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Research Notes: Inheritance of fatty acid composition in soybean seed oil

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1) Implications of seed set on \(ms_2 ms_2\) male-sterile plants in Raleigh.

We conducted tests in the summers of 1981 and 1982 to determine seed set on the maturity group 3 cultivar 'Williams' and its \(ms_2 ms_2\) male-sterile isolate. In these studies, Williams plants segregating for male sterility and the maturity group 5 cultivar, 'Forrest', were grown outdoors in pots at three isolated sites. We identified sterile and fertile plants immediately at flowering using standard pollen germination techniques. Seven pots of each genotype were then arranged in a randomized block design at each site. To maintain an adequate insect population for cross pollination, a honey bee hive was placed near each test. We included Forrest in the tests as an additional pollen source for the male-sterile Williams genotypes. Two tests were planted in May to simulate full-season growing conditions, while a third test was delayed until July to simulate double-cropped conditions.

Results: The \(ms_2 ms_2\) genotype yields only about 8% less than its male-fertile isolate under early-planted conditions in Raleigh (Table 1). This result is quite surprising because the male-sterile Williams genotype sets seed poorly in the midwestern environment of its origin and in our own late-planted experiment (Table 1) (Bernard and Cremeens, 1975). We do not think that male sterility "broke down" in our full season tests because male-sterile plants kept in the greenhouse (insect free) set no seed whatever in the summers of 1981 and 1982. We suspect that favorable air temperature effects on flower morphology and honey bee activity can explain our high full-season seed set versus our poor late-planted results (Robacker et al., 1982). Temperatures were high and near normal for the full-season tests, while temperatures were unseasonably low during flowering in the late-planted test. As a result, flowers did not open as fully in the late-planted test as they did in the full-season tests. In addition, low temperatures reduced the total number of bee flights in the late-planted test (our bee flight observations were casual, however). Partially closed flowers and reduced bee activity then apparently limited pollination of the male-sterile genotypes in the late-planted test, while no such restriction occurred in the full-season test. Since it is thought that the \(ms_2\) gene has no effect on female fertility, it seems reasonable that extensive pollination should increase seed set.

Implications: The seed set we observed on full-season \(ms_2 ms_2\) Williams genotypes is much greater than we have ever seen on \(ms_1 ms_1\) male-sterile genotypes. In fact, seed set may be high enough to obtain a useful measure of seed yield on a male-sterile genotype, although sampling variance tends to be greater for male-sterile plants.

The improved seed set on the \(ms_2 ms_2\) male-sterile plant apparently offers new flexibility in designing recurrent selection breeding programs. For example, phenotypic recurrent mass selection for seed yield should be practical. To date, this method has been used only for the improvement of seed composition (where few seed are required) in soybeans. Another possibility with high seed set is that one could monitor seed yield as chemical
Table 1. Means for $ms_2$ male-sterile and male-fertile traits at Raleigh, NC

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Yield</th>
<th>Pods</th>
<th>Seed weight</th>
<th>Seed per pod</th>
<th>Height</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/plant</td>
<td>No./plant</td>
<td>per 100 seed</td>
<td>per pod</td>
<td>inches</td>
<td></td>
</tr>
<tr>
<td>Over years - early planted§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Ms_2$ — †</td>
<td>40.8 NS</td>
<td>120*</td>
<td>13.7*</td>
<td>2.54*</td>
<td>40*</td>
<td>13</td>
</tr>
<tr>
<td>$ms_2 ms_2$</td>
<td>37.7</td>
<td>87</td>
<td>18.7</td>
<td>2.26</td>
<td>38</td>
<td>12</td>
</tr>
<tr>
<td>1981 - early planted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Ms_2$ —</td>
<td>44.3 NS</td>
<td>113*</td>
<td>16.2*</td>
<td>2.39*</td>
<td>38*</td>
<td>7</td>
</tr>
<tr>
<td>$ms_2 ms_2$</td>
<td>37.4</td>
<td>76</td>
<td>20.1</td>
<td>2.25</td>
<td>34</td>
<td>5</td>
</tr>
<tr>
<td>1982 - early planted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Ms_2$ —</td>
<td>37.6 NS</td>
<td>127 NS</td>
<td>10.7*</td>
<td>2.71*</td>
<td>43 NS</td>
<td>6</td>
</tr>
<tr>
<td>$ms_2 ms_2$</td>
<td>37.9</td>
<td>97</td>
<td>17.7</td>
<td>2.26</td>
<td>43</td>
<td>7</td>
</tr>
<tr>
<td>1982 - late planted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Ms_2$ —</td>
<td>24.8*</td>
<td>72*</td>
<td>14.2*</td>
<td>2.42*</td>
<td>32*</td>
<td>7</td>
</tr>
<tr>
<td>$ms_2 ms_2$</td>
<td>3.9</td>
<td>11</td>
<td>17.8</td>
<td>1.80</td>
<td>32</td>
<td>7</td>
</tr>
<tr>
<td>Sample variances ‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Ms_2$ —</td>
<td>60</td>
<td>405</td>
<td>2.55</td>
<td>.0123</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>$ms_2 ms_2$</td>
<td>117</td>
<td>762</td>
<td>4.17</td>
<td>.0219</td>
<td>12.2</td>
<td></td>
</tr>
</tbody>
</table>

