The Plant Geneticist's Contribution Toward Changing Lipid and Amino Acid Composition of Soybeans

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ABSTRACT

Interest in changing composition of soybeans focuses on linolenic acid in the oil. Available germ plasm includes no lines with less than about 3.5% linolenic acid in the oil. Some breeding lines have been obtained with iodine values of about 115, reflecting higher oleic acid and somewhat lower than normal linoleic and linolenic acid levels. Radiation and other mutagenic agents have been investigated to a limited extent for potential usefulness in inducing a mutation in the direction of low linolenic acid. No such mutant has yet been found, and the task of identifying one is formidable. There is no research known at this time with the objective of altering amino acid distribution in soybeans.

Soybean breeders routinely evaluate experimental lines for oil and protein, and consider oil and protein contents when deciding which lines merit release as varieties. However improvement in agronomic characters such as yield or disease resistance have had higher priority than changes in composition.

Among the structural units of soybean oil and protein, linolenic acid is unusual in that it is not present in significant quantities in other major edible oils. This component of soybean oil has been implicated as a major cause of flavor problems. However Smouse and Chang (1) have concluded that 2-pentyl furan is predominantly responsible for the reversion flavor of soybean oil and have postulated that it originates from linoleic acid. By contrast Chang et al. (2), reported that 2-ethyl furan, derived from linolenic acid, does not contribute to the reversion flavor of soybean oil. Smouse and Chang propose that linolenic acid catalyzes the autoxidation of linoleic acid, and possibly alters the decomposition pattern of the hydroperoxides. Thus flavor reversion is a problem in soybean oil but not in other oils which contain similar amounts of linoleic acid as soybean oil.

Most interest in quality improvement focuses on linolenic acid. The processor and refiner have procedures available for treatment of soybean oil to reduce linolenic acid. A better solution would be the development of varieties containing an inherently more stable oil. We assume that an oil with much less linolenic acid than present varieties–ideally no linolenic acid–would be such an oil. What progress has been made toward such a goal and what can be expected?

The first step in a plant breeding program is a search of available germ plasm for the desired trait. The U.S. soybean germ plasm collection has been searched for zero or low linolenic acid. No available genotype produces an oil with less than about 3.5% linolenic acid. An oil with 3.5% linolenic acid would represent about a 50% reduction from the 7-8% that characterizes present soybean oil. But we have been advised that a much greater reduction–to 1% or less–would probably be needed to be of much significance to oil quality. If, as Smouse and Chang (1) suggest, the role of linolenic acid in flavor reversion is essentially a catalytic one, a very low level may indeed be enough to cause trouble.

Thus we do not have in our present germ plasm collection, numbering about 4000 entries, the ideal germ plasm for a routine breeding program to eliminate linolenic acid. Our study of the germ plasm collection, however, has revealed some variability in oleic acid. Higher oleic acid percentage seems to be associated with lower percentages of linoleic and linolenic acids. Table I presents data which demonstrate an increase in oleic acid as the percentages of linoleic and linolenic acids are reduced through breeding. The selections from the cross of PI 90,406 x PI 92,567, shown in Table I, have 10-15 point lower calculated iodine values than the parental lines.

Distribution of oleic acid and linolenic acids in the F_2 population from a different cross, PI 157,406 x Peking, is shown in Figure 1. The distribution is unimodal, suggesting that inheritance of oleic and linolenic acids in soybeans is not simple. One might expect a fairly simple pattern of inheritance, however, from genetic analogies to other crops and biochemical considerations. Poneleit and Alexander (3) presented data on fatty acids in corn which suggested that desaturation of oleic at the 12 position is under simple Mendelian control. Two genes are thought to be involved in the change from high to low erucic acid in rapeseed oil (4). One might expect one enzyme to be involved in the

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

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TABLE I

Fatty Acid Composition, %, and Iodine Values of Parents and Progeny

Genotype	Oleic	Linoleic	Linolenic	IV[a]
Parents				
PI 90,406	32.8	46.4	7.0	132.7
PI 92,567	34.9	44.4	6.4	130.1
F_4 Selections from PI 90,406 x PI 92,567				
N70-3432	42.0	38.3	4.8	120.3
N70-3010	37.9	40.6	5.2	121.9
N70-3001	37.4	37.1	5.4	115.6

[a]Calculated.

