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# Inheritance of reduced linolenic acid content in soybean seed oil

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**Abstract** Linolenic acid is the unstable component of soybean [*Glycine max* (L.) Merr.] oil that is responsible for the undesirable odors and flavors commonly associated with poor oil quality. Two mutants, M-5 and KL-8, have been identified that have lower linolenic acid levels in the seed oil than the 'Bay' cultivar. Our objective was to determine the relationships between the genetic systems controlling linolenic acid in these mutants. Reciprocal crosses were made between the mutants and 'Bay', and between the two mutants. No maternal effect for linolenic acid content was observed from the analysis of $F_1$ seeds in any of the crosses. The data for linolenic acid content in $F_2$ seeds of M-5 × 'Bay' and KL-8 × 'Bay' crosses satisfactorily fit a 1 : 2 : 1 and 3 : 1 ratio, respectively. For the M-5 × KL-8 cross, segregation observed from the analysis of $F_2$ seeds for linolenic acid content satisfactorily fit a ratio of 3 more than either mutant: 12 within the range of the two mutants: 1 less than either mutant. The segregation ratio of $F_2$ seeds and the segregation of $F_3$ seeds from $F_2$ plants indicated that M-5 and KL-8 have alleles at different loci that control linolenic acid content. The allele in KL-8 has been designated as *fanx* (KL-8) to distinguish it from *fan* (M-5). The low linolenic acid segregates with the genotype *fanfanfanxfanx* provide additional germplasm to reduce the linolenic acid content from the seed oil of soybean.

**Key words** Soybean mutants · Reduced linolenic acid · Inheritance · *fan* and *fanx* alleles · *Glycine max* (L.) Merr

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## Introduction

Fatty acid composition is an important determinant of soybean oil quality. Linolenic acid has been identified as an unstable component of soybean oil that is responsible for the undesirable odors and flavors commonly associated with poor oil quality (Dutton et al. 1951; Smouse 1979). As a result, attempts have been made to isolate soybean lines with linolenic acid levels substantially below those found in commercial varieties (Wilson et al. 1981; Wilcox et al. 1984; Hammond and Fehr 1983; Rennie et al. 1988; Rennie and Tanner 1989; Takagi et al. 1990).

A mutant line, M-5, with a reduced linolenic acid content was screened at Saga University following X-ray irradiation of the seeds of cv. 'Bay' (Takagi et al. 1990). Rahman et al. (1994) found that the low linolenic acid content in M-5 was controlled by alleles at a single locus that acted in an additive manner. The mutant M-5 was crossed with the mutant C1640, and no transgressive segregation for linolenic acid content was found in the $F_2$ generation (Rahman et al. 1996). The allele for the low content of linolenic acid found in C1640 was designated *fan* (C1640) (Wilcox and Cavins 1987). On the basis of the M-5 × C1640 cross, the low linolenic acid content found in M-5 was assumed to have been conferred by the *fan* (M-5) allele (Rahman et al. 1996). The *fan* alleles were also found in two plant introduction lines (PI 123440 and PI 361088B) and one mutant (A5), and were also designated *fan* (PI 123440) (Rennie and Tanner 1989), *fan* (PI 361088B) (Rennie et al. 1988), and *fan* (A5) (Rennie and Tanner 1991). Two lines, A16 and A17, with the least linolenic acid content (2.5%) known to date were unexpectedly developed from the cross between low linolenic acid mutant A5 and high palmitic acid mutant A23. The linolenic acid content in A16 and A17 was found to be controlled by two alleles, and both lines were assumed to have the genotype *fanfanfan2fan2*, in which the *fan*

and *fan2* alleles were derived from A5 and A23, respectively (Fehr et al. 1992).

The low linolenic acid mutant KL-8 was selected from another population of 'Bay' seeds treated with X-ray irradiation (Takagi and Rahman 1995). Since KL-8 was developed independently from M-5, it was assumed that KL-8 might have a different genetic system controlling linolenic acid than the *fan* allele in M-5. The objective of this study was to determine the relationships between the genetic systems controlling linolenic acid in these mutants.

