Improving the Fatty Acid Composition of Soybean Oil[1]

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ABSTRACT

Efforts to improve the composition of soybean oil by breeding the beans for low linolenic acid in the oil have continued since 1968. This paper reports recent work using hybrid crosses and induced mutations. No lines are yet available that contain oil having less than 3% linolenic acid.

It is generally agreed that the stability of soybean oil to oxidative flavor deterioration would be much improved if it contained less linolenic acid. Commercial strains of soybean have oil with 8 to 9% linolenic acid. Reduction of linolenic acid to at least 3% and preferably less than 1% seems desirable for good stability.

We have been attempting to improve soybean oil composition at Iowa State University since 1968. Our first approach was to select for low linolenic acid genotypes in populations obtained from hybridization between parents that had the lowest available content of the fatty acid. In 1975, we began to use mutation breeding. Since then, the fatty acid composition of 28,500 plants obtained from mutagen-treated seeds has been analyzed. To date, the line with lowest linolenic acid that we have identified is A5 (1), which is compared with its parent and two cultivars in Table I. 'Corsoy' is a cultivar that tends to have relatively low linolenic acid percentage, whereas 'Weber' tends to have a relatively higher value. The linolenic acid content of A5 is considerably below the cultivars but represents only a modest gain over its parent, which was selected from the hybridization program and was fairly low in linolenic acid. Table I also reveals that the relative percentage of linolenic acid can vary by 20 to 30%, depending on the environment in which the genotypes are grown.

Because the gain indicated in Table I is rather typical of the small improvement we find with breeding and because there is an environmental effect on fatty acid composition, we have had to make repeated comparisons to verify small differences in linolenic acid among genotypes. We recently addressed the question, what is the best allocation of resources to detect small changes in fatty acid composition (2). Besides the environmental variation associated with different locations of production, there also is variation in fatty acid composition among seed samples on a single plant, among plants of the same genotype, and among the plots in which lines are grown in the field. Analytical error also must be considered when comparing genotypes. We grew a number of strains of soybeans in Iowa and Puerto Rico and measured their fatty acid compositions. Table II shows heritability estimates for linolenic acid when selection is based on varying numbers of analyses (injections) per sample, seed samples per plant, plants per plot (replication), replications per location (environment), and environments. Heritability is the proportion of the total variation among samples that is attributable to genetic differences. Heritability can range from 0 to 100%, with the higher values indicating that nongenetic variation has been

TABLE I

Percentage of Linolenic Acid for Lines and Cultivars Grown in Five Locations

	Ave.	Range
A5	3.8	3.3-4.2
FA 9525[a]	5.9	5.5-6.7
Corsoy	7.7	6.4-8.8
Weber	9.9	8.9-11.0

[a]Parent line from which A5 was produced by mutation.

TABLE II

Heritability Estimates for Linolenic Acid with Different Resource Allocations

No. of units	No. of Units 1	2	3	4
		%		
Seed samples[a]		Injections		
1	53	53	53	53
2	61	61	61	61
3	64	65	65	65
4	67	67	67	67
Plants[b]		Seed Samples		
1	53	61	64	67
2	64	70	72	74
3	69	72	75	76
4	72	75	77	77
Replications[c]		Plants		
1	53	64	69	72
2	64	72	75	77
3	70	76	78	79
4	72	77	79	79
Environments[d]		Replications		
1	53	64	70	72
2	69	78	82	84
3	77	84	87	88
4	82	87	90	92

[a]Estimates based on one plant, replication and environment.
[b]Estimates based on one injection, replication and environment.
[c]Estimates based on one injection, seed sample and environment.
[d]Estimates based on one injection, seed sample and plant.

