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Inheritance of low linolenic acid content of the seed oil of a mutant in *Glycine max* *

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Summary. Linolenic acid content of the oil from F_1, F_2, and F_3 seeds was compared with the parental values from a cross between a soybean cultivar with high (7.0%) and a mutant line with low (3.4%) linolenate (18:3). Linolenic acid content of F_1 seeds was intermediate to that of selfed seeds from the two parents and values from reciprocal crosses were essentially the same. This demonstrated that in this cross, linolenic acid content of the oil was controlled by the embryo rather than by the maternal parent. The distribution of linolenic acid in F_2 seeds from F_1 plants was trimodal and extended across the range of parental values. High and low linolenate F_2 plants bred true for 18:3 content and the F_3 distribution of seeds from F_2 plants with intermediate levels of 18:3 was similar to the F_2 distribution. The data were consistent with a model for two alleles with additive effects at a single locus controlling percent linolenic acid in these progenies. The simply-inherited alleles for low linolenate could be readily transferred to agronomically superior soybean cultivars, which would improve the fatty acid composition of the oil.

Key words: Soybean – Linolenic acid – Fatty acids – Oil quality

Introduction

Fatty acid composition of the oil from several oilseed crops generally is simply inherited and controlled by the genotype of the embryo rather than the genotype of the maternal parent.

Erucic acid content of rapeseed, *Brassica napus* L., oil is controlled by two independent genes acting in an additive manner (Harvey and Downey 1964). Analyses of the distribution of oleic and linoleic acid in the oil of backcross and F_2 populations of *Zea mays* L. suggested monohybrid inheritance for these two fatty acids (Poneleit and Alexander 1965). Knowles and Hill (1964) reported three alleles at one locus that controlled the oleic and linoleic acid contents of safflower, *Carthamus tinctorius* L., oil. The homozygous gene *ol* resulted in low oleic acid (10–15%) and high linoleic acid (75–80%). The gene *ol* in the homozygous condition resulted in high oleic acid (64–83%) and low linoleic acid (12–30%). Seeds homozygous for ol^1 contained intermediate levels of both fatty acids (34–50% oleic, 42–54% linoleic). Seeds heterozygous for the above alleles were intermediate to the homozygotes for levels of the two fatty acis. The fatty acid composition of seed oil in flax, *Linum usitatissimum* L., is determined largely by the genotype of the seed itself and to a limited extent by the genotype of the maternal sporophyte (Yermanos and Knowles 1962).

Two studies with soybean, *Glycine max* L. Merr., have indicated that fatty acid composition of the oil is determined primarily by the genotype of the maternal parent (Brim et al. 1968; Martin et al. 1983). White et al. (1961) evaluated F_2 and F_3 progenies from crosses between soybean strains with high and low levels of linolenic acid. Their data indicated that inheritance of both linoleic and linolenic acids was quantitative rather than qualitative.

A low linolenic acid mutant of *Glycine max* was identified as an M_2 plant from a population of Century soybeans treated with ethyl methanesulfonate (Wilcox et al. 1984). The linolenic acid contents of progenies from two generations of selfing the M_2 plant remained consistently low. The objective of this study was to determine the inheritance of linolenic acid in this mutant.

Materials and methods

In 1982 at Lafayette, Indiana, adjacent rows, spaced 1 m apart, were planted to 'Century' and to 'C1640', the low linolenic acid mutant. Emerged seedlings were thinned to a within-row spac-

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ing of 10 cm. Reciprocal crosses were made between the two parents and self-pollinated flowers were tagged on each parent at the sixth through the eighth nodes. At a single node on a plant, one flower bud was emasculated and pollinated 1 day prior to anthesis. A second, self-pollinated flower at the same node was retained and all other flowers and buds at the node were removed. Nine or 10 cross- and self-pollinations were made on each parent. At maturity, the crossed and selfed seeds were identified and harvested by node. Nine or ten individual seeds were destructively analyzed for fatty acid composition of the oil.