## Materials and methods

Mutants M-5 and KL-8 and cv. 'Bay' were grown in the greenhouse at 20°–30°C with a 12-h daylength at Saga University in 1992. Reciprocal crosses were made between the mutants and 'Bay', and between the two mutants. The seeds of the parents and reciprocal $F_1$s were planted in the field at Saga University in July, 1992. The seeds were planted 25 cm apart within rows, with 60 cm between rows. Reciprocal crosses were made in the field identical to those made 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 collected from a node adjacent to one from which the $F_1$ seed was obtained. Individual $F_1$ and parent seeds were analyzed for fatty acid composition with 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 analyses of parent and $F_1$ seeds indicated no maternal effect for the linolenic acid content in any of the crosses. This made it easy to determine the phenotype of individual $F_2$ seeds for linolenic acid content in each cross. However, seeds of the parents and $F_2$ seeds of the $F_1$ plants were collected from the pod with the same maturity period at the fifth through seventh nodes of the main stem. Fatty acid composition was determined from 69 and 100 $F_2$ seeds of the KL-8 × 'Bay' and M-5 × Bay crosses, respectively, while 144 $F_2$ seeds were analyzed for the M-5 × KL-8 cross. Thirty seeds of each parent were also analyzed individually.

For determination of phenotypic ratio, the whole $F_2$ seed was used for all crosses, except for M-5 × KL-8, where the $F_2$ seed was divided into two parts with a razor blade. The part with the embryonic axis, used for planting, was two-thirds of the seed, and the part with the cotyledon, used for fatty acid analysis, was one-third of the seed. The identity of all $F_2$ seeds and their progeny for M-5 × KL-8 was maintained during fatty acid analysis, planting and harvesting.

The portion of the seeds with the embryonic axis and seeds from the mutants were planted in the field at Saga University in July, 1993. The seeds were planted 20 cm apart within the rows with 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 20 individual seeds from each of 3 plants of each mutant for fatty acid composition.

The linolenic acid contents of the parents grown under the same field conditions as the $F_1$ and $F_2$ plants were used to classify $F_2$ and $F_3$ seeds. In M-5 × 'Bay', the $F_2$ seeds for linolenic acid content were classified as similar to 'Bay', greater than M-5 to less than 'Bay', or similar to M-5. In KL-8 × 'Bay', the $F_2$ seeds were classified as similar to 'Bay' or similar to KL-8. In M-5 × KL-8, $F_2$ seeds were classified as greater than any seed of either mutant, within the range of the two mutants, or less than any seeds of either mutant. The same classification was used to evaluate the segregation of $F_3$ progeny from individual $F_2$ plants.

For further confirmation of the genotypes of segregates with a linolenic acid content similar to that of 'Bay' and less than that of the M-5 mutant, 6 $F_2$ plants from both classes were selected. Ten $F_3$ seeds from each $F_2$ plant and from each of 6 plants of both 'Bay' and M-5 were planted in the field in 1994. The $F_3$ plants of each line and plants of 'Bay' and M-5 were harvested individually. A 15-seed sample from 8 $F_3$ plants for each line and from 8 plants for each line of 'Bay' and M-5 was analyzed for fatty acid composition.

Fatty acid composition was determined by gas chromatography, as described earlier by Takagi et al. (1989). Chi-square analyses were calculated to test the best fit of the data to the expected genetic ratio. A single gene model (1:2:1 and 3:1) was used to evaluate the segregation ratio for linolenic acid content in $F_2$ seeds of M-5 × 'Bay' and KL-8 × 'Bay' crosses, except for the M-5 × KL-8 cross, where a two-gene model (3:12:1 and 1:4:2:4:4:1) was used for the evaluation of $F_2$ seeds and plants.

## Results and discussion

Maternal effect for linolenic acid content was not observed in any of the crosses (Table 1). The $F_1$ seeds from reciprocal crosses did not differ for linolenic acid content, indicating that the genotype of the embryo of the seed determined its linolenic acid content and not the genotype of the maternal plant.