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TABLE III

Ranking of Soybean Lines for Linolenic Acid Percentage When Grown in Iowa and Puerto Rico

Line	Iowa[a]	Puerto Rico[b]	Combined
Pella	1	1	1
Harcor	3	4	2
L73-4673	2	6	3
Corsoy	6	3	4
L75-3674	4	5	5
A2	5	7	6
Beeson 80	14	2	7
A77-212006	7	8	8
Nebsoy	8	9	9
Gnome	9	10	10
H75-5605	16	11	11
V 11239	11	13	12
V 20235	10	12	13
Amcor	12	13	14
Century	12	15	15
Wells	15	17	16
A77-211021	17	16	17
H7703	20	18	18
Beeson	18	19	19
Weber	19	20	20

[a]Average value for 1979, 1980, and 1981.
[b]Average for 1980 and 1981 in both unlighted fields and fields lighted to adjust the day length.

minimized. The results in Table II show that heritability increased little with multiple gas chromatographic analyses on the same sample and increased modestly when more than one seed sample was analyzed from each plant. Evaluation of one sample from multiple plants in a plot was more effective than multiple samples from one plant. Replication of genotypes did not increase heritability substantially. Analyzing multiple plants from one plot was as effective as analyzing one plant from multiple plots. The use of multiple environments for testing genotypes increased the heritability.

Only one soybean crop can be produced in Iowa each year, but it is possible to grow two crops during the winter in Puerto Rico, where the environment is substantially different from that in Iowa. We wanted to know if selection of lines for low linolenic acid could be done as effectively in Puerto Rico as in Iowa (3). Table III shows the average rank of 20 genotypes when grown in multiple plantings at the two locations. The rank correlation coefficient between the two environments was 0.82. Thus, performance in Puerto Rico was a good indication of performance in Iowa.

Typical results from a mutation experiment are illustrated in Figures 1 and 2. Seeds of our best strain, A5, were treated with either ethyl methanesulfonate or sodium azide by using the procedures detailed previously (4). A total of 360 M_2 plants from the population were analyzed for fatty acid composition. The distribution of linolenic acid percentage among plants from treated seeds was similar to that of untreated A5 plants, except for a tendency to revert to higher linolenic acid. The plants in the low tail of the distribution will be grown again to determine if they continue to produce lower linolenic acid than A5.

Some investigators have reported more striking gains by mutagenesis than we have experienced. Wilcox et al. (5) obtained a mutant from the cultivar 'Century' that contained 3.8% linolenic acid, compared with its parent at 8.0%. In their experiment, only 3,000 seeds were treated with a mutagen. Australian plant breeders have reported treating 70,000 seeds of linseed with mutagens and obtaining two mutants that contained 28% linolenic acid compared with 45% in the parent. Crosses between the two mutants resulted in a line with less than 1% linolenic acid (6).

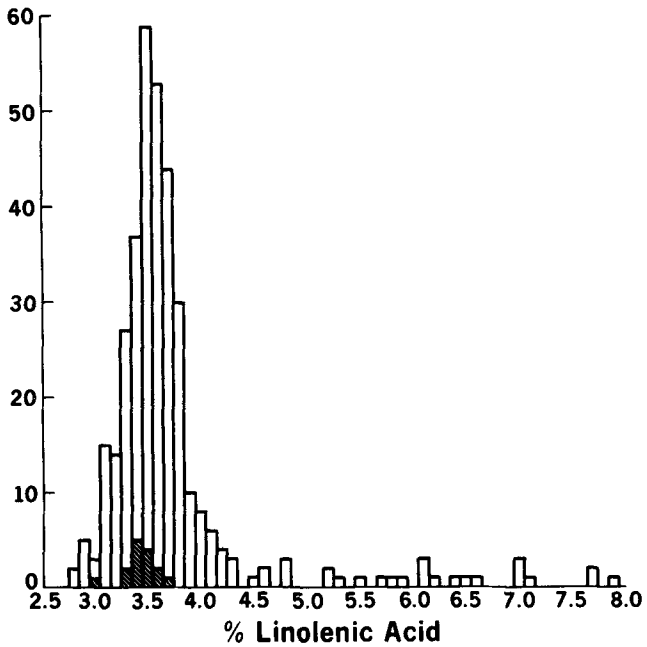

FIG. 1. Distribution of linolenic acid percentages in M_2 plants obtained by treating strain A5 with ethyl methanesulfonate. Solid bars show the distribution of untreated A5.

