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**Keywords**

germination, Glycine max (L.) Merr., membrane permeability, neutral lipids, phospholipids, saturated fatty acids, seedling growth rate

**Disciplines**

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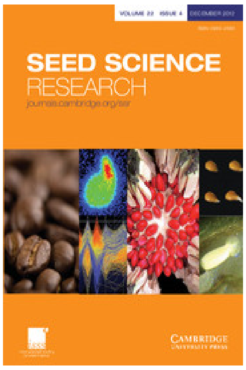


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SHORT COMMUNICATION

# Seed physiological performance of soybeans with altered saturated fatty acid contents

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**Abstract**

Soybean (*Glycine max* (L.) Merr.) seeds with elevated or reduced percentages of palmitate and elevated percentages of stearate were compared with seeds of typical composition in tests for germination, seedling growth rate and leachate conductivity. In general, seeds with altered compositions did well in these physiological tests, but their vigour tended to be negatively correlated with the percentages of stearate and palmitate in various lipid classes.

**Keywords: germination, *Glycine max* (L.) Merr., membrane permeability, neutral lipids, phospholipids, saturated fatty acids, seedling growth rate**

## Introduction

Modification of the fatty acid composition of soybean oil to make it more competitive in various segments of the food and industrial oil markets (Hammond, 1992) and to make it more nutritional (Hu *et al.*, 1997) has been an important objective of plant breeding and molecular genetics in recent years. Altered fatty acid composition has been developed through traditional plant breeding (Wilson *et al.*, 1981) and application of chemical mutagens (Hammond and Fehr, 1975; Wilcox *et al.*, 1984; Fehr *et al.*, 1991) that have extended the range of the five major fatty acids normally found in soybean oil. One alteration of interest has been elevation of the saturated fatty acids, palmitate and stearate (Hartmann *et al.*, 1996; Rebetzke *et al.*, 1996, 1998; Stoltzfus *et al.*, 2000). Elevated palmitate may be used to achieve a desirable crystal structure in plastic fats, and soybeans with elevated saturated fatty acids may be used to make margarine and shortening stocks without hydrogenation, thereby reducing processing cost and avoiding the formation of *trans* fatty acids (Kok, 1998). Soybean oil with elevated palmitate also exhibits improved oxidative stability when used as frying oil (Shen *et al.*, 1997).

Among the lipid components of soybean seeds, 0.3–0.6% phospholipids (PL) are present, with phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) as the major classes (Hui, 1996; Wang *et al.*, 1997). These PLs are structural components of the plasma membrane. Membrane PLs must have the correct balance of saturated and unsaturated fatty acids (Chapman, 1973) to be in the proper physical state for cells to perform their metabolic tasks. In soybean mutants whose triacylglycerol (TAG) saturate percentages are elevated, PL saturate percentages also are increased (Mounts *et al.*, 1996; Wang *et al.*, 1997). Soybean seeds with elevated stearate frequently have poor field germination and reduced yield (observed by W.R. Fehr, Agronomy Department, Iowa State University, data not published). When canolas were genetically modified to produce oils with greater than 30% stearate, their germination rates were lower, and seedlings were less vigorous. Accumulation of stearate in membrane lipids may have made membranes less adaptable to changes in temperature and moisture content (Thompson and Li, 1997).

The standard germination test is the most widely accepted procedure for estimating seed viability, but it is an inadequate predictor of field emergence (Dornbos, 1988). Tests of seedling vigour were developed to complement viability tests. The seedling growth rate (SGR) test gives a reproducible and objective evaluation of differences in growth rate. The conductivity test is a measurement of electrolyte leakage from plant tissues, and the leachate

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conductivity has been negatively correlated with seed viability and vigour (McKersie and Senaratna, 1983).

In this study, we report the effect of altered saturated fatty acid content in soybean seed lipids on germination, seedling growth rate, and seed electrolyte retention.

## Materials and methods

### Soybean sample selection for physiological tests

Commercial soybean cultivars and experimental soybean lines were provided by W.R. Fehr at Iowa State University and Pioneer Hi-Bred International Inc. of Des Moines, IA, USA. One hundred individual seeds from each soybean line with altered palmitate or stearate content were analysed by gas chromatography (GC) for fatty acid composition (Hammond, 1991), and 12 soybean lines were selected for the widest possible ranges of palmitate and stearate percentages and for the least variation in seed-to-seed composition.

### Lipid compositional analysis

PL isolation, class separation and fatty acid composition analysis were performed as described by Wang *et al*. (1997).