No dominance for linolenic acid content was observed in the M-5 × 'Bay' cross. The mean of $F_1$ seeds was similar to the midparent value, indicating no dominance for linolenic acid content (Table 1). The segregation for linolenic acid content observed in the $F_2$ seeds satisfactorily fit the ratio of 1:2:1 ($x^2 = 0.51$,

**Table 1** Mean linolenic acid content of $F_1$ seeds from mutant × Bay and mutant × mutant cross, and of seeds from the parents

| Parent or cross | Linolenic acid (%) | Parent or cross | Linolenic acid (%) |
|---|---|---|---|
| M-5 | 5.1 | M-5 | 5.0 |
| M-5 × Bay | 7.0 | M-5 × KL-8 | 6.6 |
| Bay × M-5 | 6.9 | KL-8 × M-5 | 6.5 |
| Bay | 9.3 | KL-8 | 7.5 |
| LSD[a] | 0.24 | LSD | 0.25 |
| Midparent | 7.2 | Midparent | 6.3 |
| $F_1$ mean[b] | 7.0 | $F_1$mean | 6.6 |
| LSD[c] | 0.24 | LSD | 0.25 |
| Replications | 15 | Replications | 15 |
| KL-8 | 7.5 | | |
| KL-8 × Bay | 8.8 | | |
| Bay × KL-8 | 8.7 | | |
| Bay | 9.2 | | |
| LSD | 0.23 | | |
| Midparent | 8.4 | | |
| $F_1$ mean | 8.8 | | |
| LSD | 0.23 | | |
| Replications | 15 | | |

[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 mid parent value

[c] Least significant difference ($P = 0.05$) for comparison of the mid-parent value with the $F_1$ mean

$P > 0.70$). The result of this cross was the same as previously reported by Rahman et al. (1994). When M-5 was crossed to C1640, there were no transgressive segregates for linolenic acid in the $F_2$ generation, which was attributed to the presence of the *fan* (M-5) allele in the M-5 (Rahman et al. 1996).

There was a partial dominance for high linolenic acid content in the KL-8 × 'Bay' cross. The $F_1$ seed value was significantly different from that of either parent or the midparent value. The mean linolenic acid content of $F_1$ seeds was higher than the midparent value, indicating partial dominance for high linolenic acid content (Table 1). Segregation for linolenic acid content in the $F_2$ seeds corresponded with the inheritance of a single gene. The $F_2$ seeds were separated into two phenotypic classes: high and low linolenic acid content. The division between the two classes was based on $F_2$ seeds similar to 'Bay' or similar to KL-8. Thus, linolenic acid content in the high class ranged from 8.0% to 9.8%, and in the low class, from 6.8% to 7.6%. The observed ratio of 51 high: 18 low satisfactorily fit a 3:1 ratio ($x^2 = 0.04$, $P > 0.75$) (Fig. 1). The results of KL-8 × 'Bay' and that of M-5 × 'Bay' indicate that alleles at a single locus controlled linolenic acid content in M-5 and KL-8.

To determine if the alleles in M-5 and KL-8 were at different loci, the $F_2$ seeds and their $F_3$ progeny from reciprocal crosses between these mutants were evaluated. The linolenic acid contents of the mutants grown under the same field conditions as the $F_1$ plants were used to evaluate the segregation pattern among $F_2$ seeds. The seeds of M-5 had a mean linolenic acid content of 5.0% and a range of 4.3% to 5.6%, and seeds of KL-8 had a mean of 7.6% and a range of 6.8% to 8.2%. The $F_2$ seeds were classified as greater than any seed of either mutant ( >8.2%), within the range of the two mutants (4.3–8.2%), or less than any seed of either mutant (<4.3%). When the $F_2$ seeds from reciprocal crosses were considered together, there were 22 $F_2$ seeds with a linolenic acid content greater than that of either mutant, 115 $F_2$ seeds within the range of the mutants, and 7 $F_2$ seeds less than either mutant, which satisfactorily fit a 3:12:1 ratio ($x^2 = 1.82$, $P > 0.30$) (Fig. 2). The transgressive segregation indicates that the mutants M-5 and KL-8 possessed alleles at different loci that control linolenic acid content.

