

Tocopherol Content and Agronomic Performance of Soybean Lines with Reduced Palmitate

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ABSTRACT

Soybean [*Glycine max* (L.) Merr.] cultivars have been developed with reduced palmitate content that is controlled by the *fap1* and *fap3* alleles. One objective of our study was to compare the tocopherol content of reduced and normal palmitate lines with similar genetic backgrounds. A second objective was to compare the agronomic and seed traits of the two types of lines. Three single-cross populations segregating for palmitate content were developed from which 20 reduced and normal palmitate lines were selected for evaluation at three environments during 2004. The mean total tocopherol of the reduced palmitate lines was 15% greater than the normal palmitate lines, and the line with the greatest total tocopherol in each population contained reduced palmitate. The mean seed yield of the reduced palmitate lines was 5.5% lower than the normal palmitate lines. There were significant differences between the two types of lines for other agronomic and seed traits; however, the significant genetic variation among lines of each type would permit the selection of reduced palmitate lines that were similar to normal palmitate lines for those traits. Use of the reduced palmitate trait would be advantageous to maximize the tocopherol content of soybean oil.

SOYBEAN OIL is an important source of the tocopherols used in supplements to increase vitamin E in the human diet. γ -Tocopherol has been associated with reduced prostate cancer and coronary heart disease (Ohrvall et al., 1996; Helzlsouer et al., 2000). Tocopherols also are known to inhibit lipid peroxidation by quenching lipid peroxy radicals, which contributes to oil stability (Stone and Papas, 2003).

Modifications in the palmitate content of soybean oil have been associated with changes in tocopherol content (Mounts et al., 1996; McCord, 2003). Mounts et al. (1996) observed that soybean lines with elevated saturated fatty acid content were significantly lower in total tocopherol compared with Hardin 91, a conventional soybean cultivar. McCord (2003) found that lines with reduced palmitate had greater tocopherol content than lines with normal palmitate. However, Dolde et al. (1999) evaluated soybean lines with modified fatty ester profiles and found no association between palmitate and tocopherol content. The three previous studies made comparisons among lines that had diverse genetic backgrounds; therefore, the associations observed between palmitate and

tocopherol content may have been due to genetic factors other than those related to palmitate content. One objective of our study was to evaluate the tocopherol content of reduced and normal palmitate lines with similar genetic backgrounds.

Reducing the palmitate content of soybean lines may be associated with a reduction in seed yield (Ndzana et al., 1994; Rebetzke et al., 1998). Ndzana et al. (1994) reported the mean seed yield of reduced palmitate lines was 7.2% less than normal palmitate lines averaged across three populations. Rebetzke et al. (1998) reported a 10% lower seed yield for reduced palmitate lines compared with normal palmitate lines averaged across two populations. In contrast, Horejsi et al. (1994) found reduced palmitate lines within a backcross population whose yield was not significantly different than the recurrent parent, which indicated that the reduced palmitate trait was not detrimental to yield. The second objective of this research was to compare the agronomic and seed traits of reduced and normal palmitate lines from segregating populations.

MATERIALS AND METHODS

The three reduced palmitate lines used as parents in this study were B01769B019, B01472B013, and B01447B013 developed jointly by Pioneer Hi-Bred International, Inc. (Johnston, IA) and Iowa State University. The reduced palmitate trait in the lines was controlled by the *fap1* and *fap3* alleles (Fehr et al., 1991). The high yielding parents with normal palmitate content were the experimental lines 98938 and XB29D01 and the cultivar S25-J5. The line 98938 was developed by Dairyland Seed Co., West Bend, WI; XB29D01 was developed by Pioneer Hi-Bred International Inc.; and S25-J5 was developed by Syngenta, Washington, IA.

The crosses of 98938 \times B01769B019 (Population 1), S25-J5 \times B01472B013 (Population 2), and XB29D01 \times B01447B013 (Population 3) were made in July 2001 at the Agricultural and Agronomy Research Center near Ames, IA. The F₁ seeds from each cross were planted in October 2001 at the Iowa State University–University of Puerto Rico soybean breeding nursery at Isabela, PR. The soil type is a Coto clay (very-fine, kaolinitic, isohyperthermic Typic Eutruxox). The F₁ plants of each population were confirmed as hybrids by flower color, and the plants of each population were harvested in bulk.