*Fertile and sterile genotypes significantly different at 0.05 level.

† $Ms_2$ — male fertile; $ms_2 ms_2$ = male sterile.

‡ Includes only early planted tests. Pooled df are 10 and 11 for fertile and sterile genotypes, respectively.

§ Year x fertility interaction detected for the traits seed weight and seed per pod.
or other seed traits are improved using mass selection. In this way, a
detrimental or negative correlation between a chemical trait under selection
and seed yield could be detected early on in a selection study. As a case
in point, we detected a negative relationship between seed yield and oleic
acid content of seed oil only after several cycles of mass selection, where
the \( ms_1 \) gene had been employed for crossing purposes. The relationship
would probably have been detected (and dealt with) much earlier if the \( ms_2 \)
rather than the \( ms_1 \) gene had been used.
The high seed set on the \( ms_2 ms_2 \) sterile plant must surely offer other
possibilities for adaptation of the \( ms_2 \) gene to recurrent selection. The
final utility of the gene in our program depends on the repeatability of
male-sterile yield levels over several full-season environments and in later
maturing lines than Williams. We are optimistic at present that the gene
will be useful.


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2) Seed set on \( G. falcata \) and a proposal to use \( ms_2 \) male sterility in its
hybridization with \( G. max \).

Seed set: Limited seed supply has severely curtailed research on \( G.
falcata \). Seed supply is limited primarily because \( G. falcata \) sets few seed
in the greenhouse, even though flower production is rather profuse. We
noticed this past summer that seed set is quite high when we grow this spe-
cies out-of-doors near a honeybee hive. At the present, we have recovered
over 3000 seed from two plants through honeybee pollination of the acces-
sion PI 246591. Upon close examination of flower morphology, we found that
the stigma protrudes beyond the anther heads, spatially separating the male
and female flower parts. Thus, insect or some other physical activity is
apparently required to transfer pollen from the anthers to the stigma.
This information should prove useful in the future studies of \( G. falcata \).
We have no evidence that self-incompatibility is a factor in seed set at
present, because an isolated individual \( G. falcata \) plant will set seed well
in the presence of honeybees.
The requirement of insect pollination for seed set (at Raleigh) leads
us to speculate that the species may be often cross-pollinated rather than
self-pollinated in its natural habitat, the dry regions of Australia. In
this respect, \( G. falcata \) may be unusual in the Glycine and Soja subgenera
of \( Glycine \); the other species appear to be primarily self-pollinating. The
cross-pollinating nature of \( G. falcata \) suggests that hybrid vigor may be
more substantial in this species than in \( G. max \). Currently, there are too
few accessions of \( G. falcata \) to test this last hypothesis properly. A last
implication from our observations is that \( G. max \) may be more distant from
the *G. falcata* than from the other *Glycine glycinene* species, in an evolutionary sense. Hybridization of the two species may be difficult as well.

**Hybridization with *G. max***: Reported attempts to obtain viable hybrids between *G. falcata* and *G. max* have failed thus far. Hood and Allen (1980) obtained 52 F₁ pods from 461 artificial fertilizations, but no hybrid plants could be recovered. Similarly, Newell and Hymowitz (1982) obtained six possible hybrid pods, but no hybrid plants, from 253 manual attempts at cross-pollinations, indicating that development of interspecific hybrid plants may be difficult. Since parasexual techniques are not yet well-developed for the *Glycine* genus, successful hybridization of the two species may depend on the advance and refinement of embryo rescue techniques.

A prerequisite to the study and refinement of embryo rescue techniques is the generation of large numbers of interspecific embryos. Such embryos have been obtained (Hood and Allen, 1980) by artificial cross-pollination, but this method is both time-consuming and labor-intensive. Realizing that honeybees are attracted to both *G. max* and *G. falcata*, we propose the use of honeybees and the *ms₂*-conditioned male sterility in *G. max* to help generate large numbers of interspecific embryos. Our rather simple scheme should provide a continual supply of embryos for study from June through August (in our area) without the need for manual cross pollination.

