Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 102 (2007) 138–145
Food Chemistry
www.elsevier.com/locate/foodchem

The dynamics of tocopherol and the effect of high temperature in developing sunflower (*Helianthus annuus* L.) embryo

Guijun Dong [a,b], Xiaoli Liu [a], Zhongyue Chen [a], Weidong Pan [c], Hongjie Li [d], Gongshe Liu [a,*]

[a] *Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, 100093 Beijing, PR China*
[b] *Graduate School of the Chinese Academy of Sciences, 100039 Beijing, PR China*
[c] *Research Department of Biomedical Engineering, Institute of Electrical Engineering, Chinese Academy of Sciences, 100093 Beijing, PR China*
[d] *Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, 100081 Beijing, PR China*

Received 19 December 2005; received in revised form 30 March 2006; accepted 4 May 2006

Abstract

To improve sunflower (*Helianthus annuus* L.) oil quality, the pattern of tocopherol and the effect of brief interval of high temperature on tocopherol accumulation during embryo development were investigated. Total tocopherol content increased linearly from 12 to 33 days after anthesis (DAA) and then remained stable until maturity. γ-Tocopherol content reached a maximum on 33 DAA and then decreased. δ-Tocopherol was not detected until 19 DAA. The effect of brief interval of high temperature ($\geqslant$35 °C for seven consecutive days) on tocopherol accumulation was studied. The accelerating effect of high temperature on tocopherol accumulation on a dry weight basis was detected, but the reduction of tocopherol yield occurred from 12 to 19 DAA because the embryo dry weight was reduced significantly. However, the embryos exposed to a temperature of 35 °C from 12 to 19 DAA significantly increased the tocopherol yield per embryo, with no effect on the dry matter weight. These results are useful for evaluating the tocopherol content in sunflower oil and may help recognizing high quality of the oil.

Keywords: Sunflower; Tocopherol; High temperature; Tocopherol accumulation

1. Introduction

As an important nutrient for human beings, vitamin E has been well known for its antioxidative properties (Kamal-Eldin & Appelqvist, 1996). It consists of tocopherols and tocotrienols in which a chromanol ring system is common and a polyprenyl side chain is distinct (Munné-Bosch & Alegre, 2002). Vitamin E is also an indispensible vitamin that can be useful in food and drug products (Biesalski, Hemmes, Hopfenmuller, Schmid, & Gollnick, 1996; Sergi et al., 2004). Furthermore, natural vitamin E, which has higher vitamin E vigor, comes from plant species. Therefore, it is necessary to understand the accumulation of vitamin E for enhancement of this vitamin in crops (Kornsteiner, Wagner, & Elmadfa, 2006; Kriese et al., 2004). Tocochromanols and lipids in barley accumulated in parallel until 80% of the final dry weight of the kernels was reached; tocochromanols did not change when lipid content decreased (Falk, Krahnstöver, van der Kooij, Schlensog, & Krupinska, 2004). Accumulation of tocopherol in oilseeds was proved to be related to the plastid development rather than to the lipid accumulation (Beringer & Northdurft, 1979; Brunia et al., 2002). As an important oil plant, sunflower (*Helianthus annuus* L.) is also an important source of tocopherol, hence their high vitamin value (Velasco, Fernández-Martínez, García-Ruíz, & Domínguez, 2002). The main tocopherols of sunflower occur as a family of four derivatives, which are known as α-, β-, γ-, and δ-Tocopherols (Morrison, Conventry, & Barnes, 1982). As reserve pool of nutriments, sunflower seed is rich in tocopherol, oil and other nutritional components. In sunflower seeds, oil content was not correlated with tocopherol content

* Corresponding author. Tel.: +86 10 6283 6227; fax: +86 10 8259 2310.
E-mail address: liugs@ibcas.ac.cn (G. Liu).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.05.013

(Alpaslan & Gunduz, 2000; Demurin, Skoric, & Karlovic, 1996). In sunflower cell cultures, vitamin E content can be enhanced with a special medium (Caretto et al., 2004). However, whether tocopherol content in sunflower embryos can be regulated has not been explored before.

Heat stress is becoming an important abiotic stress for crops with increasing global temperature (Chimenti, Hall, & Sol LoÂpez, 2001; Savin & Nicolas, 1996, 1999; Savin, Stone, Nicolas, & Wardlaw, 1997). In many regions of the world, short-time high temperature occurs frequently and affects crop development (Conroy, Seneweera, Basra, Rogers, & Nissen-Wooller, 1994). For example, high temperature affected embryo-growth rate and duration, floret and grain number and nutrients, such as oil, fatty acids, protein and starch. A brief interval of high temperature inhibited kernel development and altered starch and protein compositions (Jenner, 1994; Savin et al., 1997; Wrigley, Blumenthal, Gras, & Barlow, 1994). In addition, Rondanini, Savin, and Hall (2003) examined oil, embryo weight and fatty acids by exposing the capitulate of cv. HA89 growing at period interval of high temperature and concluded that oil content was reduced and final oil composition proved to be sensitive to the timing of heat stress (i.e., 19–26 days after anthesis). However, no information on dynamic change of vitamins (such as tocopherols) is available in response to high temperature stress.

