Tocopherols in Breeding Lines and Effects of Planting Location, Fatty Acid Composition, and Temperature During Development

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ABSTRACT: As the use of tocopherols as natural antioxidants increases, it is economically and agronomically important to determine the range, composition, and factors that affect their levels in oilseed crops, a major commercial source. In this study, tocopherols were quantified from seeds of wheat, sunflower, canola, and soybean. The breeding lines analyzed possessed a broad range of economically important phenotypic traits such as disease or herbicide resistance, improved yield and agronomic characteristics, and altered storage oil fatty acid composition. Complete separation of all four native tocopherols was achieved using normal-phase high-performance liquid chromatography with ultraviolet detection. Total tocopherol concentration among wheat germ oil samples ranged from 1947 to 4082 μg g^{-1}. Total tocopherol concentration ranges varied from 534 to 1858 μg g^{-1} in sunflower, 504 to 687 μg g^{-1} in canola, and 1205 to 2195 μg g^{-1} among the soybean oils surveyed. Although the composition of tocopherols varied substantially among crops, composition was stable within each crop. Total tocopherol concentration and the percentage linolenic acid were correlated positively in soybean oils with modified and unmodified fatty acid compositions. Tocopherol concentration and degree of unsaturation were not correlated in sunflower or canola seeds with genetically altered fatty acid composition. These findings suggest that breeding for altered storage oil fatty acid composition did not negatively impact tocopherol concentrations in sunflower and canola as they apparently did in soybeans. When 12 soybean breeding lines were grown at each of five locations, significant correlations were observed among planting location, breeding line, tocopherol concentration, and fatty acid composition. Analysis of seeds that matured under three different controlled temperature regimes suggests that the relationship between tocopherol concentration level and unsaturated fatty acids in commodity (not genetically modified for fatty acid composition) oil types is due to temperature effects on the biosynthesis of both compounds.

Paper no. J8930 in *JAOCS 76,* 349–355 (March 1999).

KEY WORDS: Fatty acid composition, fatty acid modification, genetic effects, location effects, temperature effects, tocopherol, vegetable oil.

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Tocopherols are important natural antioxidants that inhibit lipid oxidation in foods and biological systems by stabilizing hydroperoxy and other free radicals (1). Since commercial interest in tocopherols and their antioxidative properties has increased in recent years, we have evaluated oilseed breeding lines as potential higher-yielding sources of this value-added component. Duration of storage and/or abuse, as well as processing, of seed and oil, especially deodorization, can have major impacts on the tocopherol concentration of refined, bleached, and deodorized oils (2). However, the initial concentration of tocopherol in the seed determines primarily the tocopherol concentration in the crude oil. It is also valuable to understand if and how the growing environment influences tocopherol accumulation in oilseeds.

It has been suggested recently that chemical mutagenesis of soybean lines leading to altered seed oil fatty acid composition has had more than an incidental impact on tocopherol levels (3). This line of reasoning is bolstered by a general tendency for tocopherol content in oilseeds to be positively correlated with the level of triacylglycerol unsaturation that is associated with oil instability (4). However, since the seeds used in many such studies were obtained from plants grown in different locations, the environmental conditions prevailing during seed development represent an uncontrolled variable. It is well documented that the degree of fatty acid unsaturation in seed oil is inversely proportional to growing temperature (5). Although the environmental factors affecting tocopherol synthesis in oilseeds have been previously investigated, they are not clearly understood (6,7). The objectives of our research were to determine tocopherol concentrations in seeds from selected germplasm and to investigate the effects of genetic background, planting location, and temperature on tocopherol quantity and quality.

EXPERIMENTAL PROCEDURES

Materials. Wheat, sunflower, canola, and soybean seeds of experimental and commercial breeding lines developed through conventional mutation/selection breeding were used. Several of the soybean lines were obtained from a licensing agreement with Iowa State University (Ames, IA). Seeds

were stored at 10°C and 50% relative humidity prior to analysis or planting. Fatty acid methyl ester and tocopherol standards were from Matreya, Inc. (Pleasant Gap, PA).

