where a large increase in oleate and a large decrease in linoleate accompanied the decrease in linolenate. Thus, whereas the genetic lesion in N78-2245 appears to have a major effect on the conversion of oleate to linoleate, the lesion responsible for C1640 seems to act elsewhere in the pathway. Comparison of the two types of mutants may provide new insight into the desaturase pathway.

Treatment of a high yielding variety such as Century with EMS has the advantage that only a few genes may be altered while leaving the majority of the genome intact. This could facilitate development of a low 18:3 line with acceptable agronomic properties. With this in mind, the 10

TABLE I

Fatty Acid Composition of C1640 Derived Lines Compared with that of Century Soybeans in Two Environments

Strain		Fatty acid				
		16:0	18:0	18:1[a]	18:2	18:3[a]
	no.	%				
Greenhouse						
C1640-M_3	10	11.9 ± 0.09	3.0 ± 0.04	22.0 ± 0.86	59.7 ± 0.73	3.4 ± 0.07
Century	3	11.8 ± 0.12	3.0 ± 0.09	19.1 ± 0.55	59.2 ± 0.38	6.9 ± 0.18
Field						
C1640-M_4	10	11.2 ± 0.04	3.6 ± 0.02	24.2 ± 0.26	57.2 ± 0.23	3.8 ± 0.03
Century	5	11.2 ± 0.06	3.4 ± 0.05	21.5 ± 0.69	55.8 ± 0.59	8.0 ± 0.14

[a]The increase in 18:1 content and decrease in 18:3 content of C1640 compared to Century was significant at or above the 95% confidence level in both greenhouse- and field-grown plants.

C1640-M_4 plant rows and 5 rows of Century controls were compared during their growth in the field. All M_4 plant rows were similar to Century in days to maturity, mature plant height and resistance to lodging. There was insufficient seed to evaluate the yield potential of the C1640-M_4 line compared to Century. In 7 of the 10 M_4 plant rows, ca. 25% of the plants were partially sterile and were apparently expressing residual heterozygosity from EMS treatment. When these partially sterile plants were rogued from the rows, the remaining plants were uniform and visually indistinguishable from Century plants.

The data demonstrate that mutagenesis by EMS effectively increased the variability of fatty acids in soybean oil. These data represent one year of a continuing study in which a total of ca. 15,000 M_2 plants were evaluated, so the success rate of developing low linolenic acid lines is very low. The 3.4% linolenic acid line obtained in this study represents the best combination of consistently low 18:3 content and good agronomic characteristics that has been identified in soybeans to date. It is hoped that these favorable combinations of characteristics will facilitate the incorporation of low linolenic acid content into adapted cultivars.

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