For each population, 425 F₂ seeds were planted at Isabela in February 2002. One pod from each plant of a population was harvested and the pods were threshed in bulk. A total of 850 F₃ seeds from each population were planted near Ames in May 2002. The F₃ plants were harvested individually based on maturity. The maturity of the plants used in this study ranged from late maturity group II to early maturity group III. A five-seed bulk from each plant was evaluated for palmitate content by gas chromatography as described by Hammond (1991). The 50 plants with the lowest palmitate (30–40 g kg⁻¹) and the 50 plants with the highest palmitate (90–122 g kg⁻¹) content were selected for testing as F_{3,4} lines in 2003.

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For each population, one set of 110 entries was planted in May 2003. Each set consisted of the 50 F_{3,4} lines with reduced palmitate, the 50 F_{3,4} lines with normal palmitate, the parents of the population, five conventional cultivars as maturity checks, and three cultivars as fatty acid checks. Each set was grown as a randomized complete-block design with one replication at the Agronomy Farm and one replication at the Burkey Farm of Iowa State University near Ames. The soil type at both locations is a Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludoll). For each plot, 20 seeds were planted in rows 0.76 m long with a spacing of 1.02 m between rows and an alley 1.07 m wide between the ends of plots. Maturity was recorded for each entry. The entries were harvested with a single-row self-propelled combine (Almaco, Nevada, IA). After harvest, two five-seed bulk samples from each plot were analyzed for palmitate content. The 27 lines with the lowest palmitate (33–40 g kg⁻¹) and the 27 lines with the highest palmitate (95–121 g kg⁻¹) content were selected for testing in 2004.

In 2004, one set of 60 entries for each population was planted at Carlisle, Rippey, and Ames, IA. The soil type at Carlisle is a Tama silty clay loam (fine-silty, mixed, superactive, mesic Typic Argiudoll) and at Rippey is a Nicollet loam. The 60 entries of each set consisted of the 27 reduced palmitate lines, the 27 normal palmitate lines, the two parents of the population, two reduced palmitate cultivars, and two normal palmitate cultivars. A randomized complete-block design with two replications at each location was used for each set. The plots were two rows 3.05 m long with 0.69 m between rows within a plot and 1.02 m between rows of adjacent plots. The seeding rate was 30 seeds m⁻².

Each plot was evaluated for maturity, lodging, plant height, yield, seed weight, protein content, oil content, and fatty ester content. Maturity was recorded as days after 31 August when 95% of the pods had reached their mature color. Lodging was a visual score on a scale of 1 (all plants erect) to 5 (all plants prostrate). Plant height was measured as the distance from the ground to the terminal node. All plots were harvested with a self-propelled two-row combine (Almaco, Nevada, IA) and weight and moisture were recorded. Yield of the plots was adjusted to 130 g kg⁻¹ moisture. Seed weight was measured with 200 whole seeds. Protein and oil content were measured using an Infratech 1221 near-infrared whole grain analyzer (Tecator AB, Hooganas, Sweden) and adjusted to 130 g kg⁻¹ moisture. The plots were analyzed for palmitate, stearate, oleate, linoleate, and linolenate content with two five-seed bulk samples. The original goal was to analyze the tocopherol content of all of the lines in the experiment; however, the time and cost associated with tocopherol measurement was considered to be too great. Consequently, the 20 lines with the lowest palmitate and the 20 lines with the highest palmitate in each population were chosen for tocopherol analysis.