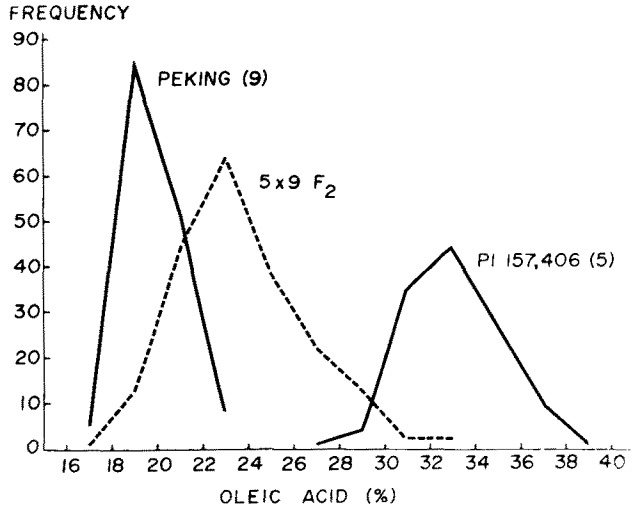

FIG. 1. Oleic acid distribution in soybean oil from Peking, PI 157,406, and in the F_2 progeny from PI 157,406 x Peking cross.

oleic-linoleic conversion and another in the linoleic-linolenic conversion.

$$\text{Oleic} \xrightarrow{E_1} \text{Linoleic} \xrightarrow{E_2} \text{Linolenic}$$

Inkpen and Quackenbush (5) showed that cell-free preparations of immature soybean cotyledons contain a 9-desaturase which actively desaturates stearate or palmitate to produce 9,10-unsaturated acids. Fractions which produced monoenoic acids also effected further desaturation to polyunsaturated compounds. Inkpen and Quackenbush did not report whether both dienoic and trienoic acids were formed. Of the major oilseed crops, only soybeans and flax have substantial amounts of linolenic acid in the oil. It would seem therefore that a desaturase specific for the 15 position is required to form linolenic from linoleic acid.

This suggestion is supported by genetic analysis of the data in Figure 1. The data do not fit any simple model of inheritance. Our conclusion at this time is that more than three genes are involved in linolenic acid synthesis and that they act in an additive, possibly sequential, way. Such a genetic system would be consistent with a pathway such as

$$\text{Precursor} \xrightarrow{E_n} \text{saturated} \xrightarrow{9_{DS}} \text{oleic} \xrightarrow{12_{DS}} \text{linoleic} \xrightarrow{15_{DS}} \text{linolenic}$$

and with the observation of Inkpen and Quackenbush that it is not necessary to consider a pathway for biosynthesis of unsaturated fatty acids separate from that for saturated acids.

Thus one or more genes would contribute to formation of the saturated acids, and one each would be associated with the 9-, 12- and 15-desaturases.

Based on results such as those shown in Table I and Figure 1, we are encouraged to try to increase selection pressure on populations to produce a genotype with low linolenic, high oleic, or both. We plan this year to increase one of our present high oleic acid lines to provide enough oil for at least a tentative evaluation of the stability and other properties of such oil.

The environmental effect on oil content and quality in soybeans is substantial. Thus a direct genetic analysis is difficult to obtain. Oil content of soybeans increases as temperature increases during the period of seed development. Similarly linolenic and linoleic acids decrease as temperature increases (Table II).

The environmental effects complicate the problem for the geneticist and breeder seeking to understand the inheritance of fatty acids and to manipulate them for his own ends. A further complication is that oil content and fatty acid composition of seed is determined by the maternal parent, even though the genotype of the seed is determined by contributions from both parents. That is, the composition of the seed is determined by the genotype of the plant on which the seed is borne rather than by the genotype of the seed itself. Brim et al. (6) and Singh and Hadley (7) concluded that selection for oil content and unsaturated fatty acid composition on individual F_2 seeds would be largely ineffective. This differs from the situation in corn where the pollen parent may significantly influence oil content of the seed (D.E. Alexander, personal communication).

TABLE II

Effect of Temperature on Fatty Acid Content of Soybean Oil[a]

Temperature	18:2, %	18:3, %
70F	49.5	11.8
80F	45.4	7.4
90F	35.3	4.7

[a]Howell and Collins (8).

The strong maternal influence in soybeans for these traits does not mean that progress from selection cannot be attained but rather that selection must proceed at a slower pace. Thus F_3 seed produced on F_2 plants, rather than the F_2 seed itself, must be used to reveal the genotypic array in the F_2 generation. The breeder must recognize the limitations imposed by the interaction of environment and genotype and make every effort to minimize the bias in genetic and selection studies.

The possibility of varietal differences in temperature sensitivity is suggested by earlier data (8), but has not been explored further.

MEANS TO INCREASE GENETIC VARIABILITY

There are means of increasing the genetic variability which may be considered. These include the use of mutagenic agents—radiation or chemical mutagens such as colchicine or EMS, and interspecific hybridization.

F.I. Collins of the U.S. Regional Soybean Laboratory, now retired, has treated soybean seeds with soft x-rays in dosages from 50-1000 kr. There were no significant effects on fatty acid composition of oil from irradiated progeny over three seasons. W.R. Fehr and E.G. Hammond of Iowa State University are also seeking a low linolenic acid soybean and have included radiation treatments in their studies. Dr. Hammond discusses their work in an accompanying paper.