The objectives of the present study were (1) to investigate the accumulation of tocopherol during embryo development of sunflower, and (2) to clarify the effect of brief treatment with high temperatures (35, 37 and 40 °C) on tocopherol accumulation in developing sunflower embryos in the sensitive periods.

2. Materials and methods

2.1. Sunflower management and temperature treatments

In 2003 and 2004, two parallel experiments were conducted in the Institute of Botany, Chinese Academy of Sciences, Beijing, China. Plants of A15, an oil type cultivar, were individually grown in 5-L plastic salvers filled with a 3:1:1 (v/v/v) mixture of soil, peat and sand. Plants were fertilized with NH_4HCO_3 (20 g per plant) every 30 days after emergence, were watered with 2.5 L water per plant in the first two weeks and 5.0 L on a weekly basis. The plants were grown in the open until 5 DAA (days after anthesis), and were then transferred to the greenhouse at 31/25 °C (day/night). Temperature was measured as close to the capitulum as possible and regulated using a calibrated thermistor. Plants were randomly arranged and rotated weekly. Embryos were randomly chosen at each of the six developmental stages, i.e., 5, 12, 19, 26, 33, and 40 DAA. The results of the two experiments were equivalent. The results in 2004 were coherent with the following experiments, so the results of the year 2004 experiment are presented here.

Brief interval of high temperature heated the capitulum in each of the three following periods, HT1: 12–19 DAA, HT2: 19–26 DAA and HT3: 26–33 DAA in the summer of 2004. The control plants were grown at 31/25 °C (day/night). Samples were grown under controlled condition until each group of samples was exposed to different mean daily temperatures (35, 37 and 40 °C, respectively). Samples were placed in an independent module within the greenhouse, differing only in their temperature region. Plants were transferred to the control condition again after high temperature treatment for 7 days, and then harvested 40 DAA. Temperature was monitored at half-hour intervals, using thermometers close to capitulums as detailed by Rondanini et al. (2003). The temperature in each interval was observed (data not show).

2.2. Determination of sunflower embryo development

Sunflower seeds, located in a band halfway between the center and the circumference of the flower head (Zimmerman & Fick, 1973), were harvested at a 7-day interval from the periphery of each capitulum. Samples were then dried at 60 °C for 48 h, and cooled over silica gel to dry the seeds (Ploschuk & Hall, 1995). Contents of lipid and protein in embryos were determined, and dry weight, fresh weight and water content were also measured to investigate the sunflower seed development.

One hundred embryos (achenes without hull and seed coat) were homogenized to a particle size smaller than 2.0 mm (Kochhar & Rossell, 1987). Oil was extracted in boiling petroleum ether and oil content was determined according to FOSFA (the Federation of Oils, Seeds and Fats Associations; 1982).

Protein content in total homogenates of embryos was measured according to Bradford (1976) using Bio-Rad protein assay dye reagent with bovine serum albumin (BSA) as a standard. Ten sunflower embryos were homogenized in 4 ml of 50 mM HEPES/NaOH (pH 7.4) using a glass homogenizer. The homogenate was centrifuged at 16,000*g* for 30 min before the assay.

2.3. Tocopherol extraction and determination during sunflower embryo development

Seeds were collected 5, 12, 19, 26, 33, and 40 DAA and cleaned with sterilized water prior to removal of seed coats. The seed meats were ground to a fine powder using a coffee grinder, weighed, and transferred to a cellulose thimble. The samples were exhaustively extracted in a Sohxlet apparatus for 3 h with hexane containing 1 g kg^{-1} BHT (butylated hydroxytoluene). Hexane was removed by rotary evaporation and the resulting crude oil was dissolved in a small amount of hexane. The tocopherol composition was analyzed with a Hewlett–Packard 1100 HPLC system (IUPAC, 1998) and fluorescence detector with 290 nm excitation and 330 nm emissions (Kriese et al., 2004). Tocopherol derivatives were identified by comparing retention times with the standards (Sigma–Aldrich, Bellefonte, PA,

USA) and the quantities were compared with the curve generated using different amounts of standards.

3. Results

3.1. Sunflower embryo development under control condition

To establish the sensitive stages of tocopherol accumulation, fresh weight, dry weight and water content during embryo development were determined (Fig. 1). Fresh weight increased progressively until 26 DAA when the fresh weight reached its highest, 6.64 g/100 embryos. From 26 to 40 DAA, the fresh weight decreased slightly. However, the highest dry weight (5.79 g/100 embryos) appeared on the 33 DAA, and then remained almost constant.

The accumulation of oil and protein, during embryo development was also determined (Fig. 2). Oil accumulated slowly during the early stage before 12 DAA. However, in the following stage from 12 to 26 DAA, oil accumulated linearly, which was the dominating period of oil accumulation. After that time, oil content did not increase, and then decreased from 33 DAA. Protein was depositing smoothly during embryo development, except for the period from 19 to 33 DAA (Fig. 2).