The tocopherol analysis was conducted using high performance liquid chromatography (HPLC) with 30 random seeds from each plot. Six of the 30 seeds were placed in each of five different wells of an aluminum plate and crushed with 2.76 × 10⁵ kPa of pressure with a hydraulic press. After the seeds were crushed, they were submerged in 2 mL of hexane and allowed to soak for 6 h. After soaking, 0.3 mL of the hexane–oil mixture from each of the five wells was pooled together in a preweighed 1.5-mL glass vial from which the sample was taken for tocopherol analysis. A second 0.3-mL sample from each well was pooled together in a second vial for fatty ester analysis. The samples for HPLC analysis were placed under a chemical hood for 8 to 12 h to allow the hexane to evaporate. To complete the solvent evaporation, the samples were transferred into a vacuum oven (National Appliance Company, Portland, OR)

equipped with a model 1300 Welch Duo-Seal vacuum pump (Sargent Welch Scientific Co., Skokie, IL) for 6 h at an ambient temperature of 22°C. After hexane evaporation, the vials were weighed again to determine the weight of the oil. The samples were redissolved with HPLC grade hexane (Fisher Scientific, Fair Lawn, NJ) to a total volume that was level with the vial neck, which averaged 1.8109 mL of the hexane–oil mixture.

The samples for each replication of a population were analyzed by HPLC in the same plot order used in the field, and the two replications of the 40 lines of a population from a location were analyzed in the same run of 80 samples. External standards of α -, γ -, and δ -tocopherol (Sigma-Aldrich, Inc., St. Louis, MO) were used with each replication with the same injection volume as the analyzed samples. The standards were used to calculate the amount of each of the individual tocopherols of each sample. A 15- μ L aliquot of each sample was injected. All samples were eluted with 1% (v/v) isopropanol in HPLC-grade hexane at a 0.650 mL min⁻¹ flow rate with a Beckman Coulter System Gold 126 solvent delivery system, 508 auto-sampler, and 168 UV detector (Beckman Coulter, Inc., Beckman, CA) at a wavelength of 292 nm. The column was 250 by 3.1 mm Lichrosorb Si60 (Merck, Whitehouse Station, NJ) with a 7- μ m particle size. The integration of β -tocopherol was included in the γ -tocopherol component due to insufficient separation during the analysis. The tocopherol data were expressed as mg kg⁻¹, and the individual tocopherol components were calculated as a percentage of total tocopherol.

The fatty ester content of each sample was determined as a normalized percentage of the five major fatty esters. The percentages were converted to grams per kilogram by multiplying the percentage by 10.

Statistical analyses for all traits were conducted for the 20 reduced palmitate and 20 normal palmitate lines used for the tocopherol analysis. All data were analyzed as a randomized complete-block design using the general linear model (GLM) procedure of SAS version 8.2 (SAS Institute, 2001). Environments and replications were considered random, and the reduced and normal palmitate lines were considered fixed. For determining the significance of the mean differences between the two types of lines, the genotype × environment interactions were used as the error estimates for the *F*-tests. Phenotypic correlations were calculated among the traits based on the line means across environments using the correlations (CORR) procedure of SAS version 8.2 (SAS Institute, 2001). Broad-sense heritability estimates on a plot and entry-mean basis were calculated based on the appropriate variance components derived from the combined analysis of variance across environments, as described by Hallauer and Miranda (1981).

RESULTS AND DISCUSSION

There was a significant difference in the mean palmitate content of the reduced and normal palmitate lines in the three populations (Table 1). The individual reduced palmitate lines were at least twofold lower in palmitate than any of the normal palmitate lines. The mean stearate content of the reduced palmitate lines was significantly lower and the mean oleate, linoleate, and linolenate contents were significantly greater than that of the normal palmitate lines. The distributions of the four fatty esters for the individual lines of the two types overlapped, which indicated that some of the reduced palmitate lines had the same stearate, oleate, linoleate, and linolenate as normal palmitate lines. A similar asso-

Table 1. Mean and range for seed and agronomic traits of 20 reduced and 20 normal palmitate soybean lines from three soybean populations grown in three environments in 2004.

