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Tocopherols in Soybean Seeds: Genetic Variation and Environmental Effects in Field-Grown Crops

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Abstract Controlled environment studies show α-tocopherol (αT) in soybean seeds increases several fold as a result of warmer temperature or drought during seed maturation, but total tocopherols (T_{tot}) stay approximately constant. To determine if natural variation in weather or climate affect T under field conditions, we analyzed soybean seeds grown at several locations in Maryland between 1999 and 2002. Weather was relatively normal during 1999–2001, whereas warmer temperatures and extreme drought were characteristic of 2002. Comparing 18 lines, there were small but significant differences in T_{tot} as well as 2- to 3-fold differences in αT during 1999–2001. Seeds from locations on the Eastern Shore of Maryland (full season crops) had higher absolute and relative levels of αT compared to seeds from a (cooler) central Maryland location or seeds from a later planting (double crop) on the Eastern Shore. Effects of location or planting date were small compared to that of genetic line when considering the normal years 1999–2001. In 2002, however, several fold increases in $\alpha T/T_{tot}$ were observed in Maturity Group III and IV seeds, especially from full season crops grown at two locations on the Eastern Shore of Maryland. We conclude weather and climate are significant factors affecting soybean seed T content.

Keywords Tocopherol · Vitamin E · *Glycine max* · Soybean · Maryland · Weather · Climate · Global change

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Introduction

α-, β-, γ-, and δ-Tocopherols (T) constitute a family of lipophilic antioxidants that protect biological membranes against peroxyl radicals, but only the 2*R* chiral form of αT is retained by humans by virtue of an αT-binding protein in the liver [1]. A recent decision to recommend increased daily intake of vitamin E and to base this intake on only 2*R*-αT [2] has resulted in calculations that over 90% of Americans are deficient in vitamin E from food sources [3]. This has led to calls to increase αT in the diet. Soybean seeds, which contain high concentrations of total T (T_{tot}, including γT, δT, αT, and βT in order of decreasing amounts), are the major source of vegetable oil in the US diet and consequently the major contributor to dietary T intake. The problem is that γT, which typically constitutes 50–60% of soybean T_{tot} and which previously was weighted and added to vitamin E totals, can no longer be included, while αT usually represents only 5–10% of soybean seed T_{tot}.

Although it is possible to increase the proportion of αT in soybean seeds through genetic engineering [4], the health consequences of concomitantly reducing the intakes of other T is unknown. Recent studies, for example, have suggested that γT may have important health benefits apart from and/or in addition to αT [5, 6]. It has also been demonstrated that consumption of certain phytochemicals such as sesamin can interfere with normal T metabolism and alter T retention in humans [7]. Consequently, it is important to understand factors influencing both the total and relative amounts of T in seeds.

Genetic variability has been reported both in total amounts and proportions of α-, β-, γ-, and/or δ-tocopherols (including tocopherols and the related tocotrienols) in soybean [8] and other species [9–14]. Although "environmental effects" are sometimes identified in these studies, the conclusions are generally based on significant effects for location and/or year without a clear explanation of specific environmental factors such as temperature or drought.

Use of controlled environments, however, has identified specific effects of temperature and/or drought on soybean phytochemicals such as tocopherols [15, 16], phytosterols [17], and isoflavones [18, 19]. For example, relatively small increases in temperature (23–28 °C) or severe drought (soil moisture reduced to 10–25% of holding capacity) during seed development caused large increases $\alpha T/T_{tot}$ [16]. Preliminary studies indicate the critical developmental period for a response to temperature occurs between the 2nd and 4th week after the start of seed fill. For most soybean crops, this corresponds to a time when it would still be normal to experience warm temperatures in the field. Therefore, we asked whether it was possible to detect differences in T in soybean seeds from the same line grown at several locations across the state of Maryland differing in average temperature. Since soybeans mature at different rates, we examined a number of lines representing different maturity groups.