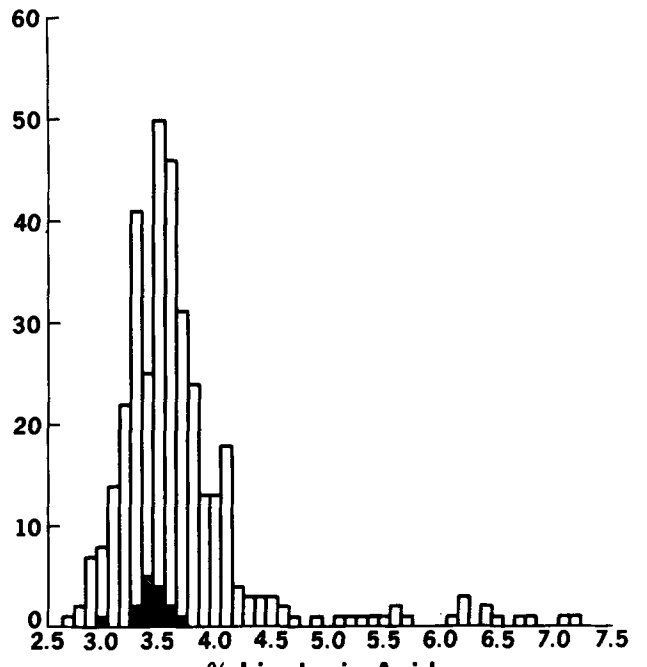

FIG. 2. Distribution of linolenic acid percentages in M_2 plants obtained by treating strain A5 with sodium azide. Solid bars show the distribution of untreated A5.

We wanted to know if the mutant gene(s) in the Century mutant would combine with favorable genes in A5 to produce progeny with a unique linolenic acid content. We crossed A5 with the Century mutant and analyzed the seed of F_2 plants. The results are shown in Figure 3. In this comparison, A5 had a linolenic acid percentage of 3.5% compared with 4.2% for the Century mutant. None of the progeny had a linolenic acid percentage substantially below that of A5.

A5 has high oleic acid and low linoleic acid percentages, whereas the Century mutant has more typical percentages of these fatty acids (Table IV). Figure 3 also shows the

