Inheritance of Altered Palmitic Acid Percentage in Two Soybean Mutants

E. A. Erickson, J. R. Wilcox, and J. F. Cavins

Palmitic acid is the major saturated fatty acid in soybean seed oil. Two mutant soybean lines with altered levels of palmitic acid in their oil were crossed and studied in F₁, F₂, and F₃ generations and in the F₄ generation of crosses to the cultivar “Century,” which was the source of the mutations. Frequency distributions for palmitic acid percentage displayed five peaks among F₁ seeds from F₁ plants and one, three, or five peaks among F₂ seeds from individual F₁ plants. Three peaks were observed in the F₂ generation of crosses between the mutants and “Century.” Chi-square analysis of these distributions indicated that alleles from two independent loci segregated for palmitic acid percentage and that the alleles were additive in gene action. We propose gene symbols for the mutant alleles at these loci: fap₁, for the allele in C1726 that acts to lower the palmitic acid percentage and fap₂, for the allele in C1727 that acts to increase the palmitic acid percentage in soybean seed oil.

The fatty acid composition of an oil, especially the ratio of saturated to unsaturated fatty acids, determines, to a large extent, the physical, chemical, and nutritional properties of the oil. Therefore, it is important to understand the variation observed in the different fatty acids. Palmitic acid (16:0) is the major saturated fatty acid in most seed oils, but little research has been done on the genetic control of this fatty acid. Palmitic acid percentages vary among the different seed oils, from 5.8% in sunflower seed oil to 46.8% in palm oil. Palmitic acid levels in soybean seed oil range from 9.3% to 17.4% within the world collection. The mean for this fatty acid within U.S. cultivars is 15%.

Depending on the extent of testing, broad-sense heritabilities observed for palmitic acid levels in soybean oil ranged from 39% to 91%, with palmitic acid levels ranging from 10.2% to 11.9% across a random sample of 20 lines. Narrow-sense heritability estimated for palmitic acid percentage in groat oil of oats were based on crosses among genotypes selected as high or low for palmitic acid and ranged from 33% to 68%, depending on the amount of testing and the method used to calculate heritability. In a diallel cross of nine maize inbreds, the general combining ability for palmitic acid percentage was highly significant, with both positive and negative effects present among the inbreds. The significant heritability and additive gene action observed for palmitic acid percentages in these species suggest the feasibility of selecting for lines with altered levels of this fatty acid.

When naturally occurring variability in fatty acid composition has been insufficient for plant-breeding goals, mutagenesis has been used to increase the variability. In a study by Wilcox et al., treatment of “Century” soybean seed with ethyl methanesulfonate (EMS) resulted in a three- to fourfold increase in the variability of palmitic, oleic, and linoleic acid percentages and a twofold increase in the variability of stearic and linolenic acid percentages over the values for the control population. The low linolenic acid percentage in a mutant line derived from this study (C1640) was shown to be inherited as a single gene in the cross C1640 × “Century.”

Lines with altered palmitic acid levels were derived from another sample of “Century” seed treated with EMS as above. Line C1726 was 3 percentage units lower than “Century” for palmitic acid and line C1727 was 6 percentage units higher (Table 1). We attempted to determine the inheritance of altered palmitic acid in the two mutant lines.

Materials and Methods
In 1982, lines C1726 and C1727 were selected as representing extreme types for
palmitic acid percentage from the M₂ generation after treatment of “Century” soybean seeds with EMS. We sowed these lines and retested them in 1983 in the M₂ generation to ensure stability of the altered fatty acid phenotype. A detailed description of the screening process has been reported.¹¹

We reciprocally crossed lines C1726 and C1727 in 1984. We grew parental and hybrid plants from this cross in a greenhouse in the winter of 1984–1985, during which time reciprocal crosses were repeated. A seed chip was removed from parental, F₁, and F₂ seeds to analyze the oil composition from each seed to be planted in the 1985 field study.

We planted seed remnants containing the embryonic axis in 0.51 cups in the greenhouse on May 5, 1985, and transplanted them to the field after 10 days in a randomized complete block design with three replications. A replication consisted of five rows of 20 plants each: one row of C1726, one row of C1727, and three rows of F₁ seedlings. Within each parental row, two F₁ and two reciprocal F₁ plants were placed randomly. We randomized rows within replications, with 1-m row spacing and 30-cm plant spacing within rows.

