JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Warm Temperatures or Drought during Seed Maturation Increase Free α-Tocopherol in Seeds of Soybean (*Glycine max* [L.] Merr.)

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Soybean seeds are an important source of dietary tocopherols, but like seeds of other dicotyledonous plants, they contain relatively little α-tocopherol, the form with the greatest vitamin E activity. To evaluate potential effects of environmental stress during seed maturation on tocopherols, soybeans were raised in greenhouses at nominal average temperatures of 23 °C or 28 °C during seed fill, with or without simultaneous drought (soil moisture at 10−25% of capacity), during normal growing seasons in 1999 (cvs. Essex and Forrest) and 2000 (cvs. Essex, Forrest, and Williams). Total free (nonesterified) tocopherols increased slightly in response to drought in Essex and Forrest. All three lines responded to elevated temperature and, to a lesser extent, drought with large (2−3-fold) increases in α-tocopherol and corresponding decreases in δ-tocopherol and γ-tocopherol. The results suggest that weather or climate can significantly affect seed tocopherols. It may be possible to breed for elevated α-tocopherols by selecting for altered plant response to temperature.

KEYWORDS: ***Glycine max*; soybean; seeds; stress; heat; temperature; drought; soil moisture; tocopherols; vitamin E; antioxidants**

INTRODUCTION

Soybean seeds contain many compounds (e.g., isoflavones, sterols, saponins, and tocopherols [vitamin E]) considered to have health-promoting effects (*1*). There is considerable interest in possible environmental effects on the content of these components and, hence, the nutritional value of soybean seeds. Although it is well-established that the amounts of protein and oil as well as fatty acid distribution in soybean seeds are affected by temperature (e.g., *2*), other seed components have scarcely been studied. Tsukamoto et al. (*3*) reported that elevated temperature dramatically decreased isoflavones in soybean seeds, but had no effect on saponins. Vlahakis and Hazebroek (*4*) reported that high temperature increased sterols in soybean seeds, with relative increases in campesterol and decreases in stigmasterol and β-sitosterol. The effect of temperature on tocopherols of soybean seeds is currently in dispute. Almonor et al. (*5*) found that elevated temperatures caused small (∼10%) increases in total tocopherols (both free and esterified), partly as a result of increased γ-tocopherol, the tocopherol species found most abundantly in soybean seed. The other main soybean tocopherols, α-tocopherol and δ-tocopherol, did not change consistently. On the other hand, Dolde et al. (*6*) observed large decreases in total free tocopherols with increased temperature with no reported change in tocopherol distribution. Since both studies reported similar values for total tocopherols, it is unlikely that esterified forms contributed significantly to the differences.

In the studies on tocopherols, elevated temperature was combined with short days and (at least in one case) reduced light levels (*5*, *6*). Therefore, experiments were conducted in 1999 and 2000 to investigate temperature effects on tocopherols in soybean seeds under more natural lighting in greenhouses. This report presents primarily data from 2000, since essentially identical results were obtained for the two years and since an additional cultivar was used in 2000.

MATERIALS AND METHODS

Plant Growth. Soybeans (*Glycine max* [L.] Merr.) were grown in large (12-L) clay pots with composted soil during 1999 and 2000 in microprocessor-controlled greenhouses at the Beltsville Agricultural Research Center, Beltsville, MD. Cultivars (cvs.) Essex and Forrest were used during both the 1999 and 2000 growing seasons; cultivar (cv.) Williams was used additionally during 2000. Planting dates in 1999 and 2000 were June 17 and June 20, respectively. All plants were grown initially in a single greenhouse section set for a nominal average daily temperature of 23 °C (**Table 1**). This temperature was chosen because it is close to average outdoor temperatures until mid-September. Actual temperatures varied diurnally between nocturnal lows near 18−19 °C and diurnal highs of 28−29 °C, depending on external weather. Aspirated air temperature was measured at canopy level in a central location in the greenhouse using a calibrated thermistor. Plants were spaced about 1 m apart.