For the evaluation of segregation among $F_3$ seeds from each of the $F_2$ plants of M-5 × KL-8, the linolenic acid content in the seeds of the mutants grown under the same field conditions as the $F_2$ plants was used. The seeds of M-5 had a mean linolenic acid content of 4.9% and a range of 4.1–5.9%, and seeds of KL-8 had a mean linolenic acid content of 7.6% and a range of 6.3–8.4%. The segregation of $F_3$ seeds from $F_2$ plants supported the theoretical model of two alleles for low linolenic acid content that occur at independent loci, with the alleles at each locus exhibiting additive gene action. With this model, the $F_3$ seeds from $F_2$ plants were classified into six segregation patterns, such as, all seeds > mutants (M); seeds > M and = M; all seeds = M; seeds > M, = M, and < M; seeds = M and < M; and all seeds < M, with an expected genotypic ratio of 1:4:2:4:4:1 for the $F_2$ plants. The observed frequency of the six segregation patterns from the 144 $F_2$ plants that were progeny-tested was 12:41:16:36:33:6, which satisfactorily fit ($x^2 = 3.17$, $P > 0.50$) the expected ratio of 9:36:18:36:36:9. The genotype of the $F_2$ plants in each phenotypic class is given in Table 2. The result indicates that the allele controlling low linolenic acid content in the KL-8 was at a different locus from the *fan* allele in M-5. The allele in KL-8 was designated *fanx* (KL-8) to differentiate it from *fan* (M-5). Two low linolenic acid lines (A16 and A17) were developed from a cross between A5 (*fan*) and a high palmitic line A23 (*fan2*), and was designated having the genotype *fanfanfan2fan2* for A16 and A17 (Fehr et al. 1992). Both A23 and the low linolenic acid lines A16 and A17 are registered to Pioneer Hi-Bred International and were not available for the genetic studies. This prevented us from determining the genetic relationship between alleles controlling linolenic acid in KL-8 and A23.