In the field study, 10 individual seeds of each parent were analyzed for oil composition from each replication. The number of hybrid and backcross seeds harvested varied between replications. We analyzed 30 F₂ seeds from one F₁ plant and 30 F₂ seeds from one reciprocal F₁ plant from each replication. We analyzed 12 to 20 F₂ seeds per plant from 27 F₁ plants representing the range in palmitic acid values. These were chosen after analyzing F₁ seed chip data.

We harvested all seeds from nodes 7 to 12 to minimize fatty acid variation associated with the position of the seed on the plant. We harvested all seeds at maturity over a one-week period in September 1985. The within-plant variability was extremely low for the saturated fatty acids, as indicated by their standard errors (Table 1).

Additionally, each of the mutant lines was crossed with “Century,” the parental source of the mutations. The F₁ generation from these crosses was grown in a greenhouse from October 1986 through January 1987. Twenty seeds of each parent and a total of 60 seeds from two F₁ plants of each cross were analyzed for fatty acid composition of the oil.

### Results and Discussion

Of the mutants screened in 1982, C1726 and C1727 diverged most widely for palmitic acid percentage. C1727 had roughly twice the palmitic acid percentage of C1726 (17.3% and 8.6%, respectively) (Table 1). The hybrid mean value for this fatty acid (12.2%) was intermediate between the values for the parents. The palmitic acid percentage found in the original line, “Century,” before mutagenesis was 11.5%.

The 180 F₂ seed chips from the cross C1726 × C1727 grown in the greenhouse segregated 10:40:79:41:10 for percentage of palmitic acid, exhibiting five distinct peaks (Figure 1). The chi-square value of 3.15 indicated a satisfactory fit to a ratio of 1:4:6:4:1, which is consistent with a model in which two major genes with additive effects determine levels of palmitic acid in this cross. In this model, C1726 contributes an allele, designated fap₁, for lowered palmitic acid, and C1727 contributed an allele, designated fap₂, for increased palmitic acid. The fap₁ allele appears to lower levels of palmitic acid by about 1.5 percentage points, and the fap₂ allele and hybrids by means of analysis of single seeds were used to establish classes in the segregating generations. We performed chi-square tests for goodness of fit for F₂ populations using one- or two-gene segregation models.¹² The F₁ fatty acid values were distinguishable from and were located between the parental values. Therefore, we used segregation ratios for additive gene action to test hypotheses concerning numbers of segregating genes.

### Table 1. Mean fatty acid percentages and standard errors in parentheses for two palmitic acid mutants and their hybrid, based on analysis of single seeds from plants grown in 1985

<table>
<thead>
<tr>
<th>Line</th>
<th>N'</th>
<th>Palmitic (%)</th>
<th>Stearic (%)</th>
<th>Oleic (%)</th>
<th>Linoleic (%)</th>
<th>Linolenic (%)</th>
<th>F₂ O.08</th>
<th>Linolenic O.14</th>
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<tbody>
<tr>
<td>C1726</td>
<td>30</td>
<td>8.6 (0.06)</td>
<td>3.3 (0.07)</td>
<td>21.4 (0.60)</td>
<td>59.0 (0.46)</td>
<td>7.2 (0.14)</td>
<td></td>
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<tr>
<td>Hybrid</td>
<td>14</td>
<td>12.2 (0.08)</td>
<td>3.3 (0.07)</td>
<td>20.2 (0.49)</td>
<td>56.8 (0.34)</td>
<td>7.4 (0.09)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1727</td>
<td>30</td>
<td>17.3 (0.08)</td>
<td>2.9 (0.07)</td>
<td>16.8 (0.25)</td>
<td>51.2 (0.20)</td>
<td>8.3 (0.14)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results and Discussion

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Table 2. Comparisons between palmitic acid percentage of F2 seed chips and their corresponding F3 progeny for nine hypothesized genotypes