Both parents and an F_1 plant from the cross 'Century' × 'C1640', from greenhouse crosses, were grown in 1982 and seed harvested from the mature plants. Fragments, about 60 mg in size, of 19 seeds of 'Century', 20 seeds of 'C1640', and 160 F_2 seeds from the F_1 plant were individually analyzed for oil composition. The fragments, distal to the embryonic axis, were sliced from the seed using a hand-held razor blade. The remnant of each seed, about 120 mg containing the embryonic axis, was planted in 0.3 l styrofoam cups of soil, then transplanted to the field at the unifoliolate stage in the spring of 1983. Seeds were harvested from the two parents and from the F_2 plants at maturity. Six seeds from 3 plants of 'Century' and of 'C1640', and 12 seeds from 30 F_2 plants were analyzed individually for fatty acid composition. Five of these 30 F_2 plants were selected from seeds with high, 5 selected from seeds with low, and 20 selected from seeds with intermediate linolenic acid (18:3) content, based on analyses of F_2 seed fragments.

To extract and esterify the fatty acids, each seed or seed fragment was cut into thin slices, macerated in the presence of 2 ml sodium methoxide (1 g sodium dissolved in 100 ml ethanol), then allowed to stand for 15 min at room temperature. One-half ml of 10% acetic acid in water was added, and the resulting solution was extracted with 2 ml heptane. The heptane solution was analyzed for methylester composition on a Varian 3,700 gas chromatograph using a 1 m × 2 mm column packed with 5% LAC-2-R-446 on 100/120 mesh Gas/chrom 0 as described previously (Wilcox et al. 1984).

Differences between means of cross- and self-pollinated seeds at the same node were evaluated using *t*-tests for paired comparisons. *T*-tests for unpaired observations were used to compare means between reciprocal crosses and to compare means of cross-pollinated seeds with self-pollinated seeds of the parental plant. Chi-square analyses were computed to test goodness-of-fit of the data to the hypothesized genetic ratio.

Results and discussion

Oil composition of 'Century' and 'C1640' did not differ significantly in palmitic (16:0), stearic (18:0), or linoleic acids (18:2) based on the unpaired *t*-tests (Table 1). Analyses of individual selfed seeds from 'Century' and 'C1640' showed that they were significantly different in both oleic (18:1) and linolenic (18:3) acid content of the oil (Table 1). Linolenic acid content of the oil from F_1 seeds was intermediate to that of the two parents and was essentially the same for reciprocal crosses. Average linolenic acid content of 160 F_2 seeds (5.25%) was approximately intermediate to that of 'Century' ($7.73 \pm 0.18\%$) and 'C1640' ($3.35 \pm 0.12\%$) (Table 2). The linolenic acid content of the oil from F_2 seeds ranged from 2.7 to 9.3%, extending across the range in linolenic acid contents of both parents (Fig. 1).

These data indicated that linolenic acid content of the oil was determined by the embryo rather than by the maternal parent. The difference in results reported in this study from those of previous studies may be associated with differences in germplasm. Previous studies have been based on naturally occurring variability for fatty acid composition in soybean (Brim et al. 1968; Martin et al. 1983). The data in this report are from a chemically induced mutant that may represent a major genetic change in control of linolenic acid synthesis in 'Century' soybeans.

The range in linolenic acid content of 5 F_2 seeds selected for low 18:3 values was from 2.7 to 3.1%; for the 5 F_2 seeds selected for high 18:3 values it was from 7.4 to 8.4%, and of the 20 F_2 seeds selected for intermediate values it was from 4.8 to 6.2%. Distributions for linolenic acid of the 360 F_3 seeds from these plants, plus the distribution of selfed seeds from the two parents are shown in Fig. 2. Selfed seeds of 'C1640' and the F_3 seeds from the low 18:3 F_2 plants averaged $3.33 \pm 0.05\%$ and $3.54 \pm 0.04\%$, respectively. Selfed seeds of 'Century' and F_3 seeds from the high 18:3 F_2 plants averaged $6.83 \pm 0.14\%$ and $6.87 \pm 0.07\%$ 18:3, respectively. The distribution of 18:3 values were similar for 'C1640' and the F_3 seeds from low 18:3 F_2 plants and for Century and the F_3 seeds from high 18:3 F_2 plants. The standard errors associated with these means appear to represent random error associated with chemical analyses of seeds with the same genotype.

Distribution of 18:3 values of the F_2 population (Fig. 1) and of the 240 F_3 seeds harvested from F_2 plants with intermediate linolenic acid content was trimodal. The lack of segregation in the parental classes and the trimodal distribution of 18:3 content among F_3 seeds from F_2 plants with intermediate 18:3 content suggests segregation for two alleles with additive effects controlling linolenic acid content in this cross. 'Century', with high linolenic acid, would be homozygous for one allele; 'C1640' with low linolenic acid, would be homozygous for the other allele, and those plants with intermediate 18:3 content would be heterozygous for the two alleles.

Data on the distribution of linolenic acid content of F_2 and F_3 seeds were analyzed for their fit to a 1:2:1 ratio (Table 3). There was little difficulty separating the low 18:3 class from the intermediate class in the two generations. However, variability was greater among seeds of 'Century' in both years (SD = 0.77 and 0.54) than among seeds of C1640 in the two years (SD = 0.54 and 0.20), resulting in some overlap of the high and intermediate 18:3 classes. The mean ($\bar{x} = 6.49\%$) of 'Century' (7.73%) and of the F_2 population (5.25%) was used to separate the intermediate and high 18:3 classes in the F_2 population. Since linolenic acid contents were lower the second year when the F_3 seeds were produced, the mean ($\bar{x} = 5.95$) of 'Century' and the se-

Table 1. Mean ($\bar{x}$) ± standard error (SE) for fatty acid composition of oil in seeds of 'Century', 'C1640', and of seeds form reciprocal crosses between them

Parent or cross	Seeds no.	Fatty acids				
		Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
		%				
		$\bar{x}$ SE	$\bar{x}$ SE	$\bar{x}$ SE	$\bar{x}$ SE	$\bar{x}$ SE
'Century'	10	11.02±0.08	2.83±0.11	21.95±0.60	57.00±0.66	7.11±0.14
'Century'×'C1640'	10	11.02±0.07	2.70±0.09	22.04±0.42	58.58±0.50	5.58±0.10
'C1640'×'Century'	9	11.26±0.12	2.72±0.13	23.67±0.79	56.46±0.78	5.79±0.16
'C1640'	9	11.38±0.17	2.77±0.10	25.81±1.04	56.34±1.02	3.60±0.13

Table 2. Mean ($\bar{x}$) ± standard error (SE) and range in fatty acid composition of oil in seeds of Century, C1640, and in F_2 seeds of 'Century'×'C1640'

Parent or cross	Fatty acids				
	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
	%				
'Century'					
No. of seeds	19	19	19	19	19
$\bar{x}$ ± SE	11.07± 0.10	2.94±0.04	20.12± 0.62	58.17± 0.55	7.73±0.18
Range	10.1 – 11.8	2.6 – 3.3	18.0 – 28.6	50.3 – 61.0	6.6 – 9.5
'Century'×'C1640'					
No. of seeds	160	160	160	160	160
$\bar{x}$ ± SE	11.01± 0.03	3.06±0.02	22.14± 0.22	58.52± 0.23	5.25±0.13
Range	9.9 – 12.2	2.0 – 3.7	17.7 – 33.6	51.3 – 63.6	2.7 – 9.3
'C1640'					
No. of seeds	21	21	21	21	21
$\bar{x}$ ± SE	10.84± 0.10	3.12±0.06	25.77± 0.59	56.89± 0.63	3.35±0.12
Range	10.0 – 11.9	2.5 – 3.6	20.6 – 30.5	52.8 – 62.7	2.3 – 4.1

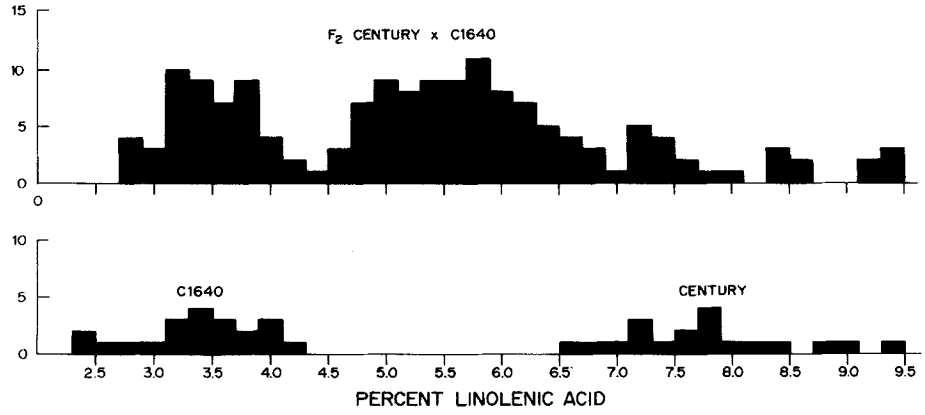

Fig. 1. Distribution of linolenic acid in seeds of 'Century', 'C1640', and in F_2 seeds of 'Century'×'C1640'

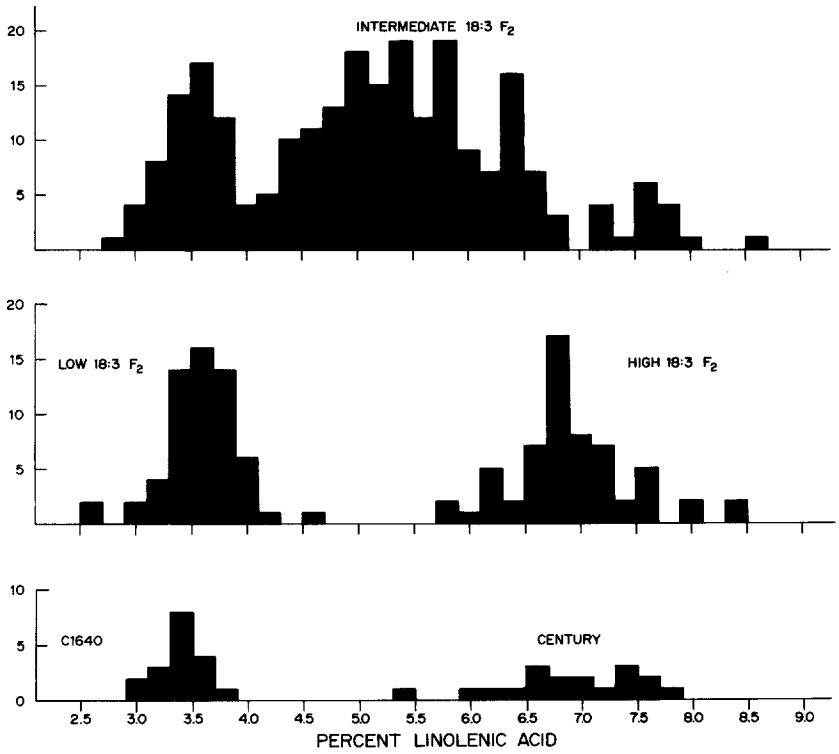

Fig. 2. Distribution of linolenic acid in seeds of 'Century', 'C1640', and of F_3 seeds produced on F_2 plants with low, intermediate, and high contents of linolenic acid

Table 3. Chi-square analyses of data on linolenic acid content of F_2 seeds and F_3 seeds of 'Century' × 'C1640'

Linolenic acid class	Expected no.	Observed no.	Chi-square 1:2:1	*P*
F_2 seeds				
2.3–3.9%	40	48		
4.0–6.4%	80	81		
6.5–9.5%	40	31	3.64	0.16
F_3 seeds				
2.9–3.9%	60	56		
4.0–5.9%	120	131		
6.0–8.5%	60	53	2.09	0.35

gregating F_3 population was used to separate the high and intermediate classes for the F_3 analyses. This effectively coded for differences in linolenic acid content of the two generations grown in successive years. Using this procedure, there was a good fit of the data to a 1:2:1 ratio for segregation of two alleles at a single locus.

The biochemical processes controlled by the alleles that result in different levels of linolenic acid are not known. Linolenic acid in soybean seeds is believed to be the final product in a sequential desaturation of oleic acid (Dutton and Mounts 1966). Genes identified in this study may regulate levels of an enzyme controlling successive desaturation in this pathway. Levels of oleic acid in the seed oil of 'C1640' were significantly higher than in the seed oil of 'Century'. Although the site of action of the gene was not clearly identified, the response was consistent with the hypothesized sequential desaturation of 18:1 to 18:3. Subsequent tests have identified no effect of this gene on oil quantity. In these tests 'Century' seeds have averaged 21.2% oil and 'C1640' seeds 21.9% oil.

This locus may be particularly useful in breeding soybeans with low linolenic acid. Because the locus is expressed in the embryo, selection for linolenic acid content based on analyses of F_2 seeds produced on F_1 plants would be effective. A fragment of an F_2 seed could be analyzed for fatty acid composition and the remainder of the seed grown only from those plants with low linolenic acid. These plants would be homozygous for the allele controlling low linolenic acid. In addition, since low linolenic acid is controlled by a single locus, this characteristic could be readily incorporated into other soybean cultivars and breeding lines. Linolenic acid has been associated with poor flavor and stability of soybean oil (Dutton et al. 1951). This gene provides the opportunity to improve the quality of soybean oil by lowering the linolenic acid content of the oil.

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