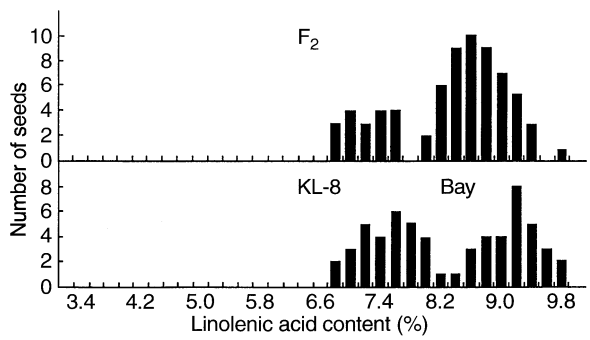


**Fig. 1** Frequency distribution of linolenic acid content in seeds of KL-8 and Bay and in $F_2$ seeds of KL-8 × Bay

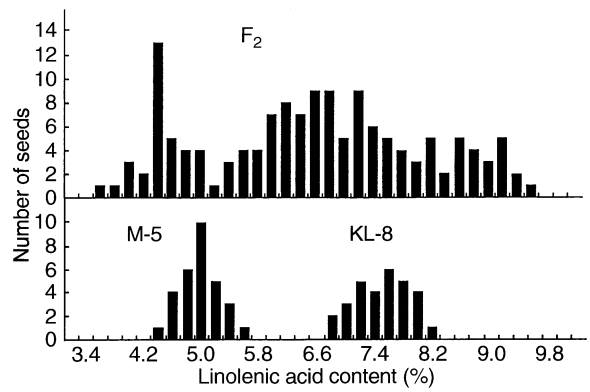


**Fig. 2** Frequency distribution of linolenic acid content in seeds of M-5 and KL-8 and in $F_2$ seeds of M-5 × KL-8

**Table 2** Classification of 144 $F_2$ plants from the cross M-5 (*fanfan Fanx Fanx*) × KL-8 (*Fan Fanfanx fanx*) based on the phenotypic pattern of 15 $F_3$ seeds from each $F_2$ plant. The expected $F_3$ phenotypic patterns are based on a model for alleles with additive gene action at two independent loci controlling linolenic acid content, which predicts a 1:4:2:4:4:1 $F_2$ genotypic ratio (*M* mutant)

| Proposed genotype | $F_2$ plants | | | | | | $F_3$ progeny | | |
|---|---|---|---|---|---|---|---|---|---|
| | Genotypic frequency | | Linolenic acid content | Observed phenotypic frequency | | | Expected phenotypes | | |
| | Expected | Observed[a] | $\bar{X}$ | > M | = M | < M | > M | = M | < M |
| | no. | | % | no. | | | | | |
| *Fan Fan Fanx Fanx* | 9 | 12 | 9.2 | 22 | | | X | | |
| *Fan Fan Fanx fanx* or *Fan fan Fanx Fanx* | 36 | 41 | 7.8 | | | | X | X | |
| *Fan Fan fanx fanx* or *fan fan Fanx Fanx* | 18 | 16 | 6.2 | | | | | X | |
| *Fan fan Fanx fanx* | 36 | 36 | 6.7 | | 115 | | X | X | X |
| *Fan fan fanx fanx* or *fan fan Fanx fanx* | 36 | 33 | 5.1 | | | | | X | X |
| *fan fan fanx fanx* | 9 | 6 | 3.8 | | | 7 | | | X |

[a] Observed genotypic frequency based on the $F_3$ progeny evaluation satisfactorily fit the expected frequency based on a Chi-square test ($x^2 = 3.17$, $P > 0.50$)

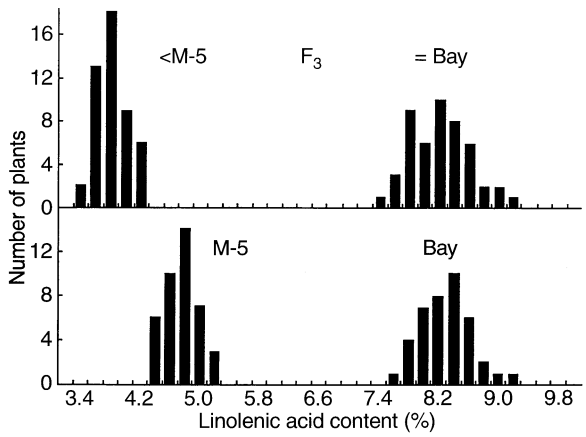


**Fig. 3** Frequency distribution of linolenic acid content in plants of M-5, Bay and in $F_3$ plants for low (< M-5) and high (= Bay) linolenic acid segregates of M-5 × KL-8

On the basis of the $F_3$ progeny test there were 12 $F_2$ plants with the genotype *FanFanFanxFanx* and 6 $F_2$ plants with the genotype *fanfanfanxfanx* in the M-5 × KL-8 cross (Table 2). The $F_3$ plants from 6 lines with the genotype *FanFanFanxFanx* had a mean linolenic acid content of 8.2% and a range of 7.4–9.3% compared with a mean of 8.3% and a range of 7.7–9.2% for the original cultivar, 'Bay'. The $F_3$ plants from 6 lines with the genotype *fanfanfanxfanx* had a mean linolenic acid content of 3.8% and a range of 3.5–4.3%, compared with a mean of 4.8% and a range of 4.4%–5.3% for the M-5 mutant (Fig. 3). The low linolenic acid segregates (3.8%) with the genotype *fanfanfanxfanx* obtained in the study provide additional germplasm that will be useful in achieving a reduction in the linolenic acid content from the seed oil of soybean.

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