The procedure is as follows: a) Grow *G. max* plants segregating for *ms₂*-conditioned male sterility in the greenhouse under insect-free conditions. Plant numbers should be high enough to insure recovery of at least 10 male-sterile plants.

b) At flowering, identify and destroy fertile *G. max* plants, retaining male-sterile plants for cross-pollination. Identification could be carried out first by "thumbnail" tests for pollen shedding, with any plant shedding pollen being discarded. Remaining plants could then be identified by pollen germination technique (Brim and Kenworthy, 1977), or simply by watching for pod formation (which usually is visible two weeks after flower initiation).

c) After male-sterile *G. max* plants have been identified with certainty, move the plants outside and surround with flowering plants of *G. falcata*. This crossing block should be kept well-isolated from other Glycine plants (Nelson and Bernard, 1979). Place an active honeybee hive near the crossing block.

d) Interspecific pollination should result in small pods on the male-sterile plants and can be used for embryo studies. Virtually no *G. max* embryos will develop in the crossing block. It should be noted, however, that male-sterile *G. max* plants form pod-like structures near the end of the flowering period, independent of fertilization. Practice is, therefore, needed to distinguish these "pseudo pods" from the pods of interest. Haploid seed may occasionally be produced on the sterile plants as well, but should not constitute a real concern.

e) The male-sterile plants will flower well for only about six weeks (under our conditions). Therefore, successive plants of *G. max* (possibly 2 or 3 weeks apart) are necessary to maintain receptive male-sterile plants. Late in the summer, photoperiod manipulation in a greenhouse may be required to obtain plants of adequate size. *G. falcata* should bloom continuously through the summer, but pods need to be picked and new plants added periodically in the crossing block to insure a profusion of flowers.
We tried the procedure on a limited basis this past fall, but little pollen was actually transferred. Cool air temperature reduced bee flights and caused the G. max flowers to remain partially closed. A favorable (i.e., summer) environment is, therefore, needed for the procedure to work.

As a final note, it may be necessary to double the chromosome number of G. falcata for successful hybridization with G. max. We do not know how such a change in chromosome number will affect pollen production and honeybee visitation.

References


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3) Inheritance of fatty acid composition in soybean seed oil.

While it has been demonstrated that the fatty acid composition of soybean oil can be changed by recurrent selection (Wilson et al., 1981), there is little information about the genetic control of oil biosynthesis in soybean seeds. In some species, such as rape (Downey and Harvey, 1963), safflower (Yermanos et al., 1967), and flax (Yermanos and Knowles, 1962), the male parent has a significant effect on the fatty acid composition of oil from F1 hybrid seeds. In corn (Jellum, 1966) and soybeans (Brim et al., 1968), the male parent has almost no effect on oil composition of F1 hybrid seeds. In these two species, the hybrid seed has oil that is phenotypically similar to that of selfed seed from the maternal plant. The purpose of the experiment reported here was to study the inheritance of fatty acid components of seed oil and also re-examine the maternal influence on fatty acid composition of oil.

Materials and methods: In the summer 1981, reciprocal crosses were made between the PI, 'Peking', which has high percent 18:3 (linolenic acid), and N78-2245 (a breeding line we developed), which has low percent 18:3. Hybrid seeds were cut in half with a razor blade and were extracted in 3 ml of chloroform:hexane:methanol (2:0:1.25:0.5 v/v/v/v), filtered and dried under N2 gas. The extracts were taken up in 2:1 chloroform:methanol, filtered once more and analyzed by a Hewlett Packard 5880 gas chromatograph. The other half of each seed was planted in the greenhouse to determine which were actually
hybrids and which were selfs. Samples of F2 seeds from mature F1 plants were ground and oil was extracted as previously described (Wilson et al., 1981). F2 soybean plants were grown to maturity in the field at Clayton, NC, in 1982. Single plants were harvested, and the fatty acid composition of the seed oil from each plant was determined.

Results and discussion: The fatty acid composition of oil from F1 hybrid seeds showed a maternal influence similar to that described previously by Brim et al. (1968). The percentages of oleic (18:1), linoleic (18:2), and linolenic (18:3) acids of the hybrid seeds were not significantly different from those of self-pollinated seed from the maternal parent in either of the reciprocal crosses (data not shown). While the fatty acid composition of seed oil was maternally determined, it was not maternally inherited, because oil from seeds produced on F1 plants had a fatty acid composition intermediate to Peking and N78-2245. In addition, the reciprocal crosses had nearly identical phenotypes (Table 1).