<table>
<thead>
<tr>
<th>F3 distribution</th>
<th>F2 genotype</th>
<th>F3 seed chip</th>
<th>F2 seed</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>x (%)</td>
</tr>
<tr>
<td>1</td>
<td>fap, fap, Fap, Fap*</td>
<td>4</td>
<td>8.5</td>
</tr>
<tr>
<td>2</td>
<td>Fap, Fap, Fap, Fap</td>
<td>1</td>
<td>11.8</td>
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<tr>
<td>3</td>
<td>Fap, Fap, fap, fap</td>
<td>1</td>
<td>12.7</td>
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<td>4</td>
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<td>17.2</td>
</tr>
<tr>
<td>5</td>
<td>Fap, fap, Fap, Fap</td>
<td>2</td>
<td>10.0</td>
</tr>
<tr>
<td>6</td>
<td>Fap, fap, fap, fap</td>
<td>2</td>
<td>15.0</td>
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<td>7</td>
<td>fap, fap, Fap, Fap</td>
<td>1</td>
<td>10.4</td>
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<td>8</td>
<td>Fap, fap, fap, fap</td>
<td>2</td>
<td>14.9</td>
</tr>
<tr>
<td>9</td>
<td>fap, fap, Fap, Fap</td>
<td>11</td>
<td>12.2</td>
</tr>
</tbody>
</table>

* Each fap allele decreases palmitic acid by 1.5 percentage points and each Fap allele increases palmitic acid by 3.0 percentage points.

An allele appears to increase palmitic acid levels by about 3.0 percentage points. Similar results were obtained from the field study, in which the F2 generation segregated 16:37:71:49:7, which also was a satisfactory fit to a 1:4:6:4:1 ratio (x² = 5.64).

Frequency distributions for palmitic acid among F1 seeds on the 27 representative F2 plants confirmed this genetic hypothesis (Figure 2). Table 2 lists F3 seed-chip values, the hypothesized F2 genotypes, and average palmitic acid values of corresponding F2 seed analyses for each of the distributions in Figure 2. Distributions 1 and 4 corresponded to the true-breeding parental types. Distributions 2 and 3 corresponded to true-breeding homozygous recombinant genotypes. In distributions 5 through 8, segregation was limited and trimodal, corresponding to segregations from self-pollination of heterozygous monohybrid genotypes. The full range of segregation, fitting a ratio of 1:4:6:4:1, was observed in distribution 9, as would be expected from self-pollination of the heterozygous dihybrid genotype. Observed F3 means were very close to expected values based on the proposed additive model (Table 2).

The F2 population from the cross CI726 × “Century” segregated 17 low, 26 intermediate, and 17 normal for palmitic acid (Figure 3). The chi-square value of 1.07 indicated a satisfactory fit to a 1:2:1 ratio for two alleles segregating at a single locus. Similarly, the F2 population from CI727 × “Century” segregated 14 normal, 28 intermediate, and 18 high for palmitic acid. The chi-square value of 0.80 for this F2 population also indicated a satisfactory fit to a ratio of 1:2:1 for two alleles at a single locus. These data further support the hypothesis of two alleles at independent loci controlling levels of palmitic acid in the oil of these soybean lines.

Polygenic inheritance for palmitic acid percentage has been observed in maize...
and oats. However, our study shows simple inheritance for this trait in soybean. The specificity of the mutation in each line, altering the palmitic acid level but not the yield or oil percentage (2-year study, data not shown), suggests that the mutations did not occur early in the fatty acid biosynthetic pathway.

The seven enzymatic functions that are required for the synthesis of palmitic acid from acetyl coenzyme A (acetyl-CoA) and malonyl coenzyme A (malonyl-CoA) are collectively termed fatty acid synthetase (FAS). In plant FAS systems, these functions appear to be nonassociated, with each function on a separate polypeptide. This differs from yeast and vertebrate FAS systems, in which the enzyme functions are associated in complex polyfunctional polypeptides.

With many separate enzymes and cofactors involved in the synthesis of palmitic acid in plants, polygenic inheritance of variability for this trait would not be surprising. The variability in our lines, however, appeared to be the result of mutations in two major genes. These mutations may affect enzymes or cofactors in the FAS system or may affect the palmitic acid elongase system that converts palmitic acid to stearic acid.

We assigned gene symbols for the mutant alleles at the two loci in CI726 and CI727 (Table 2). The alleles in our study appear to be additive in gene action, and neither maternal nor cytoplasmic effects influenced levels of palmitic acid. Similar results were reported by Graef et al.\(^\text{10}\) in a study of the inheritance of mutant alleles controlling levels of stearic acid and by Wilcox and Cavins\(^\text{13}\) in a study of the inheritance of mutant alleles controlling levels of linolenic acid in soybean oil. The simply inherited genes identified in our study could easily be incorporated into improved soybean cultivars that would have significantly different levels of palmitic acid in their seed oil.

References