### Germination evaluation

The rolled-towel germination method (AOSA, 1986) was used. Each roll consisted of two standard-weight paper towels (35.5 × 63 cm). Each towel was moistened with 30 g of water. On the lower towel, 50 seeds were oriented into two rows with 25 seeds per row. The seeds were covered with an upper towel, the two paper towels were loosely rolled, and the rolls were placed upright in plastic containers that were loosely covered with plastic bags and placed in unlighted germinators at 15, 25 and 35°C. After 7 days, germination counts were made. Three replications for each line/temperature combination were performed.

### Seedling growth rate

The seedling growth rate (SGR) vigour test, which evaluates the growth of seedlings from a standard germination test, was performed according to the procedures described in the Rules for Testing Seeds (AOSA, 1986). After germination counts, the normal seedlings were freed of cotyledons, dried at 84°C for 63 h, and weighed. SGR was expressed as mg/seedling.

### Conductivity test

A G-2000 seed analyser (Wavefront Inc., Ann Arbor, MI, USA) was used to measure the electrical conductivities of the leachates for each of 50 individual seeds at ambient temperature. Each seed was placed in a cell filled with 3.5 ml distilled water on a 100-cell tray. Conductivity readings in micro-Siemens were taken after leaching for 1, 3, 6, 12 and 24 h. Three 50-seed replicates were tested for each line.

### Statistical analysis

A general linear model procedure of SAS was used for analysis of variance, and the least significant difference (LSD) was determined for mean comparison (SAS, 1984).

## Results and discussion

### Fatty acid composition

The average fatty acid composition of the soybean lines tested and the standard deviations among 100 individual seeds are shown in Table 1. Two commercial cultivars, Sturdy and Kenwood, were included in the study. For the soybean lines with elevated stearate, there was considerably greater variation among individual seeds. The unsaturated fatty acid percentages varied to compensate for the changes in the saturated fatty acid percentages.

The fatty acid composition of the major lipid classes in the experimental lines, except for 'Sturdy', is presented in Table 2. In lines with lipids elevated in saturates, there were corresponding increases in the PLs. PC and PE contained higher palmitate percentages than TAG except for two of the lines with elevated palmitate (PAL53 and PAL82). PE generally contained more palmitate but less stearate than PC and TAG except in the reduced palmitate lines, while PI contained the highest palmitate and stearate of the PL. PI palmitate was also higher than that of TAG in all instances, but PI stearate was frequently less than that of TAG in elevated saturate lines. When one of the saturates was elevated or reduced in PL, the other saturate tended to change in the opposite direction. These observations suggest that the saturate content of soybean PL is regulated in some way.

### Seed physiological behaviour

Data for germination percentages, SGR and leachate conductivity of the 12 soybean lines are presented in Table 3. Germination percentages at 15 and 25°C were generally high except for PAL79, which had reduced

**Table 1.** Average soybean fatty acid percentages (mole) and compositional variation (SD) among 100 seeds

| Classification | Sample ID | 16:0[a] | SD | 18:0 | SD | 18:1 | SD | 18:2 | SD | 18.3 | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Typical composition | Sturdy | 10.1 | 0.3 | 4.3 | 1.5 | 24.3 | 2.2 | 52.9 | 1.8 | 8.4 | 0.7 |
| | STE07 | 10.4 | 0.6 | 4.3 | 0.4 | 31.9 | 4.5 | 46.8 | 3.6 | 6.6 | 0.6 |
| | STE15 | 11.1 | 0.5 | 4.0 | 0.3 | 28.3 | 3.8 | 49.3 | 2.7 | 7.3 | 1.1 |
| | Kenwood | 11.6 | 0.5 | 4.4 | 0.3 | 22.6 | 2.7 | 53.3 | 2.2 | 8.1 | 0.7 |
| Reduced 16:0 | PAL79 | 3.0 | 0.9 | 2.9 | 0.3 | 16.1 | 2.5 | 65.1 | 2.0 | 12.9 | 1.9 |
| | PAL22 | 3.3 | 0.5 | 4.1 | 0.5 | 26.2 | 4.0 | 56.8 | 3.6 | 9.7 | 1.3 |
| Elevated 16:0 | PAL53 | 26.5 | 0.9 | 4.8 | 0.6 | 15.3 | 1.2 | 44.5 | 1.0 | 8.9 | 0.8 |
| | PAL82 | 32.5 | 1.9 | 5.3 | 0.8 | 12.9 | 1.6 | 37.3 | 1.5 | 12.1 | 1.2 |
| Elevated 18:0 | STE39 | 10.1 | 0.5 | 15.6 | 2.6 | 16.7 | 2.0 | 48.3 | 1.9 | 9.4 | 0.9 |
| | STE62 | 9.4 | 0.6 | 21.8 | 4.0 | 17.5 | 1.7 | 43.4 | 3.7 | 8.0 | 0.8 |
| | STE73 | 8.7 | 0.8 | 25.1 | 5.9 | 17.5 | 1.9 | 40.6 | 4.7 | 8.2 | 0.7 |
| | STE71 | 9.6 | 0.5 | 26.6 | 3.6 | 16.9 | 1.0 | 39.2 | 3.5 | 7.8 | 0.7 |

[a]The number before the colon is the chain length of the fatty acid; the number after the colon is the number of double bonds in each fatty acid molecule.