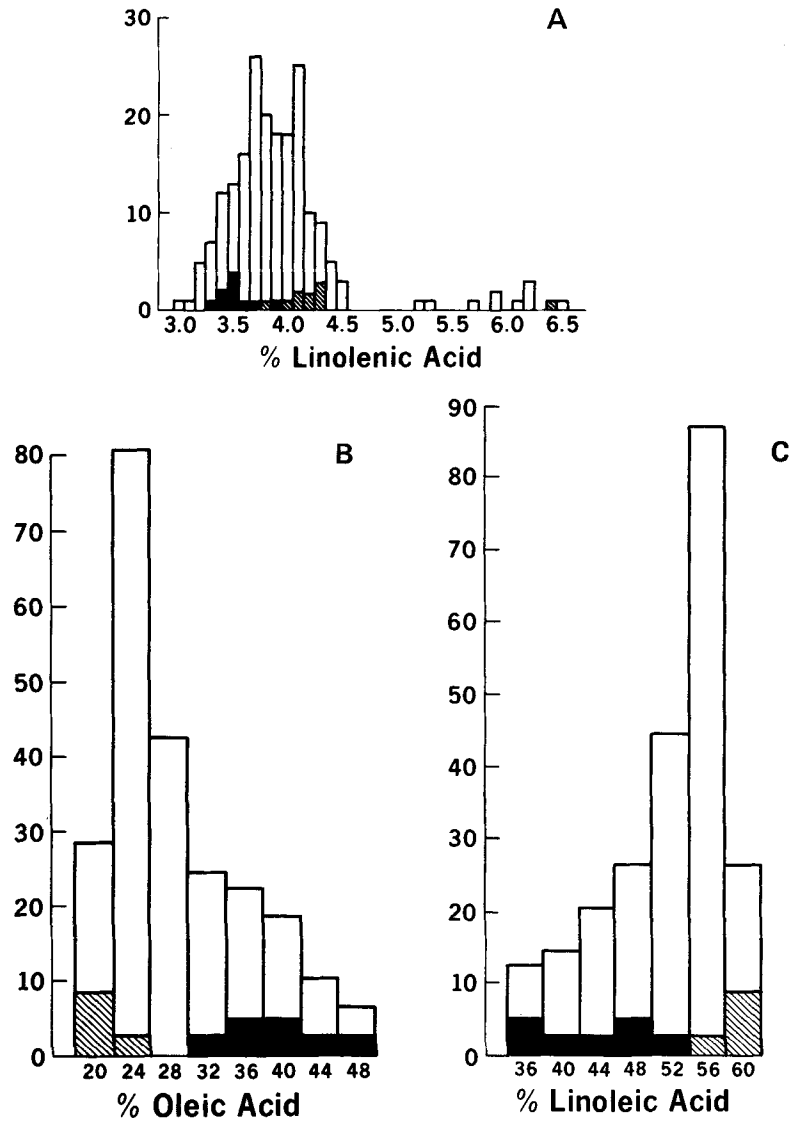

FIG. 3. Distribution of fatty acid percentages in the F_2 generation from the cross of A5 with a low-linolenic acid mutant from Century. Solid bars show distribution of the A5 parent, and the crosshatched bars those of the Century mutant.

TABLE VI

The Number of Plants Observed With Various Genotypes for Stearic Acid Percentage at F_2 Based on the Analysis of F_3 Seed

	Segregation Ratio		
	Low	Segregating	High
Observed	21	49	24
Theoretical	23.5	47	23.5

oleic and linoleic acid distributions in the progeny from the cross of A5 with the Century mutant. It seems that genotypes with a fairly broad range of fatty acid composition could be selected from the cross.

The results of mutation breeding are unpredictable, and genotypes sometimes are obtained that differ from those intended. Among the plants from mutated seeds that we have examined, several have been found that contain unusually high percentages of stearic acid. The mutant with the highest amount of stearic acid has been released under the designation A6 (7). No one has ever suggested to us that a high stearic soybean oil was desirable. Now that we have it, no one seems to know what it is good for. However, such a fatty acid composition is unique among the available commercial fats and oils (Table IV). In most other fats containing this much stearic acid, there usually are considerable trisaturated glycerides, and the melting point is quite high. Like all soybean oils, A6 has no saturated fatty acid on the sn-2 position of the glycerol, so the oil has a melting point below room temperature (4).

Figure 4 compares the stearic acid percentage in A6, during seed development, with its parent, FA 8077 (8). The difference in stearic acid between the two genotypes occurs early in seed development. The stearic acid increases primarily at the expense of oleic acid, while oleic, linoleic and linolenic acid remain relatively unchanged.

Incorporation of the unique fatty acid composition of A6 into commercial cultivars requires knowledge of the inheritance of fatty acid composition in the soybean. Crosses of A6 and its parent were made, and the analyses of the F_1 seed are shown in Table V (9). Figure 5 shows the distribution of stearic acid in the F_2 seeds, and Table VI shows the genotype of the F_2 plants. The lack of differ-

TABLE IV

Fatty Acid Composition of A6 and Its Parent FA 8077.