Field sites. Soybean seeds from 12 breeding lines were harvested from five 1995 yield trial locations throughout the Midwest: Johnston, IA; LaSalle, IL; Jasper, MI; Napoleon, OH; and Pocahontas, IA. Yield trial test plots consisted of four 15-foot rows planted at a rate of 8.5 seeds per foot. Seeds from the center two rows were harvested for evaluation.

Growth chambers. Soybean seed from the same breeding lines used in the yield test plots were planted in 1-gal pots with Universal Mix potting soil, Strong-Lite (Seneca, IL). Each pot containing one plant was grown until flowering in a greenhouse maintained at 18–35°C. The plants were top watered and treated periodically with 20:20:20 (N-P-K) commercial fertilizer. Upon flowering, two plants from each line were placed in each of three growth chambers (Coviron PGW36 model; Controlled Environments, Winnipeg, Canada). Plants of each line were placed at the same location in each chamber. Daytime temperatures of 35, 27, or 20°C were alternated with nighttime temperatures of 25, 17, and 10°C, respectively. In each chamber, daytime light was given for 12 h at an intensity of 220 μEi m^{-2} s^{-1} supplied by a combination of incandescent and fluorescent lamps. Seed samples were harvested from individual plants at maturity. Thus, for each temperature regime, two seed samples were acquired from each line.

Oil extraction. Approximately 11 g of canola or soybean seed or sunflower achene were ground to a powder in a small mill (Regal Ware, Kewaskum, WI). For wheat, sample size was increased to 110 g. Ten grams (or 100 g for wheat) ground material was combined with 40 mL (100 mL for wheat) hexane (technical grade, Fisher Scientific, Fair Lawn, NJ), stirred periodically over 4 h, and passed through a 0.2 μm cellulose acetate filter. Solvent was removed by evaporation by placing the filtrates in a fume hood at room temperature overnight under reduced light. Crude oil samples were transferred to amber vials and stored at 5°C in the dark. All oil samples were prepared and analyzed in duplicate.

Fatty acid composition. Approximately 40 mg crude oil was dissolved in 1 mL hexane [high-performance liquid chromatography (HPLC) grade, Fisher Scientific]. Transmethylation was achieved with trimethylsulfonium hydroxide according to the method of Butte *et al*. (8). Fatty acid composition (C_{14}–C_{24}) was determined by capillary gas chromatography. One microliter of extract was introduced with split (~1:130) injection on a 15 m × 0.25 mm i.d. × 0.25 μm film Supelcowax 10 (Supelco, Inc., Bellefonte, PA) column installed in a Hewlett-Packard (Palo Alto, CA) 5890 Series II chromatograph equipped with a flame-ionization detector. Peak areas of resolved methyl esters were integrated and the normalized percent recorded using Turbochrom v3.2 software, Perkin-Elmer (Cupertino, CA). Iodine values (IV) were calculated from fatty acid compositions according to AOCS official method Cd 1c-85 (9).

Tocopherols. Tocopherol contents of crude oils were determined by normal-phase HPLC using a Waters (Milford, MA) chromatography system consisting of a WISP 712 autosampler and a 510 pump. Approximately 5 mg oil in 100 μL HPLC-grade hexane was applied to a 60Å 250 × 4.00 mm LiChrosorb 5 Sil (Phenomenex, Torrance, CA) column. The column was eluted with hexane/isopropanol (99.5:0.5, vol/vol) at a flow rate of 2.0 mL min^{-1}. After 15 min, all four tocopherol isomers (α, β, γ, δ) were eluted, as detected at 292 nm by a Waters 481 ultraviolet-visible detector. Detector signal was recorded and peak areas were integrated. Individual tocopherol standards were diluted with HPLC-grade hexane to levels between 0.95 and 190.0 μg mL^{-1} to construct a six-point external standard curve. The coefficient of linearity for each tocopherol was greater than 0.999. Tocopherols in each oil were quantitated using a standard curve run on the same day as the sample.

