Theor Appl Genet (1997) 95:772–776

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Genetic control of high stearic acid content in seed oil of two soybean mutants

Received: 28 March 1997 / Accepted: 18 April 1997

Abstract Stearic acid is one of the two saturated fatty acids found in soybean [*Glycine max* (L.) Merr.] oil, with its content in the seed oil of commercial cultivars averaging 4.0%. Two mutants, KK-2 and M25 with two- and six-fold higher stearic acid contents in the seed oil than cv 'Bay', were identified after X-ray seed irradiation. Our objective was to determine the genetic control of high stearic acid content in these mutants. Reciprocal crosses were made between each mutant and 'Bay', and between the two mutants. No maternal effect for stearic acid content was observed from the analysis of F_1 seeds in any of the crosses. Low stearic acid content in 'Bay' was partially dominant to high stearic acid content in KK-2 and M25, and high stearic acid content in KK-2 was partially dominant to high stearic acid content in M25. Cytoplasmic effects were not observed, as demonstrated by the lack of reciprocal cross differences for stearic acid content in our analysis of F_2 seeds from F_1 plants. The stearic acid content in F_2 seeds of KK-2 × 'Bay' and M25 × 'Bay' crosses segregated into three phenotypic classes which satisfactorily fit a 1:2:1 ratio, indicating that high stearic acid content in KK-2 and M25 was controlled by recessive alleles at a single locus. The data for stearic acid content in F_2 seeds of the KK-2 × M25 cross satisfactorily fit a 3:9:1:3 phenotypic ratio. The F_2 segregation ratio and the segregation of F_3 seeds from individual F_2 plants indicated that KK-2 and M25 have different alleles at different loci for stearic acid content. The alleles in KK-2 and M25 have been designated as st_1 and st_2, respectively. The stearic acid content (> 30.0%) found in the $st_1st_1st_2st_2$ genotype is the highest known to date in soybean, but it was not possible to develop the line with this genotype because the irregular seeds failed to grow into plants after germination. Therefore, tissue culture methods must be developed to perpetuate this genotype.

Key words Soybean mutants · High stearic acid · Genetic control · st_1 and st_2 alleles · *Glycine max* (L.) Merr.

Communicated by P. L. Pfahler

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Introduction

Fatty acid composition and distribution differ in oil from different oilseed crops. The ratio and amount of saturated and unsaturated fatty acids determine the physical, chemical, and nutritional values of an oil (Gunstone and Norris 1983). Consequently, it is desirable to understand the variation observed in the different fatty acids. Stearic acid is one of the major saturated fatty acids in most seed oils. The percentages of stearic acid vary among the different oilseed crops, from 1.0% in rape seed oil to 3.6% in sesame and corn seed oils (Yasumoto et al. 1983). The average stearic acid content of soybean oil is 4.0%, with a range from 2.2% to 7.2% for the genotypes available in the world germplasm collection (Hymowitz et al. 1972; Downey and McGregor 1975).

Investigations are being carried out on soybean oil with a high content of stearic acid in order to determine possible uses. Oils with a higher content of saturated fatty acids generally have an increased melting temperature. Peroxide tests indicate that the stability of soybean oil with a high content of stearic acid is superior to that of oil obtained from currently available cultivars (Lundeen et al. 1987).

Limited genetic studies have been conducted on the stearic acid found in the seed oil of soybean. Graef et al. (1985) found that high stearic acid content in different mutants was controlled by different multiple recessive

alleles at a single locus, and these were designated as *fas*[a] for A6, *fas*[b] for FA41545, and *fas* for A81-606085. Bubeck et al. (1989) identified four high stearic acid mutants (ST1, ST2, ST3 and ST4) and crossed these with A6. They found that the alleles controlling high stearic acid in ST1, ST3, and ST4 occurred at the same locus as the *fas*[a] allele in A6, whereas the allele in ST2 may occur at a different locus. Maternal and cytoplasmic effects were not observed in these studies.

The mutant M25 with a high content of stearic acid has been evaluated for this characteristic and shown to be an important germplasm for the creation of soybean cultivars with high stearic acid (Rahman et al. 1995). Another high stearic acid mutant (KK-2) was developed from different M_2 populations by treating seeds of cv 'Bay' with X-rays (Takagi and Rahman 1995). The objective of the investigation described here was to determine the genetic control of high stearic acid content in these mutants.

