Improving Soybean Quality by Plant Breeding[1,2]

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ABSTRACT

Treatment of soybean seeds with x-rays has been successful in increasing the incidence of low-linolenic-acid soybean strains both in pure breeding lines and crosses of low-linolenic-acid lines. The incidence of strains high in oleic acid also is increased by x-ray treatment. A procedure for measuring the lipoxygenase content of seed is described. There is a 2- to 3-fold variation in the lipoxygenase activity of the soybean lines tested. The lipoxygenase activity of soybean seed is influenced by the genotype of the maternal parent.

The relatively poor flavor stability of soybean oil results in its commanding a lower price than other similar vegetable oils, and limits its acceptance in products where flavors contributed from oils are particularly noticeable. In spite of this, soybean oil has captured more and more of the U.S. oil market. This growing market has resulted partly from improved technology, partly from the low price of soybean oil, and partly from a gradual acceptance by the American public of the off flavors typical of soybean oil. Other populations may be less tolerant of the flavor of soybean oil, and food preparation habits in other countries may put unusual stresses on the flavor stability of soybean oil. Undoubtedly a more stable oil would also be welcomed by the American public.

The poor flavor stability of soybean oil has been attributed to the presence of linolenic acid (1). There is usually 7-8% of this acid in soybean oil while in more stable oils, such as corn oil, there is less than 1% linolenic acid. Experiments in which the linolenic acid level of soybean oil has been varied suggest that the flavor stability of the oil increases as the linolenate level decreases below 3%, but for good flavor stability a linolenic level of 1% or less is necessary (2,3).

The use of soybeans as human food is also limited by the presence of the enzyme lipoxygenase. This enzyme rapidly oxidizes the linoleic and linolenic acids present in soybeans. It is not certain that this enzyme contributes to the off flavor and instability of soybean oil, but it does cause problems in the preparation of many soy-based foods, giving rise to beany flavors that are not welcome at least to occidental tastes. The function, if any, of this enzyme is unknown.

In a breeding program at Iowa State University, we have been investigating the possibility of breeding soybeans with low levels of linolenic acid and lipoxygenase. This paper is a progress report of our work to date.

METHODS

To obtain a representative sample of seeds for fatty acid analysis, 5-10 beans from each strain evaluated were ground in a Wiley Mill. About 150 mg of meal was measured with a standard size scoop, and 2 ml of hexane was added. The hexane and meal were left in contact for at least 2 hr, with occasional shaking. The hexane extract was then decanted and shaken with 1 drop of 1 M sodium methoxide in methanol for 5 min using a vibratory test tube mixer (4). Then 0.5 ml of water was added, and the mixture was centrifuged. About 2 μliters of the upper layer was injected into the gas chromatograph (Beckman GC-5 fitted with hydrogen flame detectors). The column was 2 m long, 3.2 mm OD, and packed with 15% EGSS-X on 100:120 mesh Gas Chrom P. The areas under the peaks were determined with a Disc integrator. The instrument was checked frequently with standard ester mixtures, but when the detector was clean no correction was required.

Lipoxygenase was measured by the rate of oxygen uptake using a Beckman oxygen analyzer. The electrode was inserted in the side of a specially modified 50 ml vessel. The substrate was a soybean oil emulsion made by blending 10 g of soybean oil and a solution containing 1.25 g of gum acacia in 100 ml of water. One milliliter of the substrate emulsion was added to the vessel, and then the vessel was filled with 0.01 M phosphate buffer at pH 8. The buffer was saturated with oxygen, and the buffer and apparatus were at 25 C. The reaction was started by the addition of 50 mg of fine (through 40 mesh) soybean meal. The output from the oxygen analyzer was plotted on a strip-chart recorder. The slope of the oxygen pressure vs. time plot was linear over a considerable portion of the range, and the linear portion was used as a measure of the lipoxygenase activity. To minimize variation the same soybean oil was used for all determinations, and the activity of the samples was expressed as the ratio of the slope of the sample to that of a standard batch of Hawkeye soybean meal.

[1]One of seven papers presented at the Symposium, "The Plant Geneticist's Contribution Toward Changing Lipid and Amino Acid Composition of Oilseeds," AOCS Meeting, Houston, May 1971.

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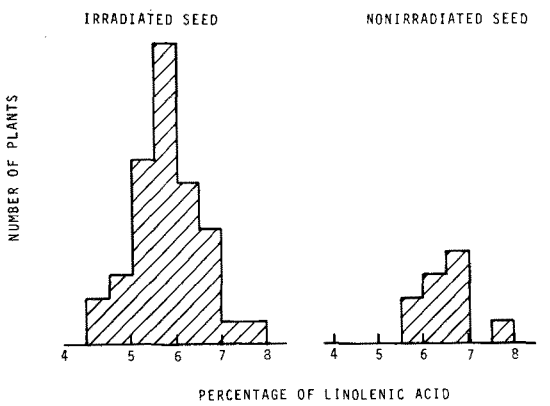

FIG. 1. The distribution of linolenic acid percentages in a pure breeding line of soybeans before and after irradiation.

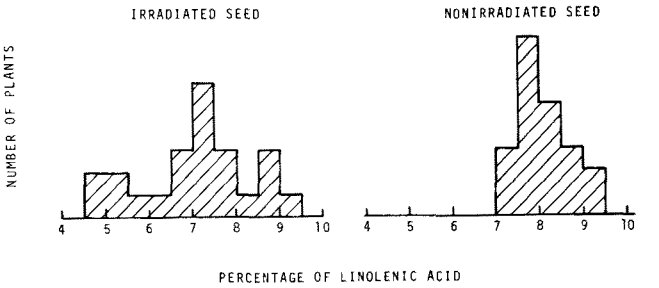

FIG. 2. The distribution of linolenic acid percentages in the progeny of irradiated and nonirradiated seed from a cross of two low-linolenic acid lines.

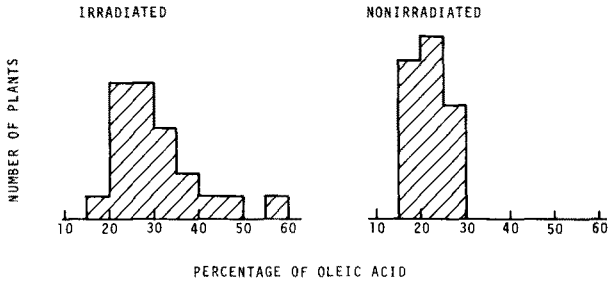

FIG. 3. The distribution of oleic acid percentages in the progeny of irradiated and nonirradiated seed from a cross of two low-linolenic acid lines.

RESULTS AND DISCUSSION

Fatty Acid Composition

We examined a number of soybean strains on hand at Iowa State University, and we asked soybean breeders throughout the U.S. for strains that they thought could be low in linolenic acid. These searches revealed that there are relatively few strains with less linolenic acid than commercial varieties. Extensive screening of commercial soybean cultivars and plant introductions by the U.S. Regional Soybean Laboratory, Urbana, Ill., had shown the same result. There are a few strains that produce as low as 5% linolenic acid and a few others that produce in the 5% and 6% region. It is easier to find strains high in linolenic acid, and it appears that it would be easier to breed for a high than a low-linolenic-acid oil.

One of the difficulties in breeding soybeans for low linolenic acid is the maternal effect on fatty composition. Hybrid soybean seeds have the genetic makeup of two parental strains, and one would expect the fatty acid composition of the hybrid seed to reflect contributions from both the maternal and paternal plant. It has been reported that the oil composition reflects the contribution of the maternal plant much more than that of the paternal parent (5). In analyzing seed from a particular genotype we are evaluating the genetic constitution of the maternal plant more than the genetic constitution of the seed. The maternal effect makes analysis of single hybrid seed a futile effort.

In Iowa we can grow only one soybean crop each year. To speed up the selection program we grow an additional two crops of beans in Puerto Rico during the winter months. But this method of speeding up selection is not without its disadvantages. The oil composition of soybeans is quite dependent on the environment in which they are grown. For example seeds of pure-breeding strains were planted in Puerto Rico in 1970. Sample 1 was planted November 1 and flowering was delayed with artificial lighting until January 1. Sample 2 was planted December 1. It did not have artificial light, so it also flowered January 1. Even though flowering and seed development occurred during the same time and in the same environment with respect to temperature and rainfall, seed harvested from the lighted areas were 1-2% lower in linolenic acid than those from the unlighted areas. So to really tell if a cross is lower in linolenic acid than its parents, it is important to grow the parental strains under the same conditions for comparison. We believe that such comparisons are valid and will predict what will happen when the plants are grown in Iowa.

Fatty acid composition also may be influenced by the source of seed planted. It has been shown that seed harvested in one environment may produce plants that perform differently from seed of the same genetic makeup harvested in another environment (6). To minimize this potential problem we plant seed of parent strains harvested from the same environment as the test genotypes.

Because relatively few low-linolenic breeding lines could be discovered among plant introductions, x-rays and ethyl methylsulfonate are being used to induce mutations. The work with x-rays has given some promising results. Seed from pure-breeding, low-linolenic-acid strains were irradiated with 10, 15, 20 and 25 kr, planted and grown to maturity. Some plants in the irradiated populations produced seed that were significantly lower in linolenic acid than those from control plants grown under the same conditions. A distribution curve of the percentage linolenic acid of plants grown from irradiated and nonirradiated seed is shown in Figure 1. So far we have been able to complete analyses on one additional generation of some of these seed. Some of the progeny of these irradiated seed have tested 2% lower in linolenic acid than the untreated control plants. We hope that the low-linolenic-acid progeny will remain low when they become true-breeding lines.

We also have tested the effect of x-ray treatment on seed from the cross between two low-linolenic-acid parents. Figure 2 shows a frequency distribution of the linolenic content of F_2 plants from irradiated and nonirradiated seed from the cross of two low-linolenic-acid lines. The fraction of plants with progeny low in linolenic acid was dramatically increased by irradiation. We do not yet know if the low-linolenic acid effect will remain when these become pure-breeding strains.

The absolute value of the linolenic acid percentage appears high in Figure 2, especially in the nonirradiated crosses. This is because the beans were grown in Puerto Rico. The parental strains in this cross were 1-2% higher in linolenic acid when grown in Puerto Rico than when grown in Iowa.

Because our most recent results are on Puerto Rican seed, and because our material is still segregating, any statement of progress in breeding low-linolenic acid lines must be tentative. But our results nourish the hope that some of our current lines will be as low as 3% linolenic acid when grown in Iowa.

One interesting sidelight is that there is considerable genetic variation in the proportions of oleic and linoleic acid in soybean oil. Those strains low in linoleic and high in oleic seem low in linolenic acid in many instances. For example, in a group of 34 strains selected for high oleic acid, 24 also were selected independently for low linolenic acid. Irradiation also raises the oleic acid percentage (Figure 3). Seed with oleic acid as high as 64% have been found. A high oleic content should also help confer stability to

TABLE I

Lipoxygenase Levels in Crosses of High and Low-Lipoxygenase Strains

	Maternal strain			Maternal strain	
Entry	C1463	Beeson	Entry	A67-3544	Amsoy
Parent	0.78[a] ± 0.02[b]	1.24 ± 0.08	Parent	0.80 ± 0.02	1.17 ± 0.05
C1463 X Beeson	0.89 ± 0.04	—	A67-3544 X Amsoy	0.92 ± 0.04	—
Beeson X C1463	—	1.20 ± 0.04	Amsoy X A67-3544	—	1.04 ± 0.03

[a]Each lipoxygenase assay value is an average of at least eight analyses.

[b]Standard error of the mean.

soybean oil.

There is less variation in the percentage of palmitic and stearic acids than that of oleic acid in soybeans. Some strains as high as 14% palmitic acid have been found. Irradiation tends to reduce the percentage of palmitic acid in the oil.

The discovery of rape seed devoid of erucic acid has led to some hope for a soybean oil that has no linolenic acid. It is unlikely that we will find soybeans without the ability to synthesize linolenic acid, as this fatty acid is probably needed in the construction of the chloroplasts (7). Soybeans and other oil seeds produce lots of linolenic acid during their early growth. Their lipid may be as high as 30% linolenic acid in the early stages. As they ripen the proportion of linolenic acid decreases. With corn it goes below 1% (8,9); with soybean it levels off at a higher value. We do not understand why or how this change in fatty acid composition occurs. Possibly the fatty acid composition of the seed is regulated by hormones secreted by the plant. This is probably the basis of the maternal effect discussed above. The fatty acid composition of the seed may be strongly influenced by the amount and kind of maternal hormone, and this may override variations in the genetic makeup of the seed. In breeding probably we are not selecting for plants that have impaired ability to synthesize linolenic acid, so much as for plants that regulate the linolenic acid content of their seed downward more rapidly and completely as the seeds mature.

Lipoxygenase

Evidence has been presented that there is more than one kind of lipoxygenase in soybeans, specifically there may be one that has a higher activity on triglycerides than on free fatty acids and another that attacks free fatty acids and has much less activity on esters (10). Since the off flavors that result from lipoxygenase activity in soybeans are probably caused by the action on triglycerides rather than the minute amounts of free fatty acids present, we devised an assay based on esterified substrate. We also wanted a test that would be rapid and simple and that could be applied to many small samples for breeding purposes. The procedure given in the method section gives results related to the amount of lipoxygenase present, although the values obtained are not a linear function of the lipoxygenase level. Survey of a modest number of soybean strains has revealed about a 2-fold variation in lipoxygenase activity. Because our assay is not linear with lipoxygenase content, this might translate into as much as a 3-fold variation in actual lipoxygenase. Interestingly most commercial strains of soybeans are quire high in lipoxygenase. It is not known if this confers any advantage on the plant.

The lipoxygenase content of hybrid soybean seed is influenced by the genetic makeup of the maternal plants. Table I shows the results of a cross of low and high lipoxygenase strains. The activity of the hybrid seed is dependent on the parent used as the female. The hybrid seed must have the same genetic content in the cell nucleus, regardless of the way the cross is made. Each of the figures in the table represents the average of several assays done on each single bean from the cross.

So far we have not tested many crosses to see if the lipoxygenase content can be reduced significantly by breeding. It would seem however that the lipoxygenase level of many commercial strains could be reduced by as much as 50% without great difficulty.

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