Statistical analysis. Statistical analysis was performed using Microsoft (Redmond, WA) Excel for Windows 95, v7. Correlation coefficients were determined and used to describe the relationship between fatty acid composition and total tocopherol concentration in each oil. Two-factor analysis of variance (ANOVA) was performed to determine the relationships between calculated iodine value, tocopherol concentration, breeding line, planting location, and temperature during seed development.

RESULTS AND DISCUSSION

Wheat. Tocopherol content and fatty acid composition of wheat seed oils are presented in Table 1. Although wheat is not considered an oilseed, it produces oil with high concentrations of tocopherol. In our study the lower limit of total tocopherols was within the ranges reported in the literature (10), although the upper limit (4082 μg g^{-1}) was greater than that reported previously. The fatty acid profiles of the oils are similar, as expected without a breeding effort to alter their composition. Total tocopherol concentration is correlated positively ($P < 0.05$) with fatty acid unsaturation, as indicated by calculated IV. It is also correlated negatively to oleic acid and positively to linoleic acid ($P < 0.01$). Tocopherols are associated with the inner plastid envelope membrane (11). However, the contribution of plastid membrane components to the total lipid pool in seeds such as wheat is relatively small (12). It is more likely that environmental factors during seed development impact, in a similar manner, the accumulation of both tocopherols and unsaturated fatty acids resulting in a spurious correlation. In this regard, planting location has been implicated as a factor governing tocopherol concentration and fatty acid composition in wheat (13).

Sunflower. A summary of the tocopherol concentration and fatty acid composition of 66 experimental sunflower oils with commodity-type fatty acid profiles is presented in Table 2. As expected, α-tocopherol was the only tocopherol found at appreciable levels. β-Tocopherol was detected at low levels in only two of these samples. Among the sunflower oils with commodity-type fatty acid compositions, total tocopherol concentration was correlated negatively with oleic acid and positively with total saturates, as reported previously (14). As in

TABLE 1
Mean Tocopherol Amount (μg g^{-1}) and Fatty Acid Composition (mol%) in 18 Wheat Oils

	Tocopherol					Fatty acid composition[a]					
Line	α	β	γ	δ	Total	16:0	18:0	18:1	18:2	18:3	$IV_{calc.}$
PVL145	1037	476	1641	n.d.[b]	3154	17.3	0.9	12.2	62.4	5.3	133.6
PVF022	1063	346	1620	n.d.	3029	18.0	1.1	16.3	58.2	4.6	127.9
FG94011	1255	437	2017	n.d.	3710	16.7	1.0	12.9	62.4	5.4	134.1
FV93001	995	229	2085	n.d.	3308	15.9	1.0	16.0	61.1	4.4	132.1
FVP0041	1396	561	2125	n.d.	4082	18.0	0.9	13.0	61.8	4.7	131.5
FVM0001	909	459	1842	n.d.	3211	18.6	0.9	10.0	63.5	5.4	133.7
RCG0078B	1297	562	1118	n.d.	2978	16.2	1.2	15.8	60.6	4.4	131.1
RCG0085B	903	327	1215	n.d.	2444	15.4	1.5	18.2	59.1	4.0	129.5
RCL0101A	741	264	1200	n.d.	2204	16.6	1.4	24.8	49.4	5.0	121.7
RBJ0221E	898	357	1135	n.d.	2391	18.0	0.9	17.6	58.0	4.1	127.1
RBK0028C2	767	420	1560	n.d.	2747	16.9	1.0	18.7	57.5	4.1	127.5
RCL0145G	601	243	1104	n.d.	1947	15.4	1.5	21.7	54.9	4.9	127.6
2510	895	464	1819	n.d.	3177	17.2	1.0	17.1	59.2	4.2	128.9
2540	612	439	1903	n.d.	2954	17.3	1.2	18.8	56.6	3.5	124.6
2548	700	433	1865	n.d.	2998	15.7	1.4	22.0	55.4	3.1	124.4
2555	607	339	1833	n.d.	2780	16.6	1.2	20.3	56.5	3.5	125.5
2571	682	404	2232	n.d.	3318	17.8	1.2	15.6	59.7	4.3	128.8
WBA416H2	579	315	1631	n.d.	2525	15.5	1.0	19.4	58.5	3.8	129.0