3.2. Tocopherol accumulation during embryo development

The tocopherol content in embryo was measured during embryo development. The total tocopherol content in 100 embryos is shown in Fig. 3a. It was very low before 12 DAA (216.6 mg/100 embryos), linearly increased till 33 DAA (2005.6 mg/100 embryos) and then remained nearly constant from 33 DAA. From 5 to 40 DAA, the tocophreol increments in each 7-day interval were 148.1, 446.7, 725.1, 617.2, and 35.3 mg/100 embryos, respectively. In addition, tocopherol content on the basis of embryo dry weight was also measured (Fig. 3b). Before 12 DAA, the increasing rate of tocopherol was equal to that of embryo dry weight, but from 12 to 33 DAA, tocopherol content on a dry weight basis of embryos increased linearly, indicating that the tocopherol accumulation rate was higher than that of embryos dry weight. This period from 12 DAA to 33 DAA was the main phase of tocopherol accumulation.

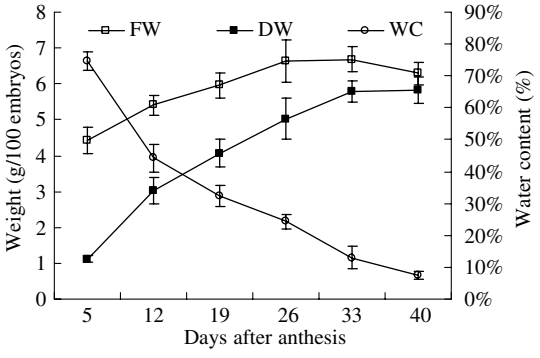

Fig. 1. Fresh weight, dry weight and water content in sunflower embryos at different development stages. Data represent means ± SE for 100 embryos for each period after anthesis.

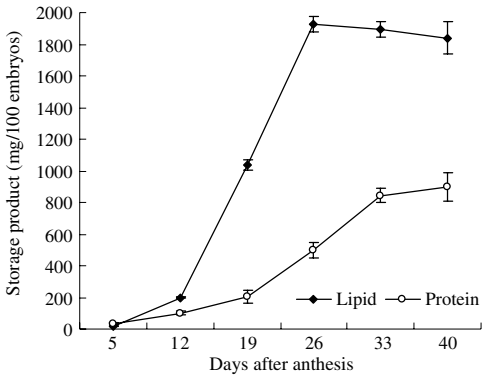

Fig. 2. Accumulation of lipid and protein during development of sunflower embryos. Each datum point represents the mean ± SE of measurements made on three separate batches of embryos.

The tocopherol content in each period is shown in Fig. 3c and d. The α-form of tocopherol shared a similar trend of accumulation with total tocopherol. The β- and γ-tocopherol contents increased before 33 DAA. Then, the β-tocopherol content went up and γ-tocopherol decreased slightly. The pattern of change of γ-tocopherol was similar to that of the oil (Figs. 2 and 3d). δ-Tocopherol was not detected until 19 DAA and then increased gradually at a slow rate (Fig. 3d).

3.3. Dynamics of sunflower embryos development affected by brief interval of high temperature

High temperature (35, 37 and 40 °C) treatment was carried out on developing embryos in HT1, HT2 and HT3. The final fresh weight was curtailed significantly (−44.3%) in HT1 after high temperature treatment (>35 °C) (Table 1). However, in HT2 and HT3, the final fresh weights were not significantly influenced by high temperature. The pericarp/embryo ratio is another index to validate embryo development. In this study, high temperature affected pericarp/embryo ratio significantly in HT1 and HT2 (Table 1). In HT1 at 40 °C, the pericarp/embryo ratio was about 2.5-folds higher than that of the control. In addition, the increment of the dry weight in each interval was determined (Table 2). The dry weight was hardly increased after treatment of at 37 and 40 °C in HT1. At the same time, the embryo hardly developed after treatment in HT1 (Table 1 and Fig. 4a). The effect of brief periods of high temperature on embryo development is shown in Fig. 4a–c. In HT1 and HT2, the embryo development was delayed significantly at 37 and 40 °C (Fig. 4a and b). However, at 35 °C, the embryo development was not affected in HT1, HT2 and HT3 (Fig. 4c). The final dry weight was significantly affected by heat treatments applied in HT1 and HT2; the decrease of the dry weight was only 2.0% at 35 °C in HT3 (Fig. 4d).