Materials and methods

Mutants KK-2 and M25 and cv 'Bay' were sown in the greenhouse at 24°–30°C with 12-h day length at Saga University in February, 1993. Reciprocal crosses were made between each mutant and 'Bay', and between the two mutants. The seeds of the parents and reciprocal F_1s were sown in the field at Saga University in July, 1993. The seeds were space-planted 20 cm apart in rows and 60 cm between rows. Reciprocal crosses were made in the field as in the greenhouse. Each parent and F_1 plant was harvested individually.

Plants of the parents used for crossing in the field were identified, and selfed seeds were harvested from a node adjacent to the one from which the F_1 seed was obtained. Individual F_1 and parent seeds were analyzed for fatty acid composition in 20 replications of a randomized complete-block design. Each cross was an independent test, and each replicate of a test consisted of 1 seed from each of the parents and 1 seed from each of the reciprocal hybrids.

Results from the analysis of parent and F_1 seeds indicated no maternal effect for the stearic acid content in the mutants. This made it easy to determine the phenotype of individual F_2 seeds for stearic acid content in each cross. However, seeds of parents and F_2 seeds of F_1 plants were also collected from the pod at the fifth through seventh nodes of the main stem. Fatty acid composition was determined from 120 individual F_2 seeds of KK-2 × 'Bay', 100 F_2 seeds of M25 × 'Bay', and 192 F_2 seeds of the cross KK-2 × M25. Forty seeds of each parent also were analyzed individually.

For determination of phenotypic ratios, the whole F_2 seed was used for all crosses, except for KK-2 × M25, where each F_2 seed was divided into two parts with a razor blade. The part with the embryonic axis, used for planting, formed two-thirds of the seed, and the part with the cotyledon, used for fatty acid analysis, formed one-third of the seed. The identity of all the F_2 seeds and their progeny for KK-2 × M25 was maintained during fatty acid analyses, planting and harvesting.

The part of the seeds with the embryonic axis and seeds from mutants were planted in the field at Saga University in July, 1994. The seeds were space-planted 20 cm apart in rows and 60 cm between rows. Each plant was harvested individually. To determine the genotype of the F_2 plants, we analyzed a random sample of 15 individual F_3 seeds from each F_2 plant and 10 individual seeds from each of 5 plants of each mutant for fatty acid composition.

The stearic acid contents of the parents grown in the same field and under the same environmental conditions as the F_1 and F_2 plants were used to classify F_2 and F_3 seeds. In both the KK-2 × 'Bay' and M25 × 'Bay' crosses, the F_2 seeds were classified as being similar to those of 'Bay', greater than those of 'Bay' to less than those of the mutant and similar to those of the mutant. In the KK-2 × M25 cross, F_2 seeds were classified as less than any seed of KK-2, similar to KK-2 through less than M25, similar to M25, and greater than any seed of M25. The F_3 seeds from individual F_2 plants were classified as less than any seed of KK-2, similar to KK-2, greater than KK-2 to less than M25, similar to M25, and greater than any seed of M25.

Fatty acid composition was determined by gas chromatography, as described by Takagi et al. (1989). Chi-square analyses were calculated to test the best fit of the data to the hypothesized genetic ratio. A single-gene model was used to evaluate the segregation ratio for stearic acid content in F_2 seeds of the KK-2 × 'Bay' and M25 × 'Bay' crosses, whereas a two-gene model was used for the evaluation of F_2 seeds and F_2 plants of the KK-2 × M25 cross.

Results and discussion

The fatty acid composition in the seeds of mutants KK-2 and M25 and cv 'Bay' is shown in Table 1. The stearic acid contents in KK-2 and M25 were two- and six-fold higher than that of 'Bay'. No maternal effect for stearic acid content was observed in our analysis of F_1 seeds from any of the crosses. In KK-2 × 'Bay', stearic acid content in the F_1 seeds and those from the reciprocal cross was 4.7%. In M25 × 'Bay' it was 7.2%; and in KK-2 × M25, it was 8.3% (Table 2). These results demonstrate that stearic acid content is controlled by the genotype of the embryo and not by the genotype of the maternal parent. The lack of maternal effect observed in this study is similar with findings previously reported by Graef et al. (1985) and Bubeck et al. (1989).

Table 1 Mean fatty acid percentages and their standard errors for the soybean lines KK-2, M25, and 'Bay'

Line	Number of plants	Fatty acid (%)				
		Palmitic	Stearic	Oleic	Linoleic	Linolenic
KK-2	40	10.7 ± 0.05	6.6 ± 0.05	25.1 ± 0.31	50.3 ± 0.24	7.3 ± 0.06
M25	40	9.0 ± 0.08	19.9 ± 0.14	17.9 ± 0.14	45.5 ± 0.16	7.7 ± 0.05
Bay	40	10.4 ± 0.04	3.5 ± 0.03	28.3 ± 0.23	50.6 ± 0.20	7.2 ± 0.09

Table 2 Mean stearic acid content of F_1 seeds from mutant × 'Bay' and mutant × mutant crosses, and of seeds from the parents

Parent or cross	Stearic acid (%)	Parent or cross	Stearic acid (%)
KK-2	6.6	KK-2	6.7
KK-2 × Bay	4.7	KK-2 × M25	8.3
Bay × KK-2	4.7	M25 × KK-2	8.3
Bay	3.5	M25	20.7
LSD[a]	0.14	LSD	0.45
Midparent	5.1	Midparent	13.7
F_1 mean[b]	4.7	F_1 mean	8.3
LSD[c]	0.14	LSD	0.45
M25	20.9		
M25 × Bay	7.2		
Bay × M25	7.2		
Bay	3.6		
LSD	0.37		
Midparent	12.3		
F_1 mean	7.2		
LSD	0.37		

[a] Least significant difference ($P = 0.05$) for comparison of parent and F_1 values
[b] Average of reciprocal crosses used for comparison with the midparent value
[c] Least significant difference ($P = 0.05$) for comparison of the midparent value with the F_1 mean

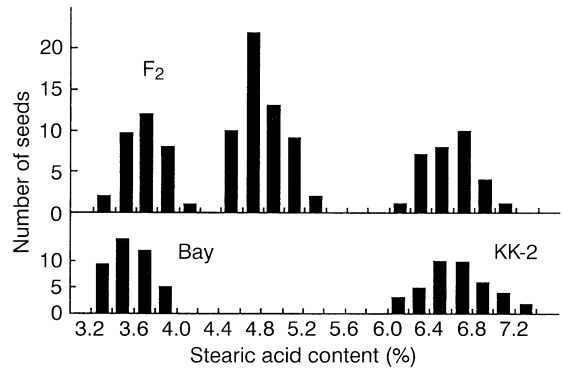

Fig. 1 Distribution of seeds having different stearic acid contents in KK-2, 'Bay' and their F_2 population

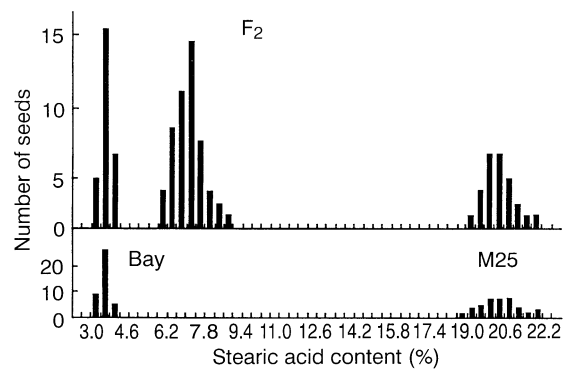

Fig. 2 Distribution of seeds having different stearic acid contents in M25, 'Bay' and their F_2 population

There was partial dominance for stearic acid content in all crosses. The values of the F_1 seeds were significantly different from those of either parents or the midparent values (Table 2). In all crosses, the stearic acid content of the F_1 seeds was lower than the midparent values. Mean stearic acid content in the F_1 seeds was 4.7%, 7.2%, and 8.3% for KK-2 × 'Bay', M25 × 'Bay', and KK-2 × M25, respectively, while the midparent values were 5.1%, 12.2%, and 13.7% for the respective crosses. These results indicate that low stearic acid content in 'Bay' is partially dominant to high stearic acid content in KK-2 and M25, and high stearic acid content in KK-2 is partially dominant to high stearic acid content in M25.

The lack of reciprocal differences in the F_2 seeds for stearic acid content indicated that there was no cytoplasmic effect in any of the crosses and consequently, the data of reciprocal F_2 seeds from each cross were combined in Figs. 1, 2 and 3.

Analysis of F_2 seeds of the KK-2 × 'Bay' and M25 × 'Bay' crosses showed three peaks for stearic acid content. In KK-2 × 'Bay', the first peak ranged from 3.2% to 4.0%, which was similar to the one in 'Bay'; the second peak ranged from 4.4% to 5.3%, which was greater than the one in 'Bay' but less than the one in KK-2; and the third peak ranged from 6.1% to 7.1%, which was similar to the one in KK-2 (Fig. 1). In M25 × 'Bay', the first peak ranged from 3.2% to 4.0%, which was similar to the one in 'Bay'; the second peak ranged from 6.1% to 8.9%, which was greater than the one in 'Bay' but less than the one in M25; and the third peak ranged from 19.1% to 22.0%, which was similar to the one in M25 (Fig. 2). In both crosses, the observed data satisfactorily fit a phenotypic ratio of 1 : 2 : 1. The chi-square values were 0.60 ($P > 0.70$) and 0.08 ($P > 0.95$) for KK-2 × 'Bay' and M25 × 'Bay', respectively. These data indicate that high stearic acid content in these mutants is controlled by recessive alleles at a single locus.

To determine whether the alleles in KK-2 and M25 were at the same locus or different loci, we evaluated the F_2 seeds and their F_3 progeny from reciprocal crosses between the two mutants. Our analysis of 192 F_2 seeds that were grown to evaluate the genotype of the F_2 plants by the F_3 progeny test showed four distinct peaks for stearic acid content. The first peak ranged from 3.2% to 5.0%, which was less than any seed of KK-2 (< 6.0%); the second peak ranged from 6.2% to 12.2%, which was similar to KK-2 but less than M25; the third peak ranged from 18.6% to 20.4%, which was similar to M25, and the fourth peak for stearic acid ranged from 22.0% to 48.2%, which was greater than any seed of M25 (> 21.7%) (Fig. 3). The observed ratio of 39 : 115 : 11 : 27 satisfactorily fit an expected ratio of 3 : 9 : 1 : 3 ($\chi^2 = 3.04$, $P > 0.25$). The presence of F_2 seeds with a stearic acid content lower than the seeds of KK-2 and higher than those of M25

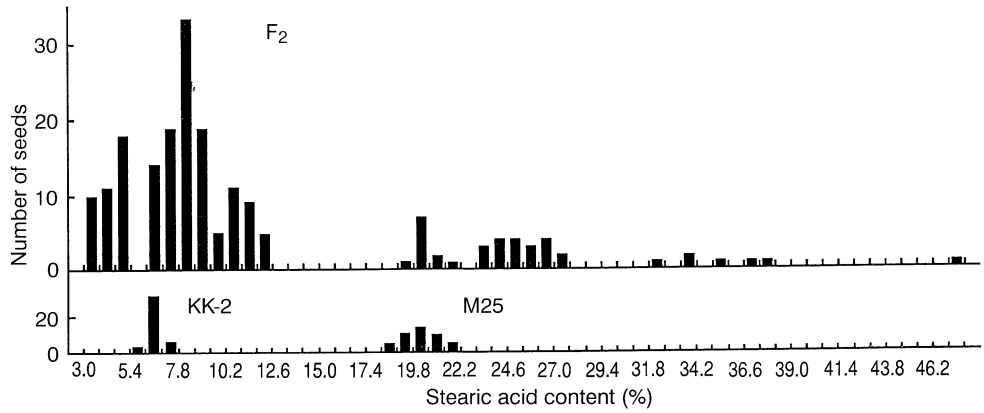

Fig. 3 Distribution of seeds having different stearic acid contents in KK-2, M25, and their F_2 population

demonstrate that different alleles at different loci control the stearic acid content in these mutants.

A progeny test was performed for stearic acid content in KK-2 × M25 by analyzing F_3 seeds from each F_2 plant. For the evaluation of individual F_3 seed from each F_2 plant, the stearic acid content of mutants grown in the same field and under the same environmental conditions as the F_2 plants was used. KK-2 had an average stearic acid content of 6.4% and a range of 6.0–7.1%, and M25 had an average stearic acid content of 19.8% and a range of 18.2–21.6%. The F_3 seeds were classified as less than any seed of KK-2 ($< 6.0\%$), similar to KK-2, greater than KK-2 ($> 7.1\%$) to less than M25 ($< 18.2\%$), similar to M25, and greater than any seed of M25 ($> 21.6\%$). With this classification, nine segregation patterns would be desired in the F_3 seeds of F_2 plants such as, all seeds $<$ KK-2; seeds $<$ KK-2, $=$ KK-2; all seeds $=$ KK-2; seeds $<$ KK-2, $>$ KK-2 to $<$ M25, $=$ M25; seeds $<$ KK-2, $=$ KK-2, $>$ KK-2 to $<$ M25, $=$ M25, $>$ M25; seeds $=$ KK-2, $>$ KK-2 to $<$ M25, $>$ M25; all seeds $=$ M25; seeds $=$ M25, $>$ M25; and all seeds $>$ M25. The data from nine segregation patterns for stearic acid content in the F_3 seeds would be consistent with the theoretical genotypic ratio of 1:2:1:2:4:2:1:2:1 for F_2 plants. The genotype of F_2 plants in each phenotypic class is shown in Table 3. The F_3 progeny test showed that there were no F_2 plants with the genotype $st_1st_1st_2st_2$. The number of F_2 plants with the genotype $St_1st_1st_2st_2$ was also smaller than expected. However, the observed frequency for the eight segregation patterns in the F_3 seeds from 169 surviving F_2 plants was 13:26:14:25:46:23:11:11, which satisfactorily fit an eight expected ratio ($\chi^2 = 7.87$, $P = 0.30$). This result further proves that two different alleles at different loci control stearic acid content in KK-2 and M25 and that