[a]Percentages for minor fatty acids are not shown in this and subsequent tables but were used to calculate iodine values ($IV_{calc.}$).
[b]n.d., Not detected.

wheat, the lower limits of total tocopherols in these oils were within the ranges found in the literature, while the upper limit (1640 μg g^{-1}) was greater than that reported previously (10,14). In our study, 12 sunflower oils with genetically modified fatty acid profiles (Table 3) also exceeded the upper published limit. Among entries with modified storage oil fatty acid compositions, tocopherol content was not correlated with percentage of any fatty acid. This finding was expected, since the genes controlling tocopherol content in sunflower achenes are not linked to those governing oleic acid percentages (15,16). The lack of such a correlation in oils with genetically modified fatty acid compositions suggests strongly that tocopherol concentration and fatty acid profile are not causally related, but may be influenced similarly by an independent parameter.

Canola. Tocopherol contents in canola oils with genetically modified fatty acid profiles are shown in Table 4. Palmitic, oleic, and linolenic acid percentages are altered from percentages found in typical commodity oils. The total tocopherol concentrations had a limited range from 504 μg g^{-1} to 687 μg g^{-1}. Tocopherol composition was consistent among the canola oils, with 63–74% γ-tocopherol and 26–35% α-tocopherol. δ-Tocopherol was present in only trace amounts, while β-tocopherol was not detected. The tocopherol concentrations we obtained are within the same range of values reported previously for commodity crude canola oils (10,17,18). As in the sunflower oils with modified fatty acid profiles, fatty acid composition was not correlated with either total or individual tocopherol concentration in these oils.

Soybean. Fatty acid composition of each soybean oil is shown in Table 5. The entries were segregated into three groups. Oils of the first group possess diverse fatty acid compositions resulting from genetic modification. Varieties of the second group produce oils with conventional fatty acid profiles and exhibit other economically important traits, such as *Phytophthora* resistance, cyst nematode resistance, sulfonylurea tolerance, and/or enhanced yield. Entry 26 is a check sample, Pioneer 9171. As expected, calculated IV of oils of the second group with conventional fatty acid profiles varied little. Also not surprisingly, the mean calculated IV for this

TABLE 2
Descriptive Statistics of Mean Tocopherol Amount (μg g^{-1}) and Fatty Acid Composition (mol%) in 66 Sunflower Oils with Commodity-Type Fatty Acid Compositions[a]

	Tocopherol			Fatty acid composition					
	α	β	Total	16:0	18:0	18:1	18:2	18:3	$IV_{calc.}$
Count	66	2	66	66	66	66	66	66	66
Mean	981.2	16.7	981.7	7.05	5.44	15.50	70.51	0.07	135.9
Standard error	27.0	0.1	27.1	0.08	0.12	0.22	0.24	0.01	0.29
Minimum	534.1	16.6	534.1	5.6	3.5	10.8	64.3	0.0	128.8
Maximum	1640.2	16.9	1640.2	8.7	8.2	19.8	74.7	0.1	140.3

[a]For abbreviation see Table 1.

TABLE 3
Mean Tocopherol Amount (μg g^{-1}) and Fatty Acid Composition (mol%) in 12 Sunflower Oils with Genetically Modified Fatty Acid Composition