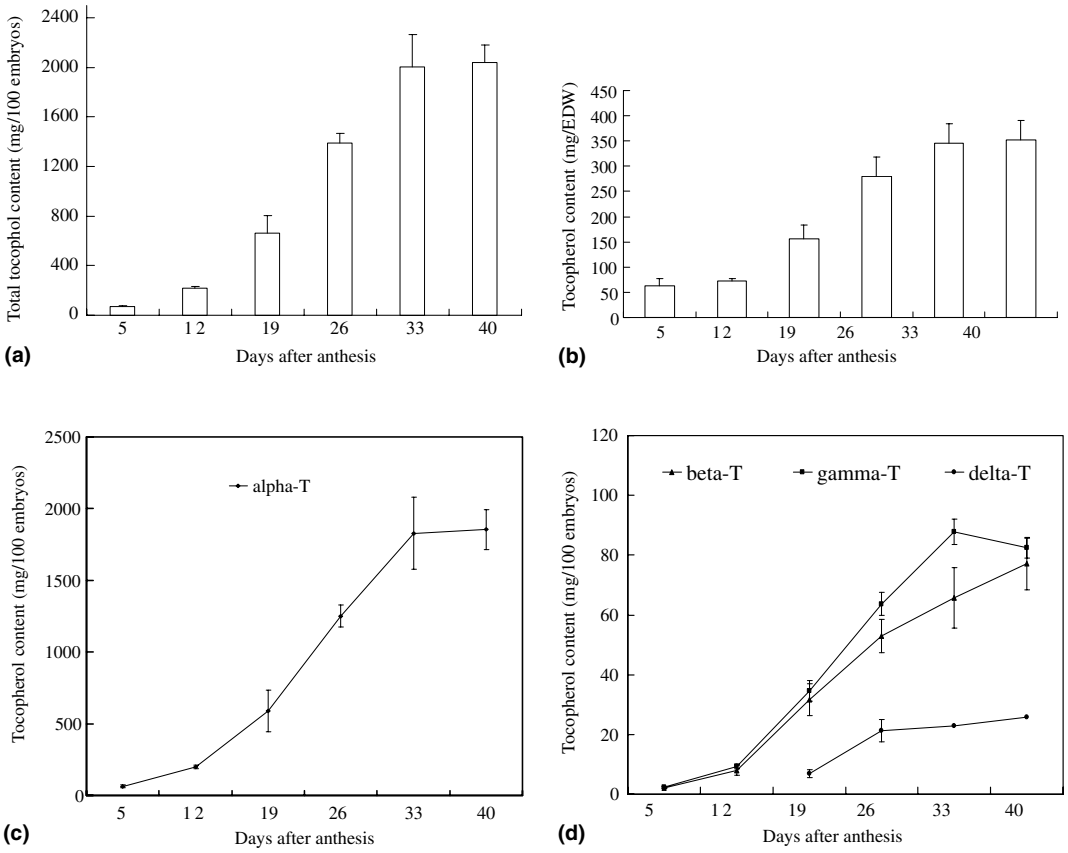

Fig. 3. Tocopherol accumulation during embryos development. Each datum point represents the mean ± SE of measurements made on three separate batches of embryos.

Table 1
Final index of embryo growth affected by increased temperature applied following the three separate 7-day-interval high temperature treatments during embryo development in sunflower (HT1 = 12–19 DAA, HT2 = 19–26 DAA, HT3 = 26–33 DAA)

Treatment	Final 100-embryo fresh weight (g)	Final 100-embryo dry weight (g)	Final 100-pericarp weight (g)	Pericarp/embryo ratio (g)	Increment of dry weight in HT1 (g)	Increment of dry weight in HT2 (g)	Increment of dry weight in HT3 (g)
HT1							
Control	6.30a	5.83a	1.17a	0.20b	1.21a		
T35	6.12a	5.71a	1.14a	0.20b	1.09a		
T37	3.42b	3.17b	1.14a	0.36a	0.14b		
T40	3.51b	3.13b	1.25a	0.40a	0.07b		
HT2							
Control	6.31a	5.83a	1.17a	0.20b		0.96a	
T35	6.18a	5.69a	1.15a	0.21b		0.92a	
T37	5.00a	4.64a	1.20a	0.26a		0.23b	
T40	4.94a	4.62a	1.33a	0.29a		0.18b	
HT3							
Control	6.31a	5.83a	1.17a	0.20a			0.78a
T35	6.09a	5.64a	1.13a	0.20a			0.57a
T37	5.92a	5.51a	1.05a	0.19a			0.44ab
T40	5.75a	5.31a	1.06a	0.20a			0.26b

[a,b] Means within a column in each part followed by the same letter are not significantly different as differentiated with the least-significant difference generated by SPSS 10.0 at $P = 0.05$.

3.4. Accumulation of tocopherol during development of embryos affected by brief intervals of high temperature

The effect of brief intervals of high temperature on dynamics of tocopherol accumulation was examined (Table 2 and Fig. 5). The dynamics of tocopherol was affected after high temperature treatment in HT1 and HT2 (Fig. 5a and c). In HT1, high temperature (>35 °C) treatment resulted in a synchronous decrease the tocopherol content. However, the tocopherol content was not affected

Table 2
Final index of tocopherol accumulation affected by increased temperature applied following the three separate 7-day-interval high temperature treatments during embryo development in sunflower