The results also suggested that dominance or epistasis effects were involved in determining fatty acid composition. In seed from the F1 plants, the percentages of all fatty acid components except palmitic acid (16:0) were between those of the midparent and Peking in magnitude (Table 1). Thus, the Peking parent may have contributed dominant genes for low percentages of 18:1 and high percentages of 18:2 and 18:3, and/or epistatic gene interactions may have been responsible. The F2 population of the crosses showed similar results with respect to 18:1 and 18:2 (Figure 1). Plants with seed oil percentages of 18:1 less than the F2 population mean of 26.5 occurred in highest frequencies. Also, a majority of F2 plants had percentages of 18:2 greater than the population mean of 50.4 (Figure 1). Percentages of 18:3 were more normally distributed (Figure 1) around the population mean of 8.1.

At this point, it is not clear which kinds of gene action are most important in the determination of fatty acid composition. Four cycles of recurrent mass selection for high 18:1 resulted in a linear increase in the percentage of 18:1 in seed oil (Wilson et al., 1981). This suggests that percent 18:1 is a typical quantitative genetic trait with additive gene effects involved in the phenotypic expression. The deviation of the F1 from the midparent may indicate dominance, but the occurrence of dominance effects with quantitative traits is unusual in self-pollinated species. Epistasis is more likely to be involved in the deviation.

Current biochemical evidence indicates that the polyunsaturated fatty acids, 18:2 and 18:3, are produced by consecutive desaturation of 18:1 (Cherif et al., 1975). A possible biochemical explanation of the data would postulate a dual-enzyme desaturase complex to perform the two reactions.

\[
\text{NADH} \rightarrow \text{NAD} \rightarrow \text{NADH} \rightarrow \text{NAD}
\]

The amount of the total enzyme complex would regulate the relative percentages of 18:1 and 18:2. More enzyme would result in more 18:1 desaturation and hence more 18:2 production. According to this hypothesis, N78-2245 and Peking would supposedly have small and large amounts, respectively, of the enzyme complex. The total amount of the complex and the amount of 18:1 formed could be controlled by several additive genes.
Figure 1. Distributions with respect to fatty acid components of seed oil from field-grown \( F_2 \) plants of the cross Peking x N78-2245.
Table 1. Mean fatty acid composition of seed oil from greenhouse-grown F<sub>1</sub> soybean plants and parents of the cross Peking x N78-2245

<table>
<thead>
<tr>
<th>Material</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peking</td>
<td>11.7</td>
<td>3.3</td>
<td>19.9</td>
<td>54.5</td>
<td>10.6</td>
</tr>
<tr>
<td>Peking&lt;sup&gt;a&lt;/sup&gt; x N78-2245 F&lt;sub&gt;1&lt;/sub&gt; plants</td>
<td>11.5</td>
<td>3.8</td>
<td>23.3</td>
<td>52.9</td>
<td>8.5</td>
</tr>
<tr>
<td>N78-2245&lt;sup&gt;a&lt;/sup&gt; x Peking F&lt;sub&gt;1&lt;/sub&gt; plants</td>
<td>11.5</td>
<td>3.9</td>
<td>22.2</td>
<td>53.7</td>
<td>8.7</td>
</tr>
<tr>
<td>N78-2245</td>
<td>9.6</td>
<td>4.0</td>
<td>46.8</td>
<td>34.7</td>
<td>4.9</td>
</tr>
<tr>
<td>Midparent</td>
<td>10.6</td>
<td>3.7</td>
<td>33.3</td>
<td>44.6</td>
<td>7.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Maternal parent.

References


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Influence of maturity date on the oil content of soybeans with genetically altered fatty acid composition.

Recurrent mass selection and within half-sib family selection for increased oleic acid percentage has been proven successful in decreasing the percentage of linolenic acid in soybean oil (Burton et al., 1983). In the first four cycles of selection, the percentage of oleic acid in the seed oil increased linearly at an average rate of $1.6 \pm 0.2\%$ per cycle whereas linoleic and linolenic acid percentages showed linear decreases. Four additional cycles of selection for increased oleic acid and two cycles for decreased oleic acid levels are currently being evaluated in a range of environments. From that investigation, it has become increasingly evident that the number of maturity days from planting has decreased with selection. A shift in maturity date in the selected populations may warrant modification of existing selection procedures if maturity effects are confounded with the effects of genes that directly control the fatty acid composition of oil. The results reported here suggest that the fatty acid composition of soybean oil may be influenced by the period of time during the growing season when the plants mature.