Table 3 Classification of 169 F_2 plants from the cross KK-2 ($st_1st_1St_2St_2$) × M25 ($St_1St_1st_2st_2$) based on the phenotypic pattern of 15 F_3 seeds from each F_2 plant. The expected F_3 phenotypic patterns for stearic acid content are based on different alleles at different loci with partial dominant gene action

Proposed genotype of F_2 plants	Expected F_3 phenotype					F_2 plants			
	$<$ KK-2[a]	$=$ KK-2	$>$ KK-2 to $<$ M25	$=$ M25	$>$ M25	Expected genotypic ratio	Observed genotypic ratio	Expected[b] number	Observed[c] number
$St_1St_1St_2St_2$	×					1	1	12	13
$St_1st_1St_2St_2$	×	×				2	2	24	26
$st_1st_1St_2St_2$		×				1	1	12	14
$St_1St_1St_2st_2$	×		×	×		2	2	24	25
$St_1st_1St_2st_2$	×	×	×	×	×	4	4	48	46
$st_1st_1St_2st_2$		×	×		×	2	2	24	23
$St_1St_1st_2st_2$				×		1	1	12	11
$St_1st_1st_2st_2$				×	×	2	2	24	11
$st_1st_1st_2st_2$					×	1		12	

[a] $<$ KK-2 was $< 6.0\%$; $=$ KK-2 was 6.0–7.1%; $=$ M25 was 18.2–21.6%

[b] Expected number of F_2 plants based on the total number of F_2 seeds analyzed

[c] Observed genotypic frequencies that satisfactorily fit the eight expected frequencies ($\chi^2 = 7.87$, $P > 0.30$)

the allele in KK-2 is partially dominant to the allele in M25.

There were only 11 F_2 plants with the genotype $St_1st_1st_2st_2$, based on the F_3 progeny test (Table 2). The F_2 seeds from which those plants arose had an average stearic acid content of 24.9% and a range of 22.0–27.6% (Fig. 3). The seed of M25 with the highest stearic acid content was 21.7%. When F_3 seeds were analyzed for the progeny test, seeds from all 11 F_2 plants were segregated into three distinct phenotypic classes: stearic acid content similar to M25, similar to F_2 seed value, and greater than F_2 seed value ($> 27.6\%$). The data for stearic acid content in 15 F_3 seeds from each of the F_2 plants was similar to a 1:2:1 ratio. The observed ratio of 44:89:32 for total F_3 seeds satisfactorily fit a 1:2:1 ratio ($\chi^2 = 2.76$, $P > 0.20$). It is important to note that when F_3 seeds ($st_1st_1st_2st_2$) with a higher stearic acid content (> 27.6) were sown, most of the 32 seeds were able to germinate but died after a few days, and that the seeds looked very irregular in shape and size. The homozygous recessive state of the alleles, st_1 and st_2 in the zygote might have brought gross changes in the physiological as well as biochemical pathway for fatty acid synthesis during the seed development stage. Thus, the seeds became irregular in shape and size and then failed to grow into the plants after germination.

The alleles in KK-2 and M25 have been designated as st_1 and st_2, respectively. Graef et al. (1985) found different multiple recessive alleles at a single locus that controlled high stearic acid content in different mutants and designated the alleles *fas*[a] for A6, *fas*[b] for FA41545, and *fas* for A81-606085. When A6 was crossed with other high stearic acid mutants (ST1, ST2, ST3, and ST4), Bubeck et al. (1989) found that alleles for high stearic acid content in three of the four mutants were at the same locus as the *fas*[a] allele in A6, whereas the allele in ST2 was assumed to be at a different locus. The genetic relationship of the new mutant alleles (st_1 and st_2) to other alleles controlling high stearic acid content is unknown. The stearic acid content ($> 30.0\%$) resulting from two different alleles in the homozygous recessive state ($s_1st_1st_2st_2$) observed in this study is the highest known to date in soybean, but it was not possible to develop the line with this genotype. Since seeds with this genotype are able to germinate, tissue culture techniques (e.g., cotyledon or hypocotyl culture) must be applied to develop such a desirable line.

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