Treatment	Final individual tocopherol content (mg/100 embryos)				Final total tocopherol content		Increment of total tocopherol content (mg/100 embryos)		
	α	β	γ	δ	mg/100 embryos	mg/DW	In HT1	In HT2	In HT3
HT1									
Control	1885.6b	77.0a	82.5a	25.8a	2040.9a	351.0b	446.7a		
T35	1998.9a	80.9a	83.5a	26.9a	2190.3a	383.9b	489.5a		
T37	1444.3c	62.9a	58.8b	21.7a	1587.7b	501.6a	169.0b		
T40	1483.0c	64.7a	62.8b	22.9a	1633.3b	522.1a	177.5b		
HT2									
Control	1855.6b	77.0ab	82.5b	25.8a	2040.9b	351.0b		725.1b	
T35	2481.4a	92.7a	96.8a	31.8a	2702.7a	476.7a		1133.5a	
T37	1556.8c	71.0b	65.5c	24.5a	1556.8c	370.5b		575.5b	
T40	1546.2c	71.1b	69.9bc	23.2a	1546.2c	370.7b		641.2b	
HT3									
Control	1855.6b	77.0a	82.5a	25.8a	2040.9b	351.0a			617.2a
T35	2481.4a	78.2a	92.7a	28.5a	2402.7a	426.7a			841.6a
T37	1556.8b	79.6a	86.4a	26.5a	2148.5b	393.4a			598.9a
T40	1546.2b	79.3a	86.9a	25.1a	2139.5b	404.2a			771.6a

(HT1 = 12–19 DAA, HT2 = 19–26 DAA, HT3 = 26–33 DAA).
[a–c] Means within a column in each part followed by the same letter are not significantly different as differentiated with the least-significant difference generated by SPSS 10.0 at $P = 0.05$.

at 35 °C (Fig. 5a). On the contrary, the rate of tocopherol accumulation was increased at 35 °C in HT2 (Fig. 5c). The tocopherol content was reduced by high temperature (>35 °C) in HT1, and the final tocopherol content was reduced 22.2% compared with the control (Table 2).

Although tocopherol content was reduced (Fig. 5a), the tocopherol accumulation, based on embryo dry weight, was activated following high temperature treatment (>35 °C) in HT1 (Fig. 5b). In HT2, the high temperature treatment resulted in a similar trend for variation of tocopherol content as HT1 (Fig. 5c). The accumulation of tocopherol in high-temperature-treated (>35 °C) embryos was not different from that of the control embryos in HT2 (Fig. 5d). However, the 35 °C-treated embryos had higher tocopherol content than other high-temperature-treated (>35 °C) embryos (Fig. 5c). In HT3, significant variation was not detected both in the tocopherol content (Fig. 5e) and in the tocopherol accumulation rate (Fig. 5f). In conclusion, tocopherol content was enhanced at 35 °C in HT2, was reduced at high temperature (>35 °C) in HT1 and HT2, and was not affected significantly in HT3 after high temperature treatment (⩾35 °C) (Table 2).

4. Discussion

Oil accumulated slowly in sunflower embryos until 14 DAA, and then followed by a second phase of rapid synthesis that lasted until 28–30 DAA (Rondanini et al., 2003). In addition, the protein deposition took place between 12 and 31 DAA (Pleite, Pike, Garcés, Martínez-Force, & Rawsthorne, 2005). In the present study, oil accumulated linearly from 12 to 26 DAA, and then decreased; proteins were mainly deposited from 19 to 33 DAA. The fresh weight of embryos accumulated gradually in parallel with oil content, while tocopherol accumulated ceaselessly until dry weight of embryo reached its peak at 33 DAA. The slight difference for results about oil and protein, compared to the previous reports, was probably due to genetic and environmental differences (Alpaslan & Gunduz, 2000; Velasco et al., 2002). These results showed that the dynamics of A15 sunflower grain development generally agree with other sunflower lines (Rondanini et al., 2003).

One aim of the present study was to investigate the dynamics of tocopherol accumulation during embryo development and to discover the sensitive period of tocopherol accumulation. Tocopherol accumulation was related to the plastid development rather than the lipid accumulation in oilseeds (Beringer & Northdurft, 1979.). In our study, total tocopherol content, on the embryo dry weight basis, increased linearly from 12 to 33 DAA, and was then stable until maturity. These suggested that the interval from 12 to 33 DAA was the sensitive period of tocopherol accumulation. This was longer than the main period (12–26 DAA) of oil accumulation (Fig. 2) (Beringer & Northdurft, 1979; Pleite et al., 2005). Furthermore, the dynamics of accumulation of individual tocopherols was different (Fig. 3a, c, and d). The α-tocopherol accumulated in parallel to the total tocopherol, probably because α-tocopherol was the dominant tocopherol in sunflower (Demurin et al., 1996). The γ-tocopherol content reached its maximum after 7 days when oil content reached its peak (Figs. 2 and 3d).

Another objective of this study was to study the effect of a brief interval of high temperature (35, 37 and 40°)