Trait	Type†	Population									
		1		2		3					
		Mean	Range	Mean	Range	Mean	Range				
Palmitate, g kg ⁻¹	R	40	36-43	ns‡	40	35-43	**	36	32-39	ns	
	N	111	106-115	**	98	95-101	ns	99	91-107	**	
Total tocopherol, mg kg ⁻¹	R	1259	1172-1494	**	1404	1223-1669	**	1378	1138-1673	**	
	N	1162	1057-1313	**	1183	1103-1346	**	1167	1041-1359	**	
α-Tocopherol, mg kg ⁻¹	R	141	124-165	**	129	90-183	**	155	123-203	**	
	N	139	112-174	**	116	85-159	**	136	120-163	**	
γ-Tocopherol, mg kg ⁻¹	R	739	682-904	**	856	719-1004	**	797	657-942	**	
	N	699	637-797	**	734	687-811	**	697	631-847	**	
δ-Tocopherol, mg kg ⁻¹	R	380	340-435	**	420	374-491	**	426	332-556	**	
	N	325	265-381	**	332	268-400	**	334	269-423	**	
α-Tocopherol, % of total§	R	11.2	10.3-12.8	**	9.2	6.8-13.8	**	11.3	9.9-13.3	**	
	N	12	9.6-14.7	**	9.8	7.7-12.8	**	11.7	9.8-13.7	**	
γ-Tocopherol, % of total§	R	58.8	56.6-60.8	**	61	57-63	**	57.9	56-60.1	**	
	N	60.2	58.3-62.7	**	62.2	59.1-64.7	**	59.8	57.2-62.9	**	
δ-Tocopherol, % of total§	R	30.1	27.6-32.3	**	29.8	27.6-32.7	**	30.8	28-33.4	**	
	N	27.9	24.7-30	**	28.1	24.3-30.8	**	28.5	24.9-31.7	**	
Stearate, g kg ⁻¹	R	35	29-40	**	33	29-40	**	31	26-36	**	
	N	42	38-46	**	40	36-44	**	41	34-44	**	
Oleate, g kg ⁻¹	R	263	247-288	**	293	264-343	**	277	233-311	**	
	N	248	222-273	**	257	234-293	**	257	231-288	**	
Linoleate, g kg ⁻¹	R	580	555-599	**	549	502-577	**	567	536-601	**	
	N	521	499-542	**	524	496-547	**	524	495-548	**	
Linolenate, g kg ⁻¹	R	82	76-86	**	85	76-98	**	89	77-107	**	
	N	78	68-83	**	81	73-91	**	79	73-89	**	
Yield, kg ha ⁻¹	R	3281	2930-3627	**	3281	2727-3474	**	3321	2784-3618	**	
	N	3418	3105-3596	ns	3504	3167-3800	**	3529	3276-3806	*	
Lodging, score	R	2.3	1.9-2.7	ns	2.4	2.2-2.6	ns	2.5	2.3-2.8	ns	
	N	2.3	1.8-2.8	**	2.3	1.9-2.7	**	2.5	2.3-2.8	**	
Maturity, d¶	R	19	14-24	**	20	16-24	**	19	12-22	**	
	N	20	15-25	**	18	15-22	**	18	14-22	**	
Seed weight, mg seed ⁻¹	R	163	147-210	**	168	152-181	**	192	175-211	**	
	N	153	145-170	ns	167	157-178	**	190	177-216	**	
Height, cm	R	92	84-100	**	88	82-96	**	85	73-93	**	
	N	90	79-102	**	83	78-91	*	83	75-91	**	
Protein, g kg ⁻¹	R	372	362-382	**	375	360-385	**	388	376-401	**	
	N	369	356-385	**	370	363-380	**	378	364-391	**	
Oil, g kg ⁻¹	R	200	195-206	**	190	175-201	**	183	171-191	**	
	N	201	193-208	**	200	191-207	**	197	186-208	**	

* Difference between the means of types or among lines within a type were significant at the 0.05 probability level.

** Difference between the means of types or among lines within a type were significant at the 0.01 probability level.

† R, reduced palmitate lines; N, normal palmitate lines.

‡ ns, differences between mean of types or among lines within a type were not significant at the 0.05 probability level.

§ Total tocopherol.

¶ Days after 31 August.

ciation of the four fatty esters with reduced palmitate was observed by Ndzana et al. (1994).