	Tocopherol					Fatty acid composition					
Line	α	β	γ	δ	Total	16:0	18:0	18:1	18:2	18:3	$IV_{calc.}$
Sun # 1	1301	<1	n.d.[a]	n.d.	1301	24.0	2.8	60.8	3.4	0.1	64.1
Sun # 2	883	14	n.d.	n.d.	898	3.2	1.6	90.8	2.7	0.0	83.2
Sun # 3	712	<1	n.d.	n.d.	712	8.4	3.8	26.1	59.7	0.1	126.4
Sun # 4	836	<1	n.d.	n.d.	836	6.8	4.9	24.8	61.5	0.1	128.4
Sun # 5	1267	2	n.d.	n.d.	1269	7.6	6.5	17.9	65.5	0.1	129.5
Sun # 6	729	<1	n.d.	n.d.	729	6.1	8.1	17.5	66.0	0.2	130.2
Sun # 7	917	<1	n.d.	n.d.	917	7.7	2.9	20.7	67.2	0.1	134.9
Sun # 8	938	<1	n.d.	n.d.	938	7.7	4.3	15.5	70.5	0.1	136.0
Sun # 9	1858	<1	n.d.	n.d.	1858	8.0	3.6	13.7	72.9	0.1	138.5
Sun # 10	1197	<1	n.d.	n.d.	1197	9.2	2.8	12.1	74.3	0.1	139.6
Sun # 11	1394	<1	n.d.	n.d.	1394	8.0	2.5	12.4	75.5	0.2	142.4
Sun # 12	778	<1	n.d.	n.d.	778	7.9	1.9	13.2	75.6	0.1	143.0

[a]See Table 1 for abbreviation.

group (132.5) was not statistically different from that (135.7) of oil produced by the check variety.

Tocopherol profiles and concentrations in these oils are also given in Table 5. Mean total tocopherol levels ranged from 1205 to 2195 μg g^{-1}. However, tocopherol composition was consistent among the oils, with γ-tocopherol being the most abundant, followed by the δ and α moieties. β-Tocopherol was detected in only trace amounts or not at all. The tocopherol concentrations in this study were within the same range as the values reported previously for crude soybean oils from commodity lines or lines that produce genetically modified storage oils (3,10,17,18).

Correlation coefficients between total tocopherol concentration and fatty acid content were calculated for each group separately. There was a high positive correlation between linolenic acid percentage and tocopherols (total, δ, and γ) in oils of both groups. Decreasing tocopherol levels with decreasing percentages of linolenic acid have been reported previously in genetically modified storage oil types (19), although an inverse relationship has been reported in both commodity-type (7) and low linolenate soybeans (3). Since the oils used in the latter study were obtained from diverse sources, it was not possible to account for the potential influence of growing conditions on expression of the two traits. Regardless, our study provides clear evidence that the low linolenate-types are associated with reduced tocopherol contents. It is likely that this association is a consequence of the very narrow gene pool of this phenotype. The positive correlation between total tocopherol and percentage linolenic acid in oils with commodity-type fatty acid profiles required an alternative explanation. Maintaining constant genetics while altering the growing environment should allow one to explore the relationship between tocopherol concentrations and fatty acid composition.

Effects of planting location and genotype. To better understand the effect of growing conditions on tocopherol accumulation, we sampled soybean seed from 12 different breeding lines that were grown at five different locations. Quantitation of tocopherols in these samples reveal that both genotype and planting location affect expression of this trait (Table 6). Genotype has the greater effect. Furthermore the location × line interaction is significant ($P < 0.01$).

Since the soybean lines in this study contained conventional fatty acid profiles, this trait is expressed as calculated IV (Table 7). ANOVA reveals that both genetic background and planting location significantly affected calculated IV, and thus fatty acid composition. Since planting location affects accumulation of both tocopherols and unsaturated fatty acids,

TABLE 4
Mean Tocopherol Amount (μg g^{-1}) and Fatty Acid Composition (mol%) in Canola Oils

	Tocopherol					Fatty acid composition					
Line	α	β	γ	δ	Total	16:0	18:0	18:1	18:2	18:3	$IV_{calc.}$
NS2309	183	n.d.[a]	391	<1	574	3.5	3.1	78.4	10.2	1.5	90.2
46A12	190	n.d.	447	<1	637	3.6	3.0	79.1	5.8	4.8	92.0
45A37	171	n.d.	333	<1	504	3.7	2.9	76.9	11.2	2.1	92.3
46A16	182	n.d.	408	<1	589	4.0	2.6	77.1	7.2	5.9	95.3
NC2304	156	n.d.	403	<1	559	2.6	1.8	78.2	8.1	6.7	99.8
NS2290	230	n.d.	454	<1	687	4.1	2.4	66.7	21.3	2.6	102.1
46A65	182	n.d.	379	<1	561	3.6	2.2	66.4	18.2	6.6	107.2
46A05	147	n.d.	418	<1	565	3.8	2.1	65.2	16.6	9.5	111.0
Goldrush	173	n.d.	452	<1	625	3.4	1.5	56.1	23.4	12.8	123.4

[a]See Table 1 for abbreviation.