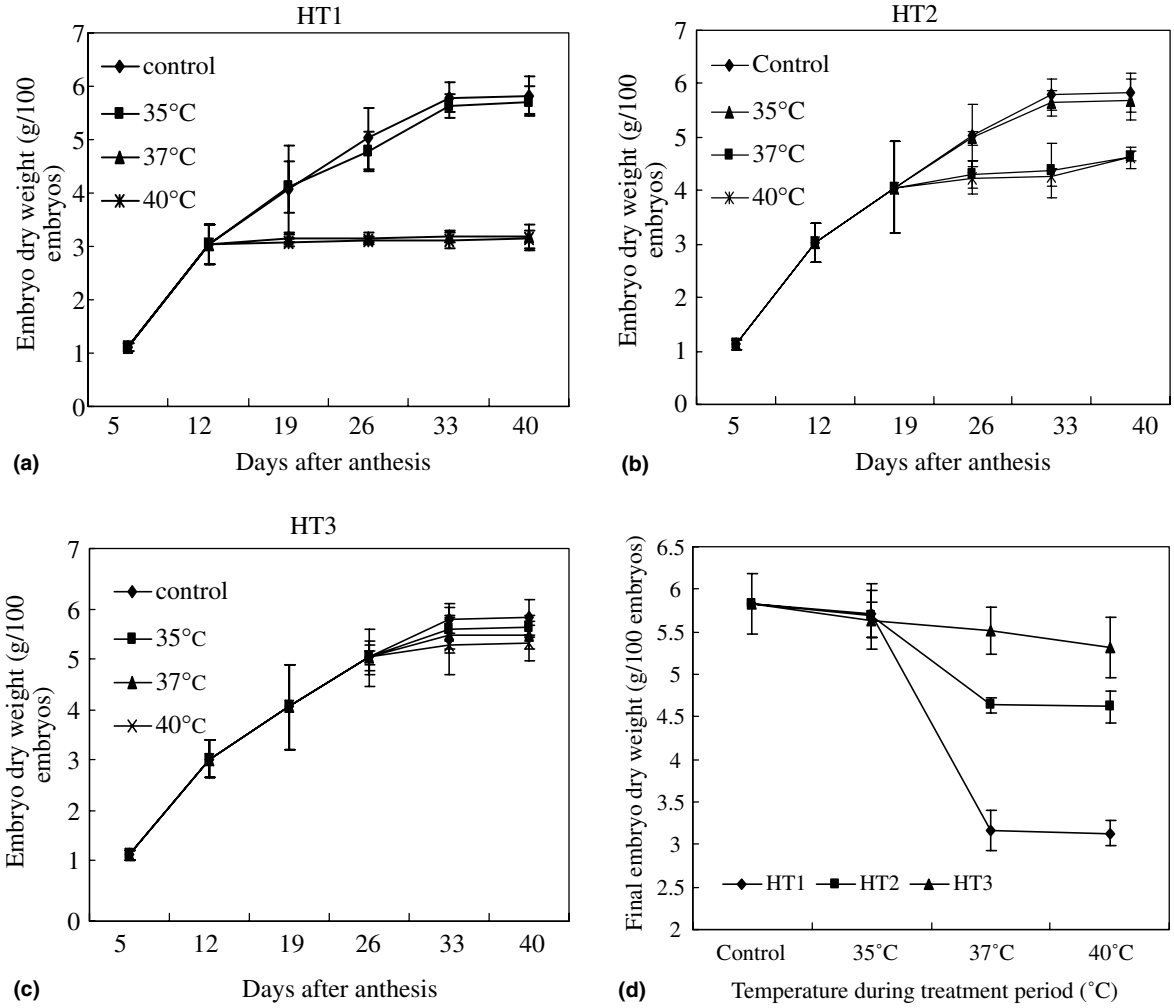

Fig. 4. Dynamics of embryo growth affected by increased temperature applied during three separate 7-day intervals during embryo development in sunflower (HT1 = 12–19 DAA, HT2 = 19–26 DAA, HT3 = 26–33 DAA).

treatment on tocopherol accumulation in developing embryo. The effect of brief interval of heat (>35 °C) was significant on grain weight in the early stages (12–19 DAA), and decreased as grain growth proceeded (Rondanini et al., 2003). In the present study, high temperature (>35 °C) treatment influenced the embryo development from 12 to 26 DAA (Fig. 4a and c), which agrees with the previous reports (Wardlaw & Wrigley, 1994). The dynamics of oil accumulation were reduced significantly by heat stress (>35 °C) in HT1 and HT2. The duration of oil accumulation was only 21.4 days in HT1 affected by 40 °C (Rondanini et al., 2003). The dynamics of tocopherol affected by brief interval of high temperature was investigated based on the above results. In this study, the duration of tocopherol accumulation was not influenced (Fig. 5a, c, and e). However, the tocopherol content in each embryo was decreased significantly at high temperature (>35 °C) in HT1 and HT2 (Table 2). Although the tocopherol content was reduced, the rate of tocopherol accumulation was enhanced in HT1 and HT2 at 37 and 40 °C (Fig. 5b, d, and f). These attested the accelerating effect of high temperature on tocopherol accumulation. High temperature led to a lower linoleic acid/oleic acid ratio in the mutants (Martínez-Force, Álvarez-Ortega, Cantisán, & Garcés, 1998) because of the loss in antioxidant defense potential and elevation in peroxidation products made under high temperature (Durak et al., 1999). Therefore, the accelerating effect of high temperature on tocopherol accumulation probably resulted from quenching reactive oxygen species and lipid radicals caused by high temperature (Kamal-Eldin & Appelqvist, 1996). However, the tocopherol content was reduced significantly by high temperature (>35 °C), due to the significant decrease of dry weight in HT1 and HT2 (Fig. 4). In this study, different dynamics of tocopherol accumulation after 7-day interval at 35 °C were observed. Embryos did not show a slower growth rate (Fig. 4a–c) and a significant decrease of dry weight at 35 °C (Table 2) was noted. However, the tocopherol accumulation was stimulated greatly at 35 °C in HT2. The yield of tocopherol treated at 35 °C in HT2 increased steadily until harvest. The tocopherol