One objective of the research was to compare the tocopherol content of reduced and normal palmitate lines. The reduced palmitate lines had significantly greater mean total tocopherol content than the normal palmitate lines in all the populations (Table 1). The total tocopherol of the reduced palmitate lines was greater by 8.3% in Population 1, 18.7% in Population 2, and 18.1% in Population 3. The individual lines with the greatest total tocopherol in the three populations had reduced palmitate. The data indicated that use of the *fap1* and *fap3* alleles for reduced palmitate would be effective for increasing the total tocopherol of soybean oil. The results support the studies of Mounts et al. (1996) and McCord (2003) that found the palmitate content of unrelated soybean lines was negatively associated with their total tocopherol content.

There was significant variation in total tocopherol content among the reduced palmitate lines of each population. The negative correlation coefficients between

palmitate and total tocopherol indicated that HPLC analysis of lines with the lowest palmitate should be effective for identifying those lines with the greatest tocopherol content (Table 2). The broad-sense heritability estimates for total tocopherol of the reduced palmitate lines ranged from 0.43 to 0.68 on a plot basis and 0.80 to 0.93 on an entry-mean basis across the three populations (Table 3). Selection for total tocopherol on an individual plot or an entry-mean basis should be effective in a breeding program.

The mean α-, γ-, and δ-tocopherols of the reduced palmitate lines were significantly greater than that of the normal palmitate lines (Table 1). The individual lines with the greatest content of the individual tocopherols had reduced palmitate, except for α-tocopherol in Population 1 (Table 1). Although the individual tocopherols generally were greater for the reduced palmitate lines, the percentages of the individual tocopherols in the total tocopherol for the two types of lines were not the same. The mean percentages of α- and γ-tocopherol in the total tocopherol were significantly less and the mean

Table 2. Phenotypic correlation coefficients among traits for reduced palmitate, normal palmitate, and all soybean lines from three populations.

Trait	Type†	Population 1							
		Tocopherol							
		α		γ		δ		Total	
Palmitate	R	0.06	ns‡	-0.31	ns	-0.10	ns	-0.22	ns
	N	0.23	ns	-0.18	ns	-0.33	ns	-0.20	ns
	A	-0.06	ns	-0.43	**	-0.69	**	-0.57	**
α -Tocopherol	R			0.41	ns	0.39	ns	0.54	*
	N			0.02	ns	-0.04	ns	0.24	ns
	A			0.19	ns	0.13	ns	0.33	*
γ -Tocopherol	R					0.67	**	0.95	**
	N					0.83	**	0.94	**
	A					0.77	**	0.94	**
δ -Tocopherol	R							0.86	**
	N							0.91	**
	A							0.91	**
Population 2									
Palmitate	R	-0.32	ns	-0.63	**	-0.50	*	-0.64	**
	N	0.14	ns	-0.35	ns	-0.74	**	-0.47	*
	A	-0.27	ns	-0.77	**	-0.81	**	-0.79	**
α -Tocopherol	R			0.23	ns	0.34	ns	0.48	*
	N			0.46	*	0.08	ns	0.60	**
	A			0.38	*	0.35	*	0.52	**
γ -Tocopherol	R					0.76	**	0.94	**
	N					0.53	*	0.90	**
	A					0.87	**	0.97	**
δ -Tocopherol	R							0.89	**
	N							0.76	**
	A							0.94	**
Population 3									
Palmitate	R	-0.38	ns	-0.55	*	-0.20	ns	-0.45	*
	N	-0.19	ns	-0.40	ns	-0.31	ns	-0.39	ns
	A	-0.56	**	-0.57	**	-0.71	**	-0.66	**
α -Tocopherol	R			0.69	**	0.47	*	0.70	**
	N			0.63	**	0.13	ns	0.55	*
	A			0.77	**	0.59	**	0.77	**
γ -Tocopherol	R					0.83	**	0.98	**
	N					0.69	**	0.97	**
	A					0.85	**	0.98	**
δ -Tocopherol	R							0.91	**
	N							0.84	**
	A							0.93	**

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† R, reduced palmitate lines; N, normal palmitate lines; A, all lines.

‡ ns, not significant at the 0.05 probability level.

percentage of δ -tocopherol was significantly greater for the reduced palmitate lines than for the normal palmitate lines.

The significant variation among the reduced palmitate lines for the individual tocopherols and their percentages of the total tocopherol indicated that it should be possible to select for the amount and proportion of each component (Table 1). There would be limits to how

much the proportion of each could be altered because of the significant correlations of the three components with total tocopherol and with each other (Table 2). The γ - and δ -tocopherols had the greatest and most consistent correlations with total tocopherol and with each other across the three populations. The correlations of α -tocopherol with total tocopherol and with γ - and δ -tocopherol generally were lowest and least consistent.

Table 3. Broad-sense heritability estimates of individual and total tocopherol content for reduced and normal palmitate soybean lines from three populations.

Population‡	Plot basis				Entry-mean basis†			
	α	γ	δ	Total	α	γ	δ	Total
Population 1								
R	0.19	0.50	0.47	0.43	0.65	0.85	0.81	0.80
N	0.19	0.13	0.41	0.19	0.87	0.42	0.78	0.53
Population 2								
R	0.45	0.75	0.71	0.76	0.96	0.93	0.93	0.93
N	0.56	0.44	0.71	0.57	0.95	0.80	0.93	0.87
Population 3								
R	0.20	0.67	0.74	0.68	0.86	0.91	0.94	0.91
N	0.17	0.72	0.76	0.71	0.79	0.92	0.94	0.92

† Based on two replications at three environments.

‡ R, reduced palmitate lines; N, normal palmitate lines.

A second objective of the research was to compare the agronomic and seed traits of reduced and normal palmitate lines. The mean yields of the reduced palmitate lines were significantly less than that of the normal palmitate lines by 4.0% in Population 1, 6.4% in Population 2, and 5.9% in Population 3 (Table 1). The highest yielding line in Population 1 had reduced palmitate, but the highest yielding line in Populations 2 and 3 had normal palmitate. Of the four highest yielding lines in each population (top 10%), only one was a reduced palmitate line in Population 1 and none of them were reduced palmitate lines in Populations 2 and 3. These results indicated that, on the average, reduced palmitate lines from a population would be expected to yield less than normal palmitate lines, which was in agreement with the research reported by Ndzana et al. (1994) and Rebetzke et al. (1998). The yield of reduced palmitate lines was more similar to that of normal palmitate lines in Population 1 than in Populations 2 and 3. These differences among the three populations for the impact of the reduced palmitate trait on yield was consistent with population differences observed by others who have evaluated the agronomic performance of lines with altered fatty ester composition (Ndzana et al., 1994; Walker et al., 1998; Ross et al., 2000). It will be important in a cultivar development program to evaluate lines from multiple populations to maximize the likelihood of identifying reduced palmitate lines that will yield comparable to lines with normal palmitate.

The mean maturity of the reduced palmitate lines was earlier than the normal palmitate lines in Population 1 and later in Populations 2 and 3 (Table 1). The mean plant height of the reduced palmitate lines was greater than the normal palmitate lines in all the populations, but the difference in lodging between the two types of lines was significant only in Population 2. The overlap in the distributions of the two types of lines for the three traits was substantial, which indicated that it should be possible to select from a population reduced palmitate cultivars that were comparable to normal palmitate cultivars.

The reduced palmitate lines had greater protein, lower oil, and greater seed weight than the normal palmitate lines in the three populations, although the differences between the means of the two types were not always significant (Table 1). The significant variation among lines within each type and the overlap in the distributions of the two types for the three traits indicated that it should be possible to select reduced palmitate cultivars from a population that were similar to normal palmitate cultivars for the three traits.

It would be advantageous to utilize the reduced palmitate trait for soybean cultivar development if the goal of the breeding program was to maximize the

tocopherol content of the oil. Selection for tocopherol content among reduced palmitate lines could be done effectively with individual or replicated plots. The number of populations used for selection should be as large as possible to maximize the likelihood of identifying reduced palmitate cultivars with seed yield comparable to those with normal palmitate.

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