**Table 2.** Saturated fatty acid percentages of neutral lipid (TAG) and phospholipids (PLs) for various lines of soybean seeds

| Sample classification | Sample ID | TAG | | PC | | PE | | PI | |
|---|---|---|---|---|---|---|---|---|---|
| | | 16:0 | 18:0 | 16:0 | 18:0 | 16:0 | 18:0 | 16:0 | 18:0 |
| Typical composition | STE07 | 10.4 | 4.3 | 14.4 | 3.7 | 20.5 | 2.9 | 29.9 | 7.5 |
| | STE15 | 11.1 | 4.0 | 15.0 | 3.7 | 20.1 | 2.8 | 32.3 | 6.9 |
| | Kenwood | 11.6 | 4.4 | 15.1 | 4.5 | 22.1 | 3.4 | 33.0 | 9.1 |
| Reduced 16:0[a] | PAL79 | 3.0 | 2.9 | 8.6 | 6.8 | 11.9 | 7.3 | 17.3 | 15.9 |
| | PAL22 | 3.3 | 4.1 | 9.2 | 5.6 | 14.9 | 6.5 | 23.2 | 14.1 |
| Elevated 16:0 | PAL53 | 26.5 | 4.8 | 21.4 | 2.8 | 26.6 | 1.6 | 40.2 | 4.6 |
| | PAL82 | 32.5 | 5.3 | 25.4 | 3.4 | 26.4 | 1.8 | 36.7 | 6.8 |
| Elevated 18:0 | STE39 | 10.1 | 15.6 | 11.7 | 8.6 | 18.2 | 5.9 | 24.8 | 15.2 |
| | STE62 | 9.4 | 21.8 | 11.5 | 11.2 | 15.2 | 7.6 | 21.6 | 20.6 |
| | STE73 | 8.7 | 25.1 | 11.2 | 11.9 | 16.0 | 8.3 | 22.2 | 19.3 |
| | STE71 | 9.6 | 26.6 | 12.3 | 11.6 | 17.5 | 8.3 | 21.1 | 22.0 |

[a]See Table 1 for explanation of the fatty acid symbols.

saturate content and shrivelled seeds. Severe seed fungal decay occurred at 35°C, making evaluation of germination at this temperature less accurate. SGR was much greater at 25 and 35°C than at 15°C, and in most instances SGR was slightly better at 25 than 35°C.

The electrical conductivity of the 24-h leachate was generally lower for typical-composition lines (300–330 $\mu$Siemens) than for those with altered compositions (360–600 $\mu$Siemens), except for STE07, which had significantly higher conductivity, and STE39, which had a relatively low conductivity compared to the lines with similar fatty acid compositions. PAL79 had an unusually high conductivity (594 $\mu$Siemens). The conductivity was also measured at 1, 3, 6 and 12 h. The values at 1 and 3 h were low (below 100 $\mu$Siemens) and similar for all lines (data not shown). At 6 and 12 h, the relative conductivity differences among lines were similar to those at 24 h.

Correlation coefficients obtained from linear correlation analysis between neutral and PL saturates and germination percentage, SGR, and conductivity are shown in Table 4. The reduced saturate line, PAL79, was not included in the analysis because of its shrivelled seeds and poor germination. Generally, germination percentages were negatively correlated with saturated fatty acid content for all classes of soybean lipids, although many of the correlation coefficients were not statistically significant at the 5% level. Elevated saturate lines had significantly lower germination at 25°C, especially for elevated stearate lines, which showed significant negative correlation for all lipid classes. At 35°C the stearate percentages of PC and PE were significantly negatively correlated with germination.

**Table 3.** Soybean seed germination, seedling growth rate (SGR) and conductivity tests of various types of soybean seeds

| Sample classification | Sample ID | Germination (%) 15°C | 25°C | 35°C | SGR (mg/seedling) 15°C | 25°C | 35°C | Conductivity ($\mu$Siemens, 24 h) |
|---|---|---|---|---|---|---|---|---|
| Typical | Sturdy | 99.3 | 98.0 | 80.0 | 21.0 | 48.7 | 40.7 | 314.5 |
| | STE07 | 96.7 | 95.3 | 76.0 | 22.3 | 48.1 | 43.4 | 431.7 |
| | STE15 | 97.3 | 97.3 | 59.3 | 23.7 | 47.6 | 44.5 | 328.8 |
| | Kenwood | 98.7 | 96.7 | 46.7 | 23.4 | 50.1 | 43.9 | 305.6 |
| Reduced 16:0[a] | PAL79 | 82.7 | 42.0 | 17.3 | 14.3 | 41.4 | 37.9 | 594.4 |
| | PAL22 | 98.7 | 94.7 | 72.7 | 22.7 | 43.1 | 40.3 | 364.6 |
| Elevated 16:0 | PAL53 | 99.3 | 91.3 | 75.3 | 21.9 | 42.1 | 41.6 | 434.8 |
| | PAL82 | 95.3 | 82.7 | 26.0 | 19.4 | 47.5 | 52.9 | 519.7 |
| Elevated 18:0 | STE39 | 96.0 | 93.3 | 64.0 | 24.4 | 46.5 | 37.2 | 329.2 |
| | STE62 | 97.3 | 92.7 | 33.3 | 23.2 | 46.3 | 43.1 | 467.7 |
| | STE73 | 98.7 | 86.7 | 6.0 | 20.9 | 45.9 | 38.1 | 504.8 |
| | STE71 | 88.7 | 88.7 | 38.0 | 17.8 | 40.5 | 42.0 | 363.5 |
| | $LSD_{0.05}$ | 3.7 | 8.2 | 19.4 | 2.2 | 4.2 | 8.0 | 37.8 |

[a]See Table 1 for explanation of the fatty acid symbols.

**Table 4.** Correlation ($R^2$) of neutral and polar lipid saturated fatty acid percentages with germination, SGR and conductivity tests

| | Germination (%) 15°C | 25°C | 35°C | SGR (mg/seedling) 15°C | 25°C | 35°C | Conductivity ($\mu$Siemens) |
|---|---|---|---|---|---|---|---|
| TG 16:0[b] | n.s.[a] | −0.85 | n.s. | −0.80 | n.s. | n.s. | n.s. |
| TG 18:0 | n.s. | −0.92 | n.s. | n.s. | −0.79 | n.s. | n.s. |
| TG 16:0 + 18:0 | n.s. | −0.84 | −0.65 | −0.63 | n.s. | n.s. | n.s. |
| PC 16:0 | n.s. | −0.82 | n.s. | −0.81 | n.s. | n.s. | n.s. |
| PC 18:0 | n.s. | −0.90 | −0.76 | n.s. | n.s. | n.s. | n.s. |
| PC 16:0 + 18:0 | n.s. | −0.85 | n.s. | n.s. | n.s. | n.s. | 0.63 |
| PE 16:0 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| PE 18:0 | n.s. | −0.91 | −0.76 | n.s. | n.s. | n.s. | n.s. |
| PE 16:0 + 18:0 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| PI 16:0 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| PI 18:0 | n.s. | −0.85 | n.s. | n.s. | −0.76 | n.s. | n.s. |
| PI 16:0 + 18:0 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |

[a]n.s. denotes not statistically significant at 5% level.
[b]See Table 1 for explanation of the fatty acid symbols.

SGRs were negatively correlated with saturate content at 15 and 25°C for all lipid classes, although only a few were statistically significant. At 15°C, palmitate in TAG and PC was significantly negatively correlated with SGR, whereas at 25°C, stearate in TAG and PI was significantly negatively correlated. More soybean lines should be included in future experiments in order to demonstrate a more definite effect of saturate percentage on seed performance.

Conductivity of seed leachate was generally not significantly correlated with saturate content, except for PC total saturates. Nevertheless, the correlations were all positive. Possibly, increased saturate in PC makes membranes more permeable.

In general, the lines with altered fatty acid compositions did well in the tests of seed viability and vigour, but under some conditions there was a significant negative correlation between elevated lipid saturates and germination, SGR and electrolyte retention. Some of these data suggest that increased stearate percentage may be more detrimental to the viability and vigour of soybean seeds than increased palmitate. These effects may be related to the changes elevated saturates had caused in the physical properties of the TAG and PL classes (Wang, 1998). Hypothetically, soybeans with high saturate percentages should perform better at a relatively high temperature because their membranes would be more fluid, and they might be too rigid at low temperatures.

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