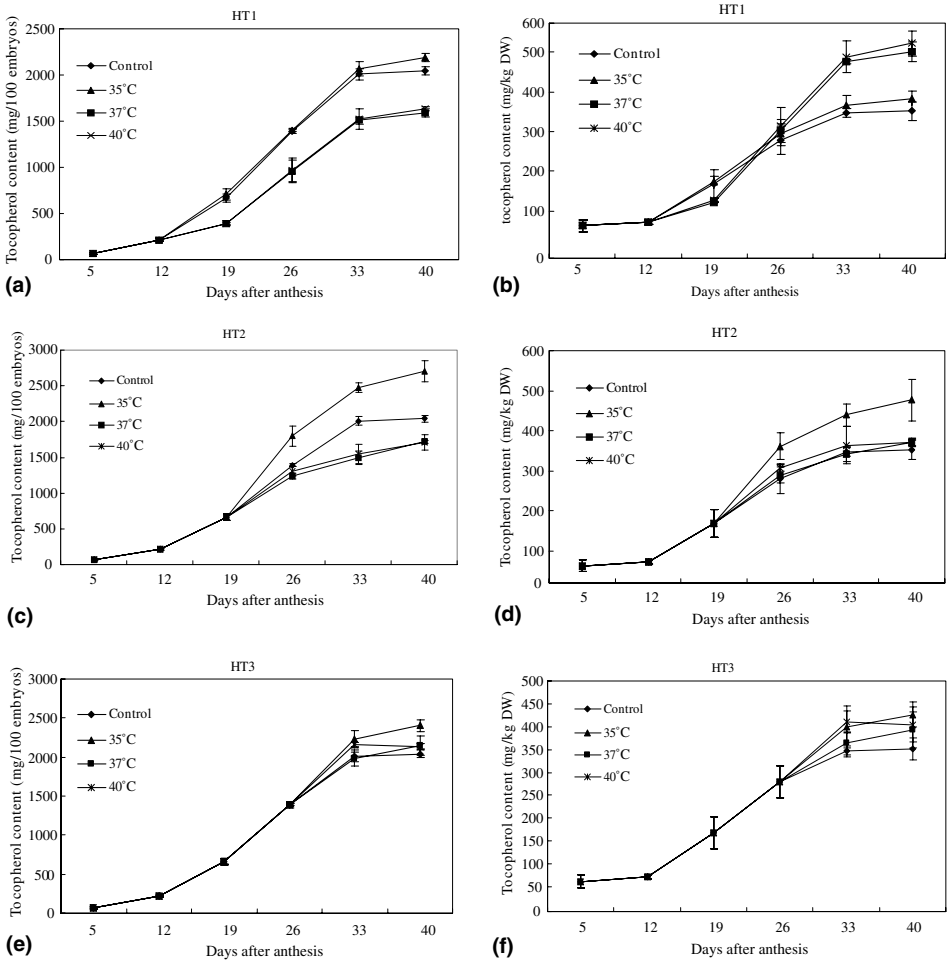

Fig. 5. Accumulation of tocopherol in developing sunflower embryo affected by increased temperature applied during three separate 7-day intervals during embryo development in sunflower (HT1 = 12–19 DAA, HT2 = 19–26 DAA, HT3 = 26–33 DAA).

content accumulated at 35 °C in HT2 was higher than those of other treatments (Fig. 5). Therefore, the mature seeds, treated at 35 °C in HT2 and harvested after 40 DAA, contained more tocopherol than other harvested seeds. Based on these results, it is possible to improve the temperature of special period during embryo development, by adjusting seedtime, to improve the tocopherol content in seeds and enhance the quality of sunflower oil (Bergmüller, Porfirova, & Dörmann, 2003).

In conclusion, our results indicate that tocopherol accumulated linearly from 12 to 33 DAA in developing embryo. The γ-tocopherol accumulation ceased when the embryo dry weight reaches its maximum. Others tocopherols accumulated continuously until harvest. Direct detrimental effects on grain filling and tocopherol yield were detected by brief treatment at high temperature (>35 °C). At 35 °C, the grain filling was not affected during the three 7-consecutive days, and the tocopherol accumulation was elevated after treatment in 19–26 DAA. Based on these results, the extent of the effects depends on the timing of stress and the degree of high temperature with differing periods of sensitivity applying for embryo weight and tocopherol accumulation. It is possible to improve tocopherol content in sunflower embryo treated with special temperature during special period (e.g., HT2).

Acknowledgements

The authors thank K.F. Li for data analysis and Dr. W.D. Zhang for manuscript preparation. The financial support provided by the Natural Science Foundation of China (NSFC: 30571305) is gratefully acknowledged.

References

Alpaslan, M., & Gunduz, H. (2000). The effects of growing conditions on oil content, fatty acid composition and tocopherol content of some sunflower varieties produced in Turkey. *Nahrung, 44*, 434–437.