Line	Fatty Acid					
	16:0	18:0	18:1	18:2	18:3	20:0
A6	8.0	28.1	19.8	35.5	6.6	2.0
FA 8077	8.7	4.4	42.8	36.7	7.6	–

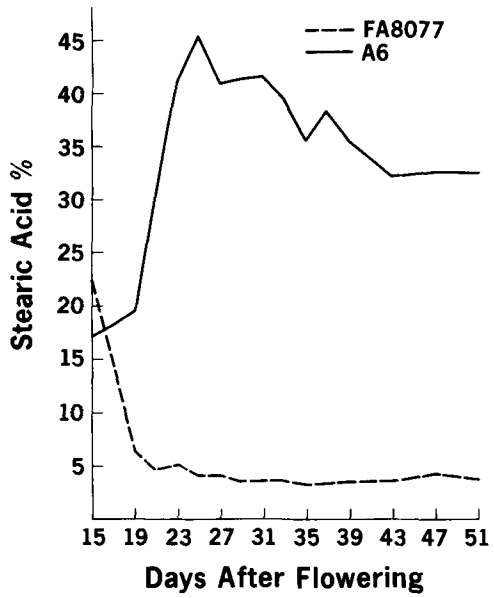

FIG. 4. The stearic acid percentage of A6 and its parent FA 8077 during seed development.

ences between the reciprocal crosses in Table V shows that there was no maternal effect and that the genotype of a seed determined its fatty acid composition. The 3:1 phenotypic ratio observed among F_2 individuals, as shown in Figure 5, indicated that the trait was controlled by alleles at a single locus. The allele for high stearic acid is recessive to the allele for low stearic acid. This was confirmed by evaluation of the progeny of F_2, which resulted in a 1:2:1 genotypic ratio (Table VI).

It is possible to manipulate the proportion of some fatty acids over a rather wide range by traditional plant breeding techniques. Success in altering the fatty acid composition of plants (5,6,10,11,12) suggests that variation can be achieved whenever adequate effort is applied to the problem. Because the fatty acid composition can be altered so drastically with no obvious ill effects to the plant, one wonders why most oil-seed plants traditionally have had such a restricted range of compositions. The possibility of altering the glyceride structure of plants seems much poorer (13,14). This may be because the glyceride distribution reflects the requirements of phosphatide synthesis, and the phosphatides have a structural role that limits the extent to which their glyceride distribution can be altered.

The substitution of fats and oils from various sources for each other has been a traditional goal of food technology. The increasing ease with which fatty acid composition can be changed by breeding will intensify this trend.

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TABLE V

The Percentage of Stearic Acid Found in A6, FA 8077, and Their Reciprocal Crosses in F_1.

Genotype	Stearic Acid(%)
A6	30.1
A6 x FA8077	7.6
FA8077 x A6	8.6
FA8077	4.9

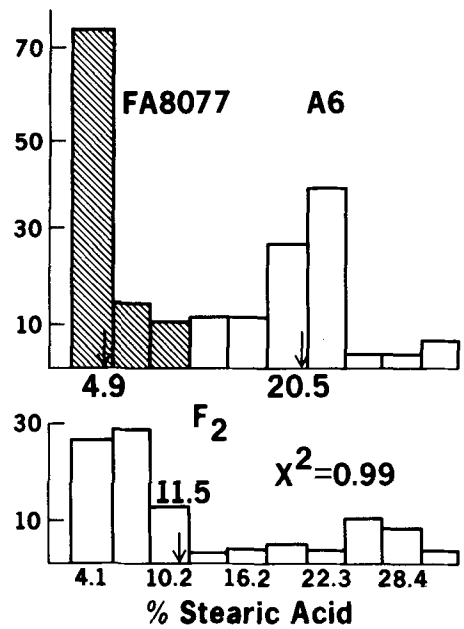

FIG. 5. The distribution of stearic acid percentages in A6, FA 8077, and F_2 progeny of the cross between them. The arrows indicate midclass percentages for each group. The Chi-square was not significant, indicating that the data satisfactorily fit a 3:1 ratio of low-stearic phenotypes to high-stearic phenotypes.

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