Experimental Procedures

Seeds from 18 soybean lines representing 4 maturity groups (MG-III, MG-IV, MG-IVS and MG-V) were obtained from 1999, 2000, and 2001 through the University of Maryland uniform field testing program from 3 locations [Clarksville, Central Maryland Research and Education Center (39.25°N, 76.93°W, 113 m above sea level); Queenstown, Wye Research and Education Center (38.92°N, 76.13°W, 6 m above sea level); and Quantico, Lower Eastern Shore Research and Education Center (38.37°N, 75.78°W, 6 m above sea level)]. In 2002, a year characterized by warm temperatures and severe drought at all three locations, only 15 of the studied lines were available.

Meteorological data were not collected at Quantico, so data were substituted from Snow Hill, MD (37.9 km southeast), the closest station in the MD-01 (Southeastern Shore) climate division. Table 1 provides a compendium of average mid-point temperatures and precipitation at all three sites during 1999–2002 growing seasons. Average precipitation was similar at all sites.

Based on 30-year averages, temperatures at Clarksville in central Maryland are ~1 °C cooler throughout the growing season than those at Queenstown on the Eastern Shore (near Chesapeake Bay). Temperatures at Queenstown are similar to those at Snow Hill (near the Atlantic Ocean) during the early part of the growing season but are about 1 °C cooler in September and October. These differences are small but important since only one soybean line with later maturation (i.e., Holladay, MG-V) was grown with economic yields at Clarksville during this period, while milder conditions at Quantico permitted a second planting (double crop) about 1 month after the first.

Table 1 Maryland environmental data

Location	Month	Average midpoint[a] temperature (°C)				
		1999	2000	2001	2002	Normal[b]
Snow Hill	June	22.3	26.2	23.2	23.6	22.7
	July	26.4	23.2	22.9	25.7	25.0
	August	24.9	23.2	24.7	25.1	24.1
	September	21.4	19.9	19.7	21.6	21.4
	October	14.3	15.0	14.8	16.1	14.7
Queenstown	June	24.0	24.0	23.1	23.1	22.4
	July	26.8	22.8	22.2	25.5	24.7
	August	24.7	24.5	24.8	25.7	23.8
	September	21.1	20.3	18.7	20.9	20.2
	October	13.4	15.3	14.2	13.7	13.9
Clarksville	June	21.4	21.9	22.3	22.2	21.5
	July	25.8	21.8	22.1	23.9	23.9
	August	23.7	21.8	23.8	24.4	22.9
	September	19.3	18.3	17.7	20.5	19.3
	October	11.7	13.5	ND[c]	12.3	12.6

	Annual precipitation[d] (mm)				
	1999	2000	2001	2002	Normal
Snow Hill	1,020	1,357	984	734[e]	1,173
Wye	1,122	1,343	1,235	637[e]	1,100
Clarksville	948	1,082	1,011	648[f]	1,149

[a] Average of daily minima and maxima; data from Maryland State Climatoglogist

[b] 1971–2000

[c] Not determined

[d] Totals for 12-month period ending August of indicated year

[e] Rainfall deficit for 11 of 12 months

[f] Rainfall deficit for 10 of 12 months

Full season soybeans were planted using conventional tillage following a corn or sweet sorghum rotation. Planting dates varied between the last week of May and the end of June and were generally 6–30 days earlier at Quantico than at Clarksville. Soybean lines and characteristics of maturity groups are discussed in annual newsletters at the Maryland Cropping Systems website (http://psla.umd.edu/extension/crops/soybeans). Average days to maturity at Queenstown and Quantico full season for MG's III (3 lines), IV (5 lines), IVS (4 lines) and V (5 lines) were, in the order, 115, 120, 130 and 136 in 2000 and 111, 115, 123, and 126 in 2001. The shorter intervals in 2001 may have resulted in part from later planting dates. Premature frosts for Clarksville and Quantico Double Crop during the years of this study make it difficult to compare relative days to maturity for later maturity groups at these locations. Double crop soybeans were planted at

Quantico using no-till into winter barley 15–29 days after the full season beans. Fertilization, liming and herbicide treatments varied between locations and years according to best management practices.

T were extracted, separated, and analyzed as described previously from freeze-dried, finely ground soybean seeds using reverse-phase HPCL with a C18 column [16]. Although this method does not discriminate between the structural isomers γT and βT, the two species have similar calibration curves using standards and T_{tot} should therefore be quantified accurately. Significance of differences was determined using three-way or two-way ANOVA and Tukey Pairwise Multiple Comparison tests (SigmaStat ver. 2.03, SPSS Science, Chicago, IL). Data were checked for adherence to normality and equal variance.

Results and Discussion

Seeds from 18 soybean lines were analyzed for the years 1999, 2000, and 2001. There were highly significant differences ($p < 0.001$) related to genetic line in $\alpha T/T_{tot}$, $\delta T/T_{tot}$, and T_{tot} (Table 2). Moreover, there were no interactions between genetic line and location or genetic line and year for $\alpha T/T_{tot}$, $\delta T/T_{tot}$ and T_{tot}, indicating relationships between lines were maintained for all locations/planting dates and for all 3 years. Note that data from Clarksville were excluded from this comparison because several of the MG-V lines were not grown there. However, performing analyses with the 14 lines all grown at Quantico, Queenstown and Clarksville yields almost identical results (data not shown).

The highest value for $\alpha T/T_{tot}$ (0.123, Hutcheson) was more than double the lowest (0.056, Wicomico). Both lines were members of MG-V. Although 5 of the 6 lines with the lowest $\alpha T/T_{tot}$ belonged to MG-IVS or MG-V, Maturity Group was not well correlated with $\alpha T/T_{tot}$, suggesting that temperature during seed maturation was less important than intrinsic genetic factors in the control of T metabolism.

On the other hand, there were significant and consistent effects of location and/or planting date ($p < 0.001$) on the mol ratios of T/T_{tot} [Table 3(A) and (B)]. Table 3(A) is constructed using data for 14 lines, MG-III, MG-IV, MG-IVS, and MG-V, grown at Quantico (both full season and double crop), Queenstown and Clarksville for the years 1999–2001 (Holladay is only MG-V line grown at Clarksville). Table 3(B) is constructed using only the 5 MG-V lines from Quantico (both full season and double crop) and

Table 2 Relative α-tocopherol, relative δ-tocopherol and total tocopherol in mature seeds of 18 soybean lines from 3 locations/plantings, 1999–2001

Line	Maturity group	Rank	$\alpha T/T_{tot}$ mol ratio	Rank	$\delta T/T_{tot}$ mol ratio	Rank	T_{tot} nmol (g seed dry matter)$^{-1}$
Hutcheson	V	1	0.123^{a}	4	0.318^{bcd}	7	612^{bcd}
MD95-5358	IV	2	0.117^{ab}	15	0.259^{hi}	14	585^{bcd}
LS93-0375	IV	3	0.104^{bc}	11	0.286^{efg}	10	608^{bcd}
IA-3010	III	4	0.103^{cd}	16	0.240^{ij}	9	609^{bcd}
Corsica	IV	5	0.097^{cde}	18	0.228^{j}	11	597^{bcd}
Stressland	IV	6	0.093^{cdef}	10	0.293^{defg}	2	644^{ab}
Clifford	V	7	0.092^{cdefg}	8	0.305^{bcdef}	16	582^{bcd}
KS-4694	IVS	8	0.091^{defg}	14	0.270^{gh}	6	615^{abc}
Macon	III	9	0.086^{efgh}	17	0.235^{ij}	1	695^{a}
General	III	10	0.081^{fghi}	13	0.271^{gh}	3	643^{ab}
MD94-5332	IVS	11	0.081^{fghi}	5	0.317^{bcd}	12	589^{bcd}
Holladay	V	12	0.080^{ghi}	6	0.309^{bcde}	5	623^{abc}
MD92-5769	IVS	13	0.073^{hij}	2	0.329^{ab}	17	560^{cd}
HS93-4118	IV	14	0.070^{ij}	9	0.301^{cdef}	15	583^{bcd}
MD96-5696	IVS	15	0.069^{ijk}	12	0.279^{fgh}	18	532^{d}
Essex	V	16	0.064^{jk}	7	0.307^{bcde}	13	585^{bcd}
Manokin	IVS	17	0.063^{jk}	3	0.324^{bc}	8	610^{bcd}
Wicomico	V	18	0.056^{k}	1	0.352^{a}	4	626^{abc}

Quantico, full season and double crop, and Queenstown, full Season

T_{tot}, total tocopherol; *$\alpha T/T_{tot}$*, ratio of α-tocopherol to total, mol basis; *$\delta T/T_{tot}$*, ratio of δ-tocopherol to total, mol basis

Significance determined by three-way ANOVA (line × year × location), $n = 9$ for each line, and Tukey Pairwise Multiple Comparison; values followed by the same letter are not significantly different ($p < 0.05$)

Table 3 Relative α-tocopherol, relative δ-tocopherol and total tocopherol in mature soybean seeds, 1999–2001

Location/ planting	$\alpha T/T_{tot}$ mol ratio	$\delta T/T_{tot}$ mol ratio	T_{tot} nmol (g seed dry matter)$^{-1}$
(A) 14 Lines, MG-III, MG-IV, MG-IVS, and MG-V[1]			
Quantico			
Full Season	0.095^{a}	0.260^{b}	608^{a}
Double Crop	0.072^{b}	0.322^{a}	605^{a}
Queenstown	0.092^{a}	0.263^{b}	607^{a}
Clarksville	0.069^{b}	0.328^{a}	599^{a}
(B) 5 Lines, MG-V			
Quantico			
Full Season	0.088^{a}	0.299^{b}	605^{a}
Double Crop	0.070^{b}	0.368^{a}	610^{a}
Queenstown	0.091^{a}	0.288^{b}	603^{a}

Soybean lines and maturity groups are listed in Table 2; other details as in Table 2

[1] Includes only cv. Holladay

Queenstown, 1999–2001, and also includes Holladay. Considering both data sets, the proportion of T/T_{tot} in full season beans from Queenstown or Quantico ranged between 0.088 and 0.095 with no significant difference between these locations. The proportion of $\alpha T/T_{tot}$ in double-cropped beans from Quantico varied between 0.070 and 0.072 and was significantly different from full season beans at either Queenstown or Quantico. Likewise, the value at Clarksville (0.069) was not significantly different from the value for the double crop at Quantico, but was significantly different from full season beans from Queenstown or Quantico. The data indicate there is a significant effect of location and/or planting date on T metabolism that is consistent with an elevation of $\alpha T/T_{tot}$ under warmer conditions during seed maturation (i.e., full season beans from Queenstown or Quantico). This effect is small, however, in comparison to genetic variation.

A completely different picture emerges in 2002, a high stress year with warm temperatures and extreme drought compared to the period 1999–2001. Although 3 of the 18 lines studied in 1999–2001 were discontinued in 2002, the remaining lines clearly show interactions between planting location/date and maturity group (Fig. 1). First, $\alpha T/T_{tot}$ was higher in 2002 for all lines at all locations and planting dates with differences between 2002 and the preceding 3 years significant at least to $p < 0.01$. Second, the increases were greatest for MG-III and MG-IV lines from full season plantings at Quantico and Queenstown and constituted as much as 3.5-fold above 1999–2001 averages (e.g., cv. General). Under more extreme conditions, it appears that $\alpha T/T_{tot}$ does increase in early maturing lines, for which seed development will coincide with warmer temperatures.

The impact of environmental stress in 2002 compared to the 3 previous years is clearly expressed as reduced seed yield (Table 4). These reductions are largest for the earlier maturity groups at Queenstown and Quantico full season. In fact, crop performance was so poor in 2002 for Quantico full season beans, that yields were not determined for MG-III and MG-IV lines. In contrast, the yield was scarcely affected at Clarksville. The apparent increase in yield in 2002 at this location for MD92-5769 and Manokin may be the result of premature frosts in 1999–2001 that could have reduced yields. Likewise, the relatively small reductions in yield for Quantico double crop beans could be affected by changes in planting density in 2002 or by premature frost in some of the earlier years. We conclude that lines expressing the largest change in tocopherol composition are also the lines with the largest impact of environment on seed yield.

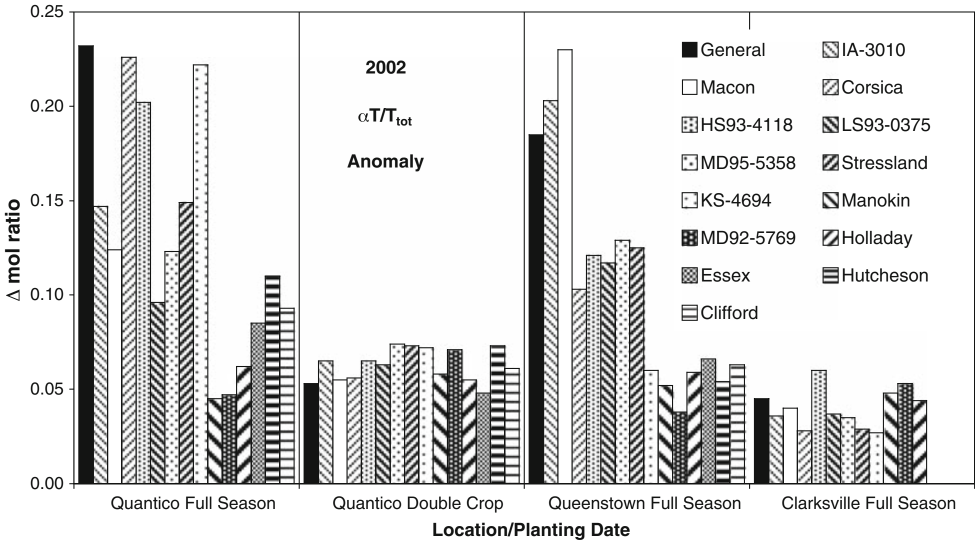

Fig. 1 Anomalous increases in the mol ratio of $\alpha T/T_{tot}$ in 2002 with respect to mean values for 1999–2001 by genetic line and location/planting date

Table 4 Anomalous changes in seed yield in 2002 with respect to mean values for 1999–2001 by genetic line and location/planting dates

Line[a]	Maturity group	Clarksville	Queenstown	Quantico full season	Quantico double crop
Relative seed yield[b]					
Macon	III	0.783	0.377	–[c]	0.750
IA-3010	III	0.832	0.412	–[c]	0.650
General	III	0.901	0.421	–[c]	0.751
Stressland	IV	0.956	0.601	–[c]	0.741
HS93-4118	IV	0.917	0.648	–[c]	0.760
LS93-0375	IV	0.922	0.672	–[c]	0.779
Corsica	IV	0.987	0.665	–[c]	0.758
MD95-5358	IV	0.984	0.583	–[c]	0.821
KS-4694	IVS	1.100	0.777	0.248	0.826[d]
MD92-5769	IVS	1.435[e]	0.904	0.645	0.989[f]
Manokin	IVS	1.345[e]	0.870	0.602	0.985[f]
Hutcheson	V	–[g]	0.717	0.669	0.969[f]
Clifford	V	–[g]	0.673	0.631	0.885[f]
Holladay	V	–[g]	0.828	0.734	0.949[f]
Essex	V	–[g]	0.662	0.677	0.934[f]

[a] Not all lines analyzed for tocopherols were harvested for yield

[b] Seed yield (kg ha^{-1} corrected for 13% moisture) in 2002 divided by the average of 1999, 2000, and 2001 yields; yield determined from the middle rows of 3 replicate plots consisting of 4 × 6.1 m rows spaced 0.61 m apart with 21.3 seeds m^{-1} for Clarksville, Queenstown and Quantico full season; for Quantico double crop, rows were spaced at 0.51 m with 26.2 seeds m^{-1} in 1999–2001 and spaced at 0.41 m with 19.7 seeds m^{-1} in 2002

[c] No yields reported in 2002

[d] Premature frost in 2000

[e] Premature frost in 1999, 2000 and 2001

[f] Premature frost in 2000 and 2001

[g] Not grown for yield determination

In studies examining temperature effects on soybean T in controlled environments, little change was noted for γT + βT while increases in αT at elevated temperature were largely matched by compensatory decreases in δT, at least within a given line [16]. Significant differences in $\delta T/T_{tot}$ were also observed in the current study, but comparison of the relative rankings for $\alpha T/T_{tot}$ and $\delta T/T_{tot}$ shows these parameters are not closely linked (Table 2). For example, Hutcheson, the line with the highest ratio of $\alpha T/T_{tot}$ has the 4th highest ratio of $\delta T/T_{tot}$. Moreover, the range of differences in $\delta T/T_{tot}$ is about twice as large as that for $\alpha T/T_{tot}$ comparing either genetic lines (Table 2) or planting location/dates [Table 3(A) and (B)]. In 2002, however, under the influence of more drastic environmental stress, the large increases in $\alpha T/T_{tot}$ are in fact compensated by decreases in $\delta T/T_{tot}$ (data not shown).

Under normal conditions (i.e., 1999–2001), it is apparent that substantial differences in γT or βT or both can contribute to differences between genetic lines and planting location/date (data not shown). Although the relative contribution of γT and βT to T_{tot} was not analyzed because the method used for T analysis did not separate these isomers, recent RP-HPLC analyses using a C30 column [20] resolve βT and γT from soybean seeds and show that βT constitutes a more or less constant proportion of T_{tot} that is uninfluenced by environmental perturbations affecting αT, δT and, in some cases, γT (Britz, unpublished).

There were significant differences between the lines in T_{tot}, but the range was relatively small. Average T_{tot} values for 16 of the 18 lines were within 1.28 standard deviations of the overall mean (605 nmol g^{-1} seed dry matter). Macon (695 nmol g^{-1} seed dry matter) and MD96-5696 (532 nmol g^{-1} seed dry matter in) were consistently the lines with the highest and lowest T_{tot} values, respectively. In addition, there was no relationship between T_{tot} and the proportion of $\alpha T/T_{tot}$. Thus, Macon and MD92-5769 were not significantly different with respect to $\alpha T/T_{tot}$. Conversely, the lines with the greatest difference in the proportion of $\alpha T/T_{tot}$ (Hutcheson and Wicomico, respectively) were not significantly different with respect to T_{tot}. In 2002, T_{tot} increased 10–20% for most lines grown at Quantico (both full season and double crop) and Queenstown but not at Clarksville (data not shown). The increases were not significant, however, when analyzed by genetic line for each location and planting date in comparison to averages for 1999–2001. The results are consistent with earlier observations that environmental stress has a relatively small effect on T_{tot}.

It should be possible to breed for lines with both elevated $\alpha T/T_{tot}$ and T_{tot}, since the two parameters are not closely linked. For example, Stressland is a variety with both high T_{tot} and high $\alpha T/T_{tot}$ resulting in a high absolute level of αT. It is likely that such lines will express this phenotype under a range of conditions, although warmer environments and earlier planting conditions may foster the expression of elevated $\alpha T/T_{tot}$. Under "normal" environments the link with environment is weak such that early maturing lines (e.g., MG-III) do not routinely express elevated $\alpha T/T_{tot}$, but under extreme stress (e.g., 2002) the link between environment and maturity group is expressed more clearly in the case of full season crops from Quantico and Queenstown, the two warmer locations (e.g., Fig. 1).

The effects reported here from field studies show how the nutritional properties of crops can be dramatically affected by weather and potentially by global environmental change, which is likely to entail increased average temperature, increased frequency of heat spells and more variable precipitation.

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