Peak natural irradiances in the greenhouse were 1200−1400 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF; 400−700 nm), as measured with a horizontal, cosine-corrected, calibrated quantum sensor (LiCor Inc, Lincoln NE). To compensate partially for shading from the greenhouse structure, supplemental high-pressure sodium lamps provided an additional 150 μmol m^{-2} s^{-1} PPF at canopy level for 12 h daily centered around solar noon. Daily integral solar radiation incident

10.1021/jf0200016
Published on Web 09/12/2002

Table 1. Average Daily Integral Solar Radiation (280–2800 nm) and Average Daily Greenhouse Air Temperatures during Soybean Seed Development, 1999 and 2000[a]

	solar radiation, MJ m^{-2}		temp, 23 °C section, °C		temp, 28 °C section, °C	
dates	1999	2000	1999	2000	1999	2000
Aug. 17–Sept. 5	14.6 ± 5.3	14.2 ± 4.7	23.9 ± 2.9	24.7 ± 3.3	29.0 ± 4.6	29.0 ± 3.9
Sept. 6–Sept. 26	14.3 ± 6.3	14.0 ± 5.7	22.9 ± 3.3	24.0 ± 3.6	28.3 ± 4.6	28.2 ± 3.9
Sept. 27–Oct. 17	10.6 ± 5.7	12.4 ± 4.4	22.5 ± 3.6[b]	23.0 ± 3.8	27.8 ± 3.8[b]	27.6 ± 3.7
Oct. 18–Nov. 11	10.2 ± 3.2	10.5 ± 2.9		22.3 ± 3.7		26.8 ± 3.4
season av			23.1 ± 3.3	23.5 ± 3.7	28.4 ± 4.4	27.9 ± 3.8

[a] Values ± 1 SD averaged over 3-week intervals. [b] Temperature data through Oct 16, 1999.

on the greenhouse (**Table 1**) was measured in the field with a pyranometer (LiCor Inc.) Plants were fertilized periodically with ~2.5 g of N−P−K (20−20−20) provided as a dilute liquid formulation and treated as needed with Orthene (Valent U.S.A. Corp., Walnut Creek, CA), Avid (Merck & Co., Inc., Rahway, NJ), Naturalis (Troy Biosciences, Phoenix, AZ), or insecticidal soaps for thrips, mites, or whitefly. Insecticides were used according to label instructions.

In 1999, plants flowered first on July 28 and August 7, respectively. Flowering dates in 2000 were July 7 (Williams), August 7 (Essex), and August 14 (Forrest). Retardation of flowering (and subsequent seed maturation) in 2000 was probably related to low light levels early in the season (**Table 1** and additional data not shown). At the onset of seed-fill [R5 stage (*7*)], one-half the plants were transferred to an adjacent greenhouse section set for a nominal average temperature of 28 °C (**Table 1**), but with otherwise identical conditions. Actual temperatures varied between nocturnal lows of 21−23 °C and diurnal highs of 33−37 °C, depending on weather. Transfer dates were August 16 (Essex and Forrest) in 1999; and August 14 (Williams) or September 10 (Essex and Forrest) in 2000. Soil moisture blocks (Soilmoisture Equipment Corp., Santa Barbara CA) calibrated against soil water capacity were inserted into the middle of each pot and were read daily prior to watering. All treatments were maintained under well-watered (W) conditions (targeted at 80−90% of soil capacity) until the start of the high temperature treatments. At that time, one-half of the plants in each temperature regime were also exposed to drought (D) stress by reducing the daily water allotment for the duration of the experiment (soil moisture maintained at 10−25% of capacity).

Mature seed were harvested October 18 (28 °C) and October 28 (23 °C) for both Essex and Forrest in 1999 and on October 18 (Williams, both 23 °C and 28 °C), October 24−31 (Essex and Forrest, 28 °C), and November 7−13 (Essex and Forrest, 23 °C) in 2000. Seed were freeze-dried, weighed to determine total plant yield and average dry matter per seed, and then ground (60 mesh) and stored at −20 °C in sealed vials until analysis.

Tocopherol Analysis. Tocopherols were extracted from lyophilized ground seed using a procedure modified from Kurilich and Juvik (*8*). Seeds (~100 mg dry matter) were extracted three times in absolute ethanol containing 0.1% butylated hydroxytoluene for 5 min in a water bath at 60 °C. After vortexing, extracts were centrifuged at low speed. Supernatants were combined, diluted with 1.5 mL HPLC-grade water, and washed three times with hexane (3.5 mL total volume). The hexane supernatants were combined and evaporated to dryness under N_2. The residue was redissolved in absolute ethanol containing butylated hydroxytoluene, clarified in a microfuge if necessary, and transferred directly to amber vials stored at 6 °C in a refrigerated autosampler prior to separation by HPLC (model 1100, Agilent Tech., Wilmington DE). Extracts were not saponified to avoid large (up to 80%) losses of tocopherols (*9*). Samples (20 μL) were injected on an Adsorbosphere HS C_{18} reverse-phase, 5 μm, 4.6 × 7.5 mm guard column followed by an Adsorbosphere HS C_{18} reverse-phase, 7 μm, 4.6 × 250 mm column (Alltech Assoc., Deerfield IL) at 25 °C. The mobile phase consisted of acetonitrile/tetrahydrofuran/water (60:25:10; v:v:v) at 1 mL min^{-1}.

Tocopherols (δ, γ, and α) were separated and identified on the basis of cochromatography with authentic standards (Sigma Chem. Co, St. Louis, MO) and by absorbance spectra (maximum, ~290 nm) measured with a photodiode array detector. Absorbance values were converted to tocopherol mass on the basis of separate standard curves for α-, γ-, and δ-tocopherol. Efficiency of recovery (70−80%) was estimated by spiking initial extracts with α-tocopherol. Values were not corrected for losses. β-Tocopherol, a structural isomer of γ-tocopherol, comigrates with γ-tocopherol on reverse-phase columns and was measured as γ-tocopherol, if present. However, soybean seeds contain low levels of β-tocopherol (*10*), and interference, if any, was assumed to be minor.

Table 2. Effects of Air Temperature and Soil Moisture during Seed Development on Soybean Whole Plant Seed Yield and Seed Size at Maturity

cultivar	av air temp °C[a]	soil moisture[b]	seed yield[c] g seed dry matter/plant (percent reduction)	seed size[c] mg dry matter/seed (percent reduction)
Williams	23	W	126.8 ± 5.2 (0)	204.1 ± 3.0 (0)
		D	83.3 ± 2.0 (34)	199.4 ± 2.2 (2)
	28	W	113.8 ± 6.6 (10)	182.1 ± 2.6 (11)
		D	67.9 ± 2.2 (46)	175.4 ± 2.8 (14)
Essex	23	W	172.0 ± 9.1 (0)	167.3 ± 2.4 (0)
		D	113.8 ± 4.9 (34)	134.7 ± 1.8 (19)
	28	W	140.4 ± 10.3 (18)	148.2 ± 2.6 (11)
		D	86.7 ± 4.6 (50)	119.7 ± 1.7 (28)
Forrest	23	W	171.3 ± 17.1 (0)	146.5 ± 2.2 (0)
		D	103.7 ± 2.1 (39)	125.2 ± 1.7 (15)
	28	W	139.3 ± 9.2 (19)	120.9 ± 1.9 (17)
		D	66.4 ± 2.5 (61)	92.9 ± 1.9 (37)

[a] Plants raised at 23 °C nominal average temperature until the R5 stage of seed development when one-half of the plants were shifted to an identical section at 28 °C for the remainder of seed development. Data are from the 2000 growing season. [b] Soil moisture for droughted (D) plants maintained at 10–25% of capacity starting at transfer. Well-watered (W) plants maintained at soil moisture near 80–90% of capacity. [c] Values are means ± 1 SE [$n = 4$ plants ($n = 3$; Forrest 28 °C × D)].

Statistics. Significance of treatment effects ($p \leq 0.05$) by cultivar was determined using two-way ANOVA and the Tukey Multiple Comparison Test (SigmaStat Ver. 2.03; SPSS Science, Chicago, IL). Data were checked for adherence to normality and equal variance. Four replicate plants of each line were used for each treatment. In the case of tocopherol measurements, two determinations were performed on each plant.

RESULTS

Calibration of Environmental Stress. Seed yield per plant and seed size (dry matter) were used as whole plant indicators of environmental stress integrated over development. Depending on the indicator, the cultivars responded differently to elevated temperature, drought, or the combination of the two (**Tables 2** and **4**).

Both drought and elevated temperatures during seed development reduced seed yield. Relative to temperature, drought treatment was a more severe stress, producing 34−52% reductions in yield in all three cultivars, as compared to well-watered conditions. In contrast, there were only 11−36% reductions in yield at 28 °C, as compared to 23 °C average air temperatures.

Table 3. Effects of Air Temperature and Soil Moisture during Seed Development on Soybean Seed Tocopherol Contents at Maturity[a]

			μg tocopherol/g seed dry matter			
			tocopherols			
cultivar	greenhouse av air temp, °C	soil moisture	δ	γ	α	Total
Williams	23	W	64.0 ± 2.2	201.7 ± 3.5	57.7 ± 3.1	323.4 ± 4.0
		D	47.2 ± 3.3	176.9 ± 8.3	88.2 ± 2.3	312.3 ± 12.8
	28	W	34.4 ± 1.5	183.0 ± 0.8	115.5 ± 5.1	333.1 ± 6.1
		D	22.7 ± 0.6	140.6 ± 3.3	172.2 ± 5.8	335.6 ± 8.0
Essex	23	W	46.5 ± 1.5	161.3 ± 4.8	54.5 ± 3.6	262.3 ± 5.1
		D	42.8 ± 2.2	167.6 ± 3.5	73.8 ± 3.0	284.1 ± 4.1
	28	W	23.7 ± 1.1	141.0 ± 4.9	114.6 ± 4.8	279.3 ± 6.4
		D	22.3 ± 1.0	133.2 ± 1.4	131.6 ± 4.9	287.2 ± 6.7
Forrest	23	W	56.9 ± 1.9	171.5 ± 6.8	58.2 ± 4.5	286.6 ± 4.9
		D	43.1 ± 2.7	162.2 ± 6.0	79.3 ± 2.0	284.7 ± 6.7
	28	W	20.9 ± 1.1	119.8 ± 5.4	120.1 ± 7.4	260.9 ± 4.6
		D	26.7 ± 3.9	158.9 ± 14.4	133.1 ± 11.9	318.8 ± 7.1

[a] See **Table 2** for details. Data are from the 2000 growing season.

Table 4. Significance of Treatment Effects (*p* Values)[a]

cultivar	comparison	seed yield	seed size	total tocopherols	α-tocopherol/total	δ-tocopherol/total
Williams	temperature	**0.02**	**0.01**	0.12	**<0.01**	**<0.01**
	soil moisture	**<0.01**	0.49	0.67	**<0.01**	**<0.01**
	temperature × soil moisture	0.82	0.889	0.50	**0.01**	0.31
Essex	temperature	**0.01**	**0.05**	0.15	**<0.01**	**<0.01**
	soil moisture	**<0.01**	**0.01**	**0.04**	**<0.01**	**0.03**
	temperature × soil moisture	0.80	0.70	0.31	0.86	0.18
Forrest	temperature	**0.02**	**<0.01**	0.55	**<0.01**	**<0.01**
	soil moisture	**<0.01**	**<0.01**	**<0.01**	0.54	**0.03**
	temperature × soil moisture	0.83	0.50	**<0.01**	0.07	**0.01**

[a] Data from **Tables 2** and **3** analyzed by two-way ANOVA. Significant treatment effects ($p \leq 0.05$) highlighted in bold.

Table 5. Total and Relative Tocopherols, 1999[a]

			total tocopherols		
cultivar	av air temp °C	soil moisture	μg tocopherol/g seed dry matter	α-tocopherol/total	δ-tocopherol/total
Essex	23	W	243.7 ± 3.6	0.130 ± 0.006	0.235 ± 0.006
		D	262.7 ± 6.0	0.152 ± 0.008	0.197 ± 0.007
	28	W	256.8 ± 4.2	0.373 ± 0.007	0.102 ± 0.004
		D	270.4 ± 3.9	0.468 ± 0.017	0.066 ± 0.003
Forrest	23	W	280.6 ± 5.0	0.128 ± 0.015	0.261 ± 0.019
		D	292.1 ± 16.6	0.166 ± 0.011	0.219 ± 0.006
	28	W	317.9 ± 13.0	0.280 ± 0.009	0.152 ± 0.010
		D	318.2 ± 2.93	0.313 ± 0.010	0.122 ± 0.006

[a] See **Table 2** for details. Data from 1999 growing season.

Treatment effects on yield were significant and generally similar for all three cultivars.

On the basis of seed size, however, the relative severity of drought was diminished and strongly dependent on cultivar. For example, drought had no effect on seed size in cv. Williams. Changes in seed size were therefore not involved in drought effects on yield in this cultivar, whereas reduction in seed size accounted for essentially all of the decrease in yield at elevated temperature (and also for well-watered Forrest). The responses in cvs. Essex and Forrest were different from cv. Williams in that drought and temperature had similar, negative effects on seed size, but smaller seed size was only a part of the yield response.

Tocopherols. Total tocopherol contents were greatest in seeds of cv. Williams and about 10−15% less in cvs. Essex and Forrest (**Tables 3** and **4**). Assuming that the seeds were ~20% oil, the levels were comparable to other published results with soybean seeds (*6*, *9*). Drought and elevated temperature had no significant effects on total tocopherols in cv. Williams, but drought caused small (8−10%) increases in cvs. Essex and Forrest. Similar results were observed for tocopherols measured in plants from the 1999 greenhouse study, but the significant interaction between soil moisture and temperature for cv. Forrest in 2000 (**Table 4**) was not observed in 1999 (**Table 5**).

Although total tocopherols were relatively constant, all three cultivars showed large increases in α-tocopherol (128, 142, and 198% for cvs. Forrest, Essex, and Williams, respectively) when comparing results obtained under drought at 28 °C with well-watered controls at 23 °C (**Table 3**). There were corresponding decreases in δ-tocopherol (53, 52, and 65% for cvs. Forrest, Essex, and Williams, respectively) and γ-tocopherol (7, 17, and 30% for cvs. Forrest, Essex, and Williams, respectively). Note that measured changes in γ-tocopherol also include possible changes in β-tocopherol.

Increases in α-tocopherol (and decreases in δ-tocopherol and γ-tocopherol) were the largest in cv. Williams. On a mole equivalent basis, the combined decreases in γ-tocopherol and δ-tocopherol accounted for 94% of the increase in α-tocopherol in cv. Williams (where total tocopherols were constant), whereas declines in γ-tocopherol and δ-tocopherol accounted for only

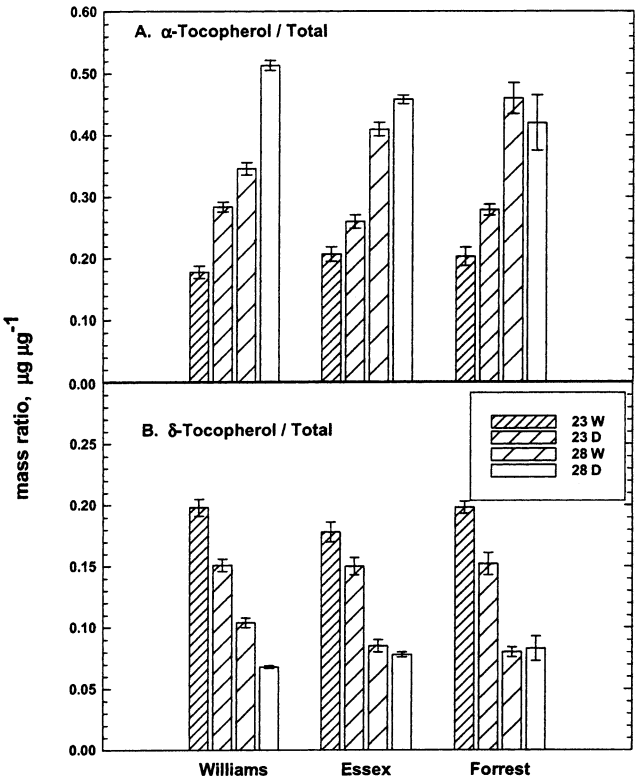

Figure 1. Mass ratio of α-tocopherol to total tocopherol (A) and δ-tocopherol to total tocopherol (B). Data from **Table 3**. See **Table 3** for explanation of treatments.

60−70% of the increase in α-tocopherol in cvs. Essex and Forrest (the remainder coming from increases in total tocopherols).

The mass ratios of α-tocopherol or δ-tocopherol to total tocopherol clearly revealed the effects of elevated temperature and drought (**Figure 1** and **Table 4**). For control conditions (plants at 23 °C and well-watered), the relative amounts of α-tocopherol (and δ-tocopherol) varied only between 18 and 20% for all 3 cultivars. Note that α-tocopherol is high, as compared to both field-grown plants [typically 5−10% (*10*)] and plants from the 1999 greenhouse study (∼13%; **Table 5**). It is not surprising that mass ratios for α-tocopherol are smaller in field-grown plants, since temperature affects the relative distribution of tocopherol species, and average outdoor temperatures during seed development are generally 1−4 °C lower than the 23 °C greenhouse. Year-to-year differences in mass ratio may also be related to temperature, since average greenhouse temperatures in 1999 were ∼1 °C lower than 2000 during the first 6 weeks of seed development. Note that the sum of the mass ratios for α-tocopherol and δ-tocopherol were similar for both 1999 and 2000 greenhouse studies (∼38% of total).

Although mass ratios for the three cultivars were quite similar at 23 °C under well-watered conditions, the change in tocopherol species in response to drought and elevated temperature was quite different, depending on cultivar. Thus, the mass ratio of α-tocopherol increased (and δ-tocopherol decreased) more in response to elevated temperature than drought for cvs. Essex and Forrest, and the combination of drought and elevated temperature had little additional effect above that observed for elevated temperature (unlike combined effects of drought and elevated temperature on seed yield or seed size in these cultivars). In cv. Williams, on the other hand, the relative magnitude of increases in α-tocopherol mass ratio (and decreases in δ-tocopherol) were similar for drought and elevated temperature, and the superimposition of drought on elevated temperature resulted in further significant shifts in mass ratios. Of the 3 cultivars, note that drought tended to have the smallest effect on stress indicators in cv. Williams (**Table 2**).

DISCUSSION

Air temperature or soil moisture had large effects on the absolute and relative amounts of the three major tocopherol species in soybean seeds. In cv. Williams, moderate increases in temperature combined with extreme drought caused a large increase in α-tocopherol that was almost precisely matched by decreases in δ-tocopherol and γ-tocopherol content (94% on a molar equivalent basis). Since total tocopherol remained constant, we consider it unlikely that these results derived from coincidental and compensatory changes in extractability or stability of two or more individual tocopherol species. It is more plausible that elevated temperature and drought increased the metabolism of δ-tocopherol or γ-tocopherol to α-tocopherol. Even significant increases in total tocopherol levels were not tightly linked to the distribution of tocopherol species (e.g., in cvs. Essex and Forrest), since drought was responsible for increases in the total amount in these cultivars, whereas temperature was the major factor associated with increased α-tocopherol. In another study, it was also observed that large increases in α-tocopherol in seeds of *Arabidopsis* were not accompanied by increases in total tocopherols (*11*). In this case, *Arabidopsis*, a model plant species, was genetically transformed to increase the expression of an enzyme that transforms γ-tocopherol to α-tocopherol.

Changes in tocopherol metabolism did not appear to be the result of nonspecific responses to stress. Thus, drought treatment, which was specifically designed to cause more stress (e.g., yield reduction) than elevated temperature, had relatively little effect on distribution of tocopherol species, as compared to temperature in cvs. Essex and Forrest. The relative ineffectiveness of drought also suggests that temperature did not affect tocopherol metabolism by inducing drought stress. Although drought did have more effect on the distribution of tocopherol species in cv. Williams than in cvs. Essex and Forrest, drought did not cause significant reduction in seed mass in cv. Williams. The results do not exclude that drought affected tocopherol metabolism partly via an increase in temperature (e.g., as a result of reduced evapotranspirational cooling). Of course, caution should be exercised when evaluating relative impacts of environmental stress on seed physiology on the basis of yield or seed size only, because the two indicators did not always agree with regard to temperature and drought, and it is not clear which indicator is a better predictor of processes occurring within the seed. Since tocopherols differ quantitatively and qualitatively in the seed coat, embryonic axis, and cotyledon (*10*), it will also be important to determine the effects of environment on the growth and development of these components as well as on the partitioning of total tocopherol amounts and species.

Tocopherols constitute an important class of lipophilic antioxidants that are reported to increase in plant leaf tissue (*12*, *13*) as well as in axes of germinating soybeans (*14*, *15*) exposed to various forms of environmental stress. Although an inability to make tocopherols and other important prenyllipids has been implicated in increased susceptibility to stress in plant leaves (*16*), the actual role of tocopherols remains unclear, since a more specific genetic lesion that eliminated only tocopherol failed to result in increased susceptibility to stress (*17*). Nonetheless, increases in α-tocopherol induced by either elevated temperature or drought during seed maturation may

be of value to the plant if seeds or seedlings are subsequently better able to tolerate stress. We note that the relatively small effects of temperature and drought on stress indicators in cv. Williams correspond with higher levels of tocopherols per seed mass in this line as well as with a relatively large increase in α-tocopherol in stressed plants. It will be important to continue these studies, comparing additional lines with respect to stress responses as well as tocopherol contents and species.

The ability to affect the distribution of tocopherol species also has important implications for nutrition, since α-tocopherol, the form with the highest vitamin E activity, is generally low in oilseeds (*18*). Although seeds of a model plant species have already been genetically engineered to produce elevated α-tocopherol (*11*), it is obvious from the results here that soybean seeds are competent to synthesize high levels of α-tocopherol when provided suitable environmental signals. We would predict elevated levels of α-tocopherol under warmer conditions (e.g., lines that mature early or that develop in warmer regions). Yearly changes in weather or differences in climate over location or time may already affect tocopherols under field conditions if differences in average air temperature of ∼1 °C (i.e., comparing 1999 and 2000 greenhouse results) are sufficient to affect tocopherol metabolism. In fact, differences in soybean seed isoflavone content in field plants that are consistent with differences in environment with respect to location or season have already been observed (*3*, *19*). Further comparison to approaches taken with isoflavones (*3*) suggests it may be possible to breed for plants with an altered response to temperature that produce increased α-tocopherol in seeds at normal growing temperatures. A key factor in developing this approach will be to obtain more precise information on the nature of the temperature response and the period during seed development that is sensitive to temperature.

Genetic differences in the response of tocopherol metabolism to temperature may explain the disparity between present results and two earlier studies (*5*, *6*), but there are also some potentially significant differences in methods among the three studies. In the current study, temperature treatments took place in greenhouses with irradiances close to those in the field and with normal seasonal changes in daylength. In contrast, Dolde et al. (*6*) initiated experiments in a greenhouse (no information on daylength or lighting) and shifted plants at flowering to growth chambers for temperature treatment during the remainder of reproductive development (12 h light periods at 220 μmol m^{-2} s^{-1} PPF). Thus, treatments started early in reproductive development and took place under shortened daylengths. We estimate the plants received one-half or less the estimated average daily light integral provided by the current study. Almonor et al. (*5*) did not report on irradiance or whether plants were raised or treated in greenhouses or chambers, but they did specify that soybeans were exposed to 15 h daylengths until seed fill whereupon daylengths were reduced to 9 h during temperature treatment. Thus, this study also involved the use of shortened daylengths as well as probable reductions in daily light integral. It is not known whether light affects tocopherol metabolism in soybean seeds, but light quality has been shown to alter fatty acid metabolism in soybean seeds (*20*, *21*).

ABBREVIATIONS

PPF, photosynthetic photon flux; cv(s)., cultivar(s); W, well-watered; D, drought.

ACKNOWLEDGMENT

We thank Roman Mirecki, Ernest Goins, and Louis Rose for assistance with greenhouse studies.

LITERATURE CITED

(1) Beecher, G. R. Phytonutrients' role in metabolism: effects on resistance to degenerative processes. *Nutr. Rev.* **1999**, *57*, S3−S6.

(2) Wolf, R. B.; Cavins, J. F.; Kleiman, R.; Black, L. T. Effect of temperature on soybean seed constituents: oil, protein, moisture, fatty acids, amino acids and sugars. *J. Am. Oil Chem. Soc.* **1982**, *59*, 230−232.

(3) Tsukamoto, C.; Shimada, S.; Igita, K.; Kudou, S.; Kokubun, M.; Okubo, K.; Kitamura, K. Factors affecting isoflavone content in soybean seeds: changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. *J. Agric. Food Chem.* **1995**, *43*, 1184−1192.

(4) Vlahakis, C.; Hazebroek, J. Phytosterol accumulation in canola, sunflower, and soybean oils: effects of genetics, planting location, and temperature. *J. Am. Oil Chem. Soc.* **2000**, *77*, 49−53.

(5) Almonor, G. O.; Fenner, G. P.; Wilson, R. F. Temperature effects on tocopherol composition in soybeans with genetically improved oil quality. *J. Am. Oil Chem. Soc.* **1998**, *75*, 591−596.

(6) Dolde, D.; Vlahakis, C.; Hazebroek, J. Tocopherols in breeding lines and effects of plant location, fatty acid composition, and temperature during development. *J. Am. Oil Chem. Soc.* **1999**, *76*, 349−355.

(7) Fehr, W. R.; Caviness, C. E. Stages of soybean development. *Special Report, Agriculture and Home Economics Experiment Station, Iowa State University of Science and Technology*. **1977**, *80*, 1−11.

(8) Kurilich, A. C.; Juvik, J. A. Quantification of carotenoid and tocopherol antioxidants in Zea mays. *J. Agric. Food Chem.* **1999**, *47*, 1948−1955.

(9) Kramer, J. K. G.; Fouchard, R. C.; Kallury, K. M. R. Determination of vitamin E forms in tissues and diets by high-performance liquid chromatography using normal-phase diol column. *Methods Enzymol.* **1999**, *299*, 318−329.

(10) Yoshida, H.; Takagi, S.; Ienaga, H.; Tsuchiya, C. Regional distribution of tocopherols and fatty acids within soybean seeds. *J. Am. Oil Chem. Soc.* **1998**, *75*, 767−774.

(11) Shintani, D.; DellaPenna, D. Elevating the vitamin E content of plants through metabolic engineering. *Science*. **1998**, *282*, 2098−2100.

(12) Munné-Bosch, S.; Schwarz, K.; Alegre, L. Enhanced formation of α-tocopherol and highly oxidized abietane diterpenes in water-stressed rosemary plants. *Plant Physiol*. **1999**, *121*, 1047−1052.

(13) Havaux, M.; Kloppstech, K. The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in Arabidopsis *npq* and *tt* mutants. *Planta*. **2001**, *213*, 953−966.

(14) Simontacchi, M.; Caro, A.; Fraga, C. G.; Puntarulo, S. Oxidative stress affects α-tocopherol content in soybean embryonic axes upon imbibition and following germination. *Plant Physiol*. **1993**, *103*, 949−953.

(15) Kozak, R. G.; Ricco, R. A.; Gurni, A. A.; Boveris, A. D.; Puntarulo, S. Antioxidant response of soybean cotyledons (*Glycine max*) to ultraviolet irradiation. *Can. J. Plant Sci*. **1999**, *79*, 181−189.

(16) Grasses, T.; Grimm, B.; Koroleva, O.; Jahns, P. Loss of alpha-tocopherol in tobacco plants with decreased geranylgeranyl reductase activity does not modify photosynthesis in optimal growth conditions but increases sensitivity to high-light stress. *Planta* **2001**, *213*, 620−628.

(17) Collakova, E.; DellaPenna, D. Isolation and functional analysis of homogentisate phytyl transferase from *Synechocystis* sp. PCC 6803 and Arabidopsis. *Plant Physiol.* **2001**, *127*, 1113−1124.

(18) Jiang, Q.; Christen, S.; Shigenaga, M. K.; Ames, B. N. γ-tocopherol, the major form of vitamin E in the U.S. diet, deserves more attention. *Am. J. Clin. Nutr*. **2001**, *74*, 714−722.

(19) Wang, H. J.; Murphy, P. Isoflavone content in commercial soybean foods. *J. Agric. Food Chem.* **1994**, *42*, 1674−1677.
(20) Britz, S. J.; Cavins, J. F. Spectral quality during pod development modulates soybean seed fatty acid desaturation. *Plant, Cell Environ.* **1993**, *16*, 719−725.
(21) Holden, M. J.; Norman, H. A.; Britz, S. J. Spectral quality during pod development affects omega-6 desaturase activity in soybean seed endoplasmic reticulum. *Physiol. Plant.* **1994**, *91*, 346−351.

Received for review January 2, 2002. Revised manuscript received July 19, 2002. Accepted July 19, 2002.

JF0200016