TABLE 5
Mean Tocopherol Amount (μg g^{-1}) and Fatty Acid Composition (mol%) in Soybean Oils

	Tocopherol					Fatty acid composition					
Line	α	β	γ	δ	Total	16:0	18:0	18:1	18:2	18:3	$IV_{calc.}$
Genetically modified fatty acid compositions											
Bulk ABC	108	30	1232	775	2145	22.3	18.1	7.4	38.3	11.2	102.3
A91-194022	50	<10	1008	454	1513	8.0	24.8	17.3	39.2	8.2	104.4
A91-200049	41	<10	1172	535	1747	8.7	22.7	16.8	40.9	8.6	108.0
Bulk DE	93	16	750	504	1362	20.5	4.4	18.0	51.6	3.5	114.9
A92-215069	52	<10	1070	287	1409	9.3	4.3	39.0	43.3	2.9	116.5
YB24ZA	113	19	1049	506	1687	24.9	3.9	12.9	45.1	11.2	119.3
9253	56	<10	876	274	1205	9.9	4.7	28.1	53.1	3.0	124.2
A92-216030	71	<10	1245	584	1901	4.1	16.0	17.8	50.7	9.7	128.7
YA7777J09	50	<10	966	269	1285	4.0	3.4	28.3	60.7	2.6	136.6
9243	49	<10	1235	535	1819	3.5	2.8	22.8	60.5	9.6	149.7
WA7343Z007	108	<10	1415	565	2089	3.3	2.7	20.0	61.2	11.9	154.6
Commodity-type fatty acid compositions											
9255	110	12	983	353	1457	10.9	5.3	23.7	50.7	8.2	129.9
9281	57	2	1040	339	1438	9.9	4.5	26.1	51.1	7.4	130.6
9182	66	8	1081	427	1582	10.7	4.5	24.9	50.5	8.3	130.8
9202	125	19	926	338	1407	10.5	3.7	25.6	51.9	7.2	131.0
9352	85	16	947	421	1469	10.0	4.1	26.0	51.4	7.4	131.0
XB33E	89	13	944	369	1415	11.5	4.6	22.2	52.2	8.4	131.7
9151	44	2	1062	405	1512	10.1	3.9	25.3	52.5	7.2	131.8
9482	108	5	1135	300	1549	11.0	4.4	22.0	54.1	7.3	132.0
9304	92	7	971	293	1363	10.0	4.8	23.9	51.8	8.2	132.0
9381	118	29	850	439	1436	10.2	4.3	23.8	52.9	7.6	132.2
9481	116	3	1171	254	1543	10.9	3.8	22.6	54.9	6.7	132.3
9231	90	8	981	328	1406	11.3	4.6	20.6	54.0	8.2	132.9
9004	158	14	1559	464	2195	10.2	3.5	20.9	54.8	9.5	138.0
9611	83	19	1015	477	1593	11.0	3.1	18.0	57.6	9.1	139.3
Check											
9171	46	<1	1022	408	1476	9.2	3.9	24.1	53.1	8.7	135.7

it is possible that the significant correlation between the two traits observed in these samples as well as those from the survey (Table 5) (and commodity-type wheat and sunflower for that matter) is the result of the coincident influence of growing conditions on the levels of both constituents. When growing conditions were kept constant (i.e., at a single location), tocopherol content and fatty acid composition were no longer correlated (data not shown), reflecting the lack of a parameter driving the relationship.

It would be valuable to know what specific environmental parameters affected tocopherol levels in these oils. In an attempt to determine this, mean temperature, number of growing degree days (accumulation of heat factors from planting date through the date of maturity for each variety), and cumulative hours of light from flowering to maturity were measured at three of the planting locations. Perhaps due to the limited data available, no significant correlation was found between these specific environmental parameters and seed oil tocopherol concentrations. Although we demonstrated that planting location significantly affected tocopherol concentration, the environmental or geographical factors responsible were not determined.