Bergmüller, E., Porfirova, S., & Dörmann, P. (2003). Characterization of an Arabidopsis mutant deficient in γ-tocopherol methyltransferase. *Plant Molecular Biology, 52*, 1181–1190.

Beringer, H., & Northdurft, F. (1979). Plastid development and tocochromanol accumulation on oil seeds. In L. A. Appelqvist & C. Liljenberg (Eds.). *Advance in Biochemistry and Physiology of Plant Lipids* (vol. 133–137). Amsterdam: Klsevier/North-Holland Biomedical Press.

Biesalski, H. K., Hemmes, C., Hopfenmuller, W., Schmid, C., & Gollnick, H. P. (1996). Effects of controlled exposure of sunlight on plasma and skin levels of β-carotene. *Free Radical Research, 24*, 215–224.

Bradford, M. M. (1976). A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Brunia, R., Medicia, A., Guerrinia, A., Scaliab, S., Polic, F., Romagnolid, C., et al. (2002). Tocopherol, fatty acids and sterol distributions in wild Ecuadorian *Theobroma subincanum* (Sterculiaceae) seeds. *Food Chemistry, 77*, 337–341.

Caretto, S., Bray-Speth, E., Fachechi, C., Gala, R., Zacheo, G., & Giovinazzo, G. (2004). Enhancement of vitamin E production in sunflower cell cultures. *Plant Cell Reports, 23*, 174–179.

Chimenti, C. A., Hall, A. J., & Sol LoÂpez, M. (2001). Embryo-growth rate and duration in sunflower as affected by temperature. *Field Crops Research, 69*, 81–88.

Conroy, J. P., Seneweera, S., Basra, A. S., Rogers, G., & Nissen-Wooller, B. (1994). Influence of rising atmospheric CO_2 concentrations and temperature on growth, yield and grain quality of cereal crops. *Australian Journal of Plant Physiology, 21*, 741–758.

Demurin, Y., Skoric, D., & Karlovic, D. (1996). Genetic variability of tocopherol composition in sunflower seeds as a basis of breeding for improved oil quality. *Plant Breeding, 115*, 33–36.

Durak, I., Yalcin, S., Kacmaz, M., Cimen, M. Y., Buyukkocak, S., Avci, A., et al. (1999). High-temperature effects on antioxidant systems and toxic product formation in nutritional oils. *Journal of Toxicology and Environmental Health A, 57*, 585–589.

Falk, J., Krahnstöver, A., van der Kooij, T. A. W., Schlensog, M., & Krupinska, K. (2004). Tocopherol and tocotrienol accumulation during development of caryopses from barley (*Hordeum vulgare* L.). *Phytochemistry, 65*, 2977–2985.

FOSFA. (1982). *Federation of Oils, Seeds and Fats Associations International Manual* 283.

IUPAC. (1998). Standard methods for the analysis of oils, fats and derivatives, determination of tocopherol and tocotrienols in vegetable oils and fats by high-performance liquid chromatography. *Method 2.432. 60*, 887–892.

Jenner, C. (1994). Starch synthesis in the kernel of wheat under high temperature conditions. *Australian Journal of Plant Physiology, 21*, 791–806.

Kamal-Eldin, A., & Appelqvist, L. A. (1996). The chemistry and antioxidant properties of tocopherol and tocotrienols. *Lipids, 31*, 671–701.

Kochhar, S. P., & Rossell, J. B. (1987). Determination of oil in sunflower seeds. *Journal of the American Chemists' Society, 64*, 865–869.

Kornsteiner, M., Wagner, K. H., & Elmadfa, I. (2006). Tocopherols and total phenolics in 10 different nut types. *Food Chemistry, 98*, 381–387.

Kriese, U., Schumann, E., Weber, W. E., Beyer, M., Brühl, L., & Matthäus, B. (2004). Oil content, tocopherol composition and fatty acid patterns of the seeds of 51 *Cannabis sativa* L. genotypes. *Euphytica, 137*, 339–351.

Martínez-Force, E., Álvarez-Ortega, R., Cantisán, S., & Garcés, R. (1998). Fatty acid composition in developing high saturated sunflower (*Helianthus annuus*) seeds: maturation changes and temperature effect. *Journal of Agricultural and Food Chemistry, 46*, 3577–3582.

Morrison, W. R., Conventry, A. M., & Barnes, P. J. (1982). The distribution of acyl lipids and tocopherols in flour millstreams. *Journal of Agricultural and Food Chemistry, 33*, 925–933.

Munné-Bosch, S., & Alegre, L. (2002). The function of tocopherol and tocotrienols in plants. *Critical Reviews in Plant Sciences, 21*, 31–57.

Pleite, R., Pike, M. J., Garcés, R., Marténez-Force, E., & Rawsthorne, S. (2005). The sources of carbon and reducing power for fatty acid synthesis in the heterotrophic plastids of developing sunflower (*Helianthus annuus* L.) embryos. *Journal of Experimental Botany, 56*, 1297–1303.

Ploschuk, E., & Hall, A. (1995). Capitulum position in sunflower affects grain temperature and duration of grain filling. *Field Crops Research, 44*, 111–117.

Rondanini, D., Savin, R., & Hall, A. