Environmental Signals Triggering Enhanced Content of Vitamin E in Seeds of Vegetable Soybean Varieties: Implications for Global Change

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Introduction and Methods

Global change encompasses both known (rising atmospheric CO_2) and hypothetical (elevated temperature, increased weather variability and environmental stress) parameters that can affect plant growth, yield and composition (Cave et al., 1981). But, relatively little attention has been given to potential effects of global change on phytochemicals with known or putative nutritional benefit. Stress has been implicated in large year-to-year variations in soybean seed isoflavones (Wang and Murphy, 1994). Temperature during seed development has also been shown to affect isoflavone (Tsukamoto et al., 1995) and sterol (Vlahakis and Hazebroek, 2000) levels in soybean seeds. Although environmental stress altered tocopherol levels in vegetative tissues (Garcia-Plazaola and Becerril, 2000), conflicting results have been reported for temperature effects on soybean seed tocopherols (Almonor et al., 1998; Dolde et al., 1999).

Tocopherols are a major class of lipid-soluble antioxidant (Shintani and DellaPenna, 1998) consisting of gamma-tocopherol (gT), delta-tocopherol (dT), and alpha-tocopherol (aT), in general order of decreasing abundance in dicot seeds. Vegetative tissues, in contrast, contain primarily aT, which has the highest Vitamin E activity. Soybean seeds are a major source of tocopherols. Since increases in dietary Vitamin E may be beneficial, there is interest in genetic and environmental factors that affect both total tocopherols and the relative proportions of aT, gT, and dT. We felt it was important to reexamine the influence of environment on tocopherols in soybean seeds during development.

Seed tocopherols were analyzed by HPLC using a modification of the method of Kurilich and Juvik (1999). A wide range of soybeans was examined, including vegetable lines (Verde and Emerald). We have no reason to believe that vegetable and field lines respond differently, so results from all lines will be discussed. Experiments were conducted primarily in greenhouses in 1999 and 2000 comparing two different temperature regimes (averages of 23 or 28 C) during seed development (post-R5). Average outdoor temperatures were close to the 23 C average until mid-September. Plants were grown in composted soil in large (12 L) clay pots fertilized periodically with N-P-K. Some plants were exposed to severe drought during seed development (ca. 25% of soil water capacity vs. 80-90% controlled with soil moisture blocks). Plants were also grown in the field in either open plots (ambient air) or in ventilated open-top chambers in which tropospheric ozone and water-stress were controlled or in acrylic enclosures in which atmospheric CO_2 was controlled. Ambient air studies were conducted at Beltsville (1999) and at the Univ. Maryland research centers at Poplar Hill, Wye Island, and Clarksville (1999 and 2000). Finally, plants were also grown in growth chambers (M-18, EGC, Chagrin Falls, OH) over a range of temperatures with controlled CO_2 and simulated solar radiation.

Results

Seed growth and accumulation of total tocopherols (µg g^{-1} seed dry weight) were generally similar for all treatments (greenhouse 23 and 28 C and field) for both Emerald and Verde, but final seed dry weights were lower at 28 C while final tocopherols were lower in field samples. The last harvest took place 68 days after flowering in the field and 61 (Emerald) or 67 (Verde) days after flowering in the greenhouse. At this stage, seeds were still green, but pods were yellowing. Seeds of Emerald and Verde had much greater levels of aT in the 28 C greenhouse conditions (Table 1). In Verde, the increase in aT initiated at about 45 days after flowering for all treatments, whereas in Emerald the increase began earlier at 28 C (38 days). aT was essentially non-detectable early in seed development, but the effects of elevated temperature on tocopherol metabolism were already manifested at the first harvest as increases in the proportion of dT relative to gT.

Table 1. Alpha-Tocopherol at Final Harvest (Percent of Total)

Cultivar	Field	Greenhouse 23 C	Greenhouse 28 C
Emerald	11	15	31
Verde	10	12	30

Similar results were obtained in the greenhouse with other soybeans (Williams, Essex, Forrest and an early-maturing dwarf line). The dwarf line is also being use in growth chambers to dissect the temperature response. Constant 28 C during seed development is almost as effective at inducing increased aT as a temperature cycle (23 C night, 33 C day; 28 C average). Since 28 C has little or no effect on plant seed yield, it probably does not act as a heat stress *per se*.

Environmental stress, however, does affect tocopherol metabolism, generally causing an increase in the proportion of aT and a decrease in dT. In greenhouse studies, severe drought (60-70% yield inhibition) affected tocopherols to a similar extent as the relatively mild increase in temperature. There did not appear to be an interaction when drought and elevated temperatures were combined. In the field, using ventilated open-top chambers, combined drought and elevated tropospheric ozone (30% yield inhibition) also affected tocopherol metabolism. Although aT levels remained low, reductions in the proportion of dT were significant.

Atmospheric carbon dioxide levels have steadily increased over the last 150 years from 280 to 360 ppm currently, with projected values reaching 700 ppm by the end of this century. No evidence for an effect of elevated CO_2 on tocopherol metabolism was detected, but differences were noted between plants in enclosures (both elevated and ambient CO_2 controls) and plants grown outdoors as ambient air controls. Since the enclosures were warmer, especially during the day, the results are consistent with known temperature effects.

It is apparent that relatively small differences in temperature can affect tocopherols in field-grown plants. We therefore obtained soybean seed from several field sites in Maryland as part of the uniform field trials studies. To date 8 lines representing MG 3, 4 and 5 were examined from 3 locations representing different environmental conditions for both 1999 and 2000. On average, soybean seeds harvested from cooler conditions were marked by elevated dT and reduced aT.

Conclusions

Tocopherol metabolism in developing seeds of vegetable and field soybean lines was examined to determine whether temperature, drought or atmospheric CO_2 influence either the total amount of tocopherols or the relative distribution of the three major forms present in seeds, alpha-, gamma-, and delta-tocopherol (aT, gT and dT, in order). Small increases in temperature caused large increases in aT, with levels increasing from 5-10% of total tocopherols to as much as 50%. There were corresponding decreases in the proportion of dT, suggesting that metabolic throughput was affected. Total tocopherol levels did not change dramatically. Under the right conditions, seeds are evidently able to synthesize large amounts of aT. It may be possible to breed for soybeans with altered response to temperature to enhance vitamin E content. For example, aT synthesis appeared to turn on earlier in Emerald when exposed to higher temperatures. Among field soybean lines, there were variations in the extent to which temperature affected aT.

Field data are also consistent with an effect of temperature on tocopherols, although other variables such as photoperiod and irradiance cannot be excluded as yet. Nonetheless, the ability to differentiate early and late plantings at a single location suggests that the nutritional value of crops can be affected by small changes in climate. Tocopherol metabolism also appears to be influenced by environmental stresses such as drought, indicating that phytonutrients such as vitamin E may be influenced by weather variability. On the other hand, elevated atmospheric CO_2, a known element of global change, did not affect tocopherols.

Further studies will include evaluation of potential molecular mechanisms affecting tocopherol metabolism, screening of additional soybean lines, and more detailed studies of temperature response, including the stage of seed development affected by temperature. For example, tocopherol levels in the 23 C greenhouse were almost identical to those in the field for Verde, suggesting either that differences between these conditions were not important or that tocopherol metabolism was regulated by events early in seed development when the greenhouse and field were similar. In contrast, there was less congruence between tocopherols in the field and greenhouse for Emerald.

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