In the absence of any apparent selection, it is probable that tocopherol content and fatty acid composition of unmodified soybean storage oils are not causally related, but rather influenced similarly by environmental factors the plants experienced during development in the field. Elevated levels of tocopherols in soybean oils with higher percentages of less oxidatively stable linolenate may be due to the effects of lower temperatures during seed filling, promoting both tocopherol synthesis and fatty acid desaturation independently. While tocopherols may convey oxidative stability to soybean oils (20), especially those enriched in linolenic acid, our findings suggest a noncausal relationship between tocopherols and linolenate. With the lack of a direct biochemical link between the synthesis of tocopherols (11) and storage lipids, altered fatty acid composition might have only a spurious effect on tocopherol levels.

Effects of temperature. To test the hypothesis that temperature effects both fatty acid desaturation and tocopherol accumulation, we controlled the temperature during development of soybean seeds by maintaining plants in growth chambers. Analysis of these samples show that both tocopherol content (Table 8) and fatty acid composition represented as calculated

TABLE 6
Mean Total Tocopherol Amount (μg g^{-1}) in Soybean Oils Produced by Plants Grown at Five Locations[a]

Line	Jasper, MI	Johnston, IA	LaSalle, IL	Napoleon, OH	Pocahontas, IA
A2396	1522	1432	1667	1515	1594
A2506	1468	1509	1492	1475	1386
A2835	1514	1496	1585	1486	1596
AP2990	1543	1519	1547	1524	1549
JACK	1550	1613	1598	1536	1567
9255	1402	1331	1404	1316	1420
9281	1514	1535	1533	1467	1567
S1990	1410	1370	1456	1396	1388
S2918	1531	1583	1515	1542	1547
ST2621	1395	1430	1421	1352	1381
ST2660	1373	1348	1390	1370	1374
YB30M	1319	1296	1287	1305	1343

ANOVA

Source of variation	SS	df	MS	F
Location	36623	4	9156	13.02***
Line	873022	11	79366	112.84***
Location × line	130978	44	2977	4.23***
Error	42201	60	703	
Total	1082823	119		

[a]ANOVA, analysis of variance; SS, sum of squares; df, degrees of freedom; MS, mean square; F, F test statistic; ***$P < 0.01$.

IV (Table 9) are highly correlated to temperature. Genetic background in this experiment was less important in determining expression of these traits. A completely different response of soybean oil tocopherol to temperature was reported recently (19). In that study, total tocopherol content in oils increased rather than decreased with increasing growth temperatures. Furthermore, the response was greater in lines that carried homozygous recessive alleles for the Δ12 desaturase gene. The biochemical basis for either of these observations is not obvious, and no suggestions were offered by the authors. As stated previously, a decline in the amount of tocopherols, especially γ-tocopherol in low linolenic acid oils, could be due to the narrow gene pool that constitutes the low linolenate phenotypes used in both studies.

It has been suggested that tocopherol synthesis in seeds is related to plastid development, not storage oil accumulation

TABLE 7
Calculated Iodine Values of Soybean Oils Produced by Plants Grown at Five Locations[a]

Line	Jasper, MI	Johnston, IA	LaSalle, IL	Napoleon, OH	Pocahontas, IA
A2396	131.4	131.0	128.4	132.5	134.4
A2506	131.2	128.1	128.3	131.0	133.5
A2835	137.4	135.4	133.2	139.0	139.6
AP2990	131.7	129.7	128.5	132.9	133.3
JACK	134.8	134.0	134.4	135.0	136.4
9255	129.6	125.0	125.0	128.7	132.5
9281	129.1	131.2	129.1	131.3	135.1
S1990	130.6	128.2	128.2	131.6	133.3
S2918	136.5	135.7	133.4	138.3	137.8
ST2621	126.5	122.9	124.0	125.7	130.5
ST2660	128.4	128.5	128.1	130.9	134.0
YB30M	132.0	131.2	129.9	132.7	134.6

ANOVA

Source of variation	SS	df	MS	F
Location	422.7	4	105.7	638.9***
Line	1123	11	102.1	617.4***
Location × line	94.32	44	2.144	12.962***
Error	9.923	60	0.1654	
Total	1650	119		

[a]See Table 6 for abbreviations. ***$P < 0.01$.