Note that Collins, and Fehr and Hammond irradiated the seed. Most evidence indicates that seeds are less sensitive to the effects of radiation than are growing plants. A paper by Bottino and Sparrow (9) reports that bean plants are most sensitive to radiation during the bud stage prior to flowering. Perhaps this should be expected since reproductive cells at that time are undergoing reductional division in

TABLE III

Fatty Acids of Oil From *Glycine* Species[a]

Species	18:1	18:2	18:3	IV
G. clandestina	29	31	13	117
G. falcata	17	37	14	120
G. tabacina	30	33	11	117
G. tomentella	28	26	9	97
G. max	24	49	8	132
G. wightii	10	38	27	151

[a]Fatty acid data of Hymowitz et al. (10). IV calculated by present authors.

the process of forming egg and sperm cells. Bottino and Sparrow used low radiation levels such as those to be expected from fallout. Use of such radiation as a mutagenic treatment of soybean plants at the reproductive stage deserves consideration.

Radiation studies to date have involved relatively small populations of plants. One may need to examine thousands, perhaps hundreds of thousands, of individuals to find a desired mutant which occurs in very low frequency. Mass screening techniques are being used to search large populations for some characters. At present we lack a technique for mass screening of large populations for fatty acid mutants. Conditions would have to be rigidly controlled to eliminate environmental effects. Analysis of 100,000 samples would require possibly 20,000 hr of gas liquid chromatography time and many more man-hours to grow and harvest the plants and to prepare samples. Thus, while we do not rule out use of mutagenic agents, we should not underestimate the task of finding a desirable mutant.

INTERSPECIFIC HYBRIDIZATION

Hymowitz et al. (10) have analyzed seed of *Glycine* species other than *G. max*, the cultivated soybean. Values which they reported are presented in Table III. None of the species had a linolenic acid level lower than *G. max*. All were lower in linoleic acid and one, *G. tomentella*, had a calculated IV of 97. Most species were represented by one or a few accessions. However *G. wightii* was represented by 84 accessions, and ranges of fatty acid values as follows: oleic, 7.3-17.7%; linoleic, 28.8-46.2%; linolenic, 17.2-33.5%.

The results of Hymowitz et al. do not favor interspecific hybridization as a means of producing a low linolenic acid soybean. Even if a low linolenic relative were found, there would be formidable barriers to transferring such a character to a variety of commercial acceptability. None of the species crosses readily with *G. max*. While greater effort could be made to find genetic bridges between species, it does not appear that doing so would increase chances of developing a low linolenic acid soybean.

AMINO ACIDS

There has been much less interest in protein or amino acid characteristics of soybeans than in fatty acids. Progress in developing high protein varieties, two of which have been released, was discussed at the Chicago AOCS meeting in September 1970. Additional high protein varieties may be released.

At a recent conference of soybean workers, there was no indication of any current soybean breeding research to modify amino acid composition of soybean protein. Soybean protein is not deficient in lysine, the amino acid of greatest concern in the cereals and most other seeds. The limiting amino acid in soybeans is methionine, which can be added to soybean feeds or products at reasonable cost. There is little incentive therefore for breeding or genetic research to modify amino acid distribution in soybeans.

POSSIBLE CHANGE IN GRAS LIST

A proposal of some interest with respect to possible changes in composition of crops through breeding is the proposed change in the list of substances generally recognized as safe (GRAS) by the Food and Drug Administration. The proposed change would impose a requirement for individual reviews of pertinent data on foods that have had a significant alteration of composition by breeding or selection, where there is reason to believe that changes in composition could affect safety or nutritional value of the food.

REFERENCES

1. Smouse, T.H., and S.S.Chang, JAOCS 44:509 (1967).
2. Chang, S.S., R.G. Krishnamurthy and B.R. Reddy, Ibid. 44:159 (1967).
3. Poneleit, C.G., and D.E. Alexander, Science 147:1585 (1965).
4. Stefansson, B.R., and F.W. Hougen, Can. J. Plant Sci. 44:359 (1964).
5. Inkpen, J.A., and F.W. Quackenbush, Lipids 4:539 (1969).
6. Brim, C.A., W.M. Schutz, and F.I. Collins, Crop Sci. 8:517 (1968).
7. Singh, B.B., and H.H. Hadley, Ibid. 8:622 (1968).
8. Howell, R.W., and F.I. Collins, Agron. J. 49:593 (1957).
9. Bottino, P.J., and A.H. Sparrow, Crop Sci., 11:436 (1971).
10. Hymowitz, T., F.I. Collins, V.E. Sedgwick and R.W. Clark, Trop Agr. 47:265 (1970).

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