A segregating $F_4$ population of 182 plants from a cross between 'Tracy' and N79-2002, an experimental line selected from the sixth cycle of mass selection, was grown in Clayton, NC, in 1982. Date of maturity was monitored on a weekly basis, and seed from single plants were analyzed for fatty acid composition at harvest maturity. Average percentages of unsaturated fatty acids of $F_4$ lines within a maturity date set were reported in Table 1. Because each set represented a random sample of lines with respect to fatty acid composition, the means should not have differed among sets beyond that expected due to sampling. Therefore, a shift in the percentages of unsaturated fatty acids would be a result due to maturity date differences if there were no linkages between genes controlling maturity and genes controlling fatty acid composition. Although there was very little change in the proportions of fatty acids among the final three maturity dates, the percentage of oleic acid decreased while the percentages of the polyunsaturated fatty acids, linoleic and linolenic acids, generally increased from September 17 to October 5. There was no consistent trend observed for the levels of palmitic and stearic acids and, thus, were not reported.

The same trends were found in another experiment in which N78-2245 was planted on three dates (May 5, June 6, and June 28, 1982) in two replications to induce a wide range of maturity dates (Table 2). N78-2245, a highly inbred experimental line, was selected on the basis of high oleic acid concentration from the fifth cycle of mass selection, followed by within half-sib family selection. Over a period of 29 days, the mean percentage of oleic acid in N78-2245 decreased by 9.2 mole% while the mean percentages of linoleic and linolenic acids increased by 6.6 and 0.9 mole%. In addition, the proportion of shriveled seeds declined dramatically over maturity dates. Probably as a result of insufficient replication, the effect of maturity date on fatty acid percentage was not statistically significant at the 5% level. The noted trends in unsaturated fatty acid composition, however, predicate a source of concern for the genetic improvement of a trait so highly influenced by environmental factors.
Previous reports have suggested that seed maturation under a warmer environment, i.e., as a result of earlier planting, may result in lower percentages of the polyunsaturated fatty acids (Howell and Collins, 1957; Unger and Thompson, 1982). From another viewpoint, the lower levels of polyunsaturated fatty acids observed at the early maturity date may have resulted from a shortened pod-filling period, as indicated by the higher proportion of shriveled seeds. In that regard, the deposition of polyunsaturated fatty acids in N78-2245 seed oil predominates late in seed development (Carver et al., n.d.). In concluding, it may prove beneficial to consider maturity date in addition to oleic acid percentage when trying to reduce linolenic acid content in a soybean population.

Table 1. Unsaturated fatty acid composition of an F₄ population from the cross Tracy x N79-2002

<table>
<thead>
<tr>
<th>Maturity date</th>
<th>No. of plants analyzed</th>
<th>Oleic acid %</th>
<th>Linoleic acid %</th>
<th>Linolenic acid %</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 17</td>
<td>21</td>
<td>38.1 ± 1.1</td>
<td>41.3 ± 0.9</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>September 24</td>
<td>35</td>
<td>37.0 ± 1.3</td>
<td>42.9 ± 1.0</td>
<td>5.9 ± 0.2</td>
</tr>
<tr>
<td>September 30</td>
<td>34</td>
<td>36.2 ± 1.3</td>
<td>42.6 ± 1.0</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>October 5</td>
<td>53</td>
<td>35.9 ± 0.8</td>
<td>43.0 ± 0.6</td>
<td>6.6 ± 0.1</td>
</tr>
<tr>
<td>October 12</td>
<td>29</td>
<td>36.1 ± 1.0</td>
<td>43.0 ± 0.8</td>
<td>6.4 ± 0.2</td>
</tr>
<tr>
<td>October 18</td>
<td>10</td>
<td>36.2 ± 1.6</td>
<td>43.0 ± 1.3</td>
<td>6.3 ± 0.2</td>
</tr>
</tbody>
</table>

*aMeans and standard error of single plant analyses within a maturity date set.

Table 2. Effect of maturity date on the unsaturated fatty acid composition of N78-2245

<table>
<thead>
<tr>
<th>Maturity date</th>
<th>Shriveling %</th>
<th>Oleic acid %</th>
<th>Linoleic acid %</th>
<th>Linolenic acid %</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 10</td>
<td>78</td>
<td>49.5</td>
<td>33.2</td>
<td>4.5</td>
</tr>
<tr>
<td>September 28</td>
<td>37</td>
<td>46.9</td>
<td>35.5</td>
<td>4.5</td>
</tr>
<tr>
<td>October 8</td>
<td>20</td>
<td>40.3</td>
<td>39.8</td>
<td>5.4</td>
</tr>
<tr>
<td>S⁻ₓ</td>
<td>7</td>
<td>4.2</td>
<td>3.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*aEach analysis = 6 samples from each of 2 reps.
References


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