TABLE 8
Mean Total Tocopherol Amount (μg g^{-1}) in Soybean Oils Produced by Plants Grown Under Three Temperature Regimes During Seed Development[a]

Line	35/25°C	27/17°C	20/10°C
A2396	318	946	1298
A2835	183	820	964
AP2990	227	1000	773
9255	153	728	1147
9281	136	649	1121
S1990	811	896	1733
S2918	324	1266	1334
ST2621	427	859	985
ST2660	86	1098	1463
YB30M	343	1169	1583

ANOVA

Source of variation	SS	df	MS	F
Temperature	9213344	2	4606672	62.36207***
Line	1737128	9	193014.2	2.612898**
Line × temperature	1340602	18	74477.88	1.008232*
Error	2216093	30	73869.77	
Total	14507166	59		

[a]See Table 6 for abbreviations. ***$P < 0.01$. **$P < 0.05$. *Not significant.

TABLE 9
Calculated Iodine Values of Soybean Oils Produced by Plants Grown Under Three Temperature Regimes During Seed Development[a]

Line	35/25°C	27/17°C	20/10°C
A2396	118.9	135.2	140.1
A2835	123.6	135.1	145.1
AP2990	120.5	130.8	142.8
9255	119.2	130.1	138.9
9281	124.2	128.3	137.8
S1990	120.5	130.0	142.7
S2918	124.0	133.7	143.9
ST2621	118.4	125.9	135.4
ST2660	120.3	132.1	138.5
YB30M	121.8	130.6	139.3

ANOVA

Source of variation	SS	df	MS	F
Temperature	3729.986	2	1864.993	593.9417***
Line	272.0734	9	30.23038	9.627427**
Line × temperature	139.3035	18	7.739082	2.464655*
Error	94.20081	30	3.140027	
Total	4235.563	59		

[a]See Table 6 for abbreviations. ***$P < 0.01$. **$P < 0.05$. *Not significant.

(21). If this is the case, tocopherol accumulation would be inversely related to seed oil content (22). Lower temperatures during soybean seed maturation lead to lower oil content and higher linolenate levels (23–25). Under these conditions, higher tocopherol levels would be expected, resulting in the positive correlation between linolenic acid and tocopherol levels, such as was observed with the soybean oils. However, others have found a negative correlation between these two parameters (3,7). Given the minor contribution of plastid lipids to overall storage oil synthesis in developing oilseeds (12), it is doubtful that modest reductions in oil content would result in consistently significant elevations in tocopherol levels. Furthermore, total oil and total tocopherol contents were not significantly correlated in the 60 soybean oils used in the planting location experiment (data not shown).

Tocopherol content and oil composition varied in the wheat, sunflower, canola, and soybean oils studied. This variability was due to both the genetic makeup of the plants and environmental factors such as temperature during seed set, at least in soybeans. Given the former, it is possible for plant breeders to develop varieties that produce seed oils with elevated or depressed tocopherol levels. Our results indicate that attention would need to be paid to the influence of environmental conditions, especially temperature in the field, on expression of this trait. Although we found little variability in tocopherol composition in our oils, selection breeding for the introgression of recessive mutant genes controlling this trait has resulted in sunflower oils with significant amounts of β-, γ-, or δ-tocopherol (16). Our results also indicate that selection for altered tocopherol quantity could be accomplished independently from selection for modified fatty acid composition. For example, altering the saturated fatty acid content in sunflower, canola, or soybean did not affect total tocopherol concentrations. However, a putative linkage between the genes in soybean controlling accumulation of both linolenic acid and tocopherols may need to be broken in order to produce an oil with the benefits of both low linolenic acid and high tocopherol content.

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[Received July 2, 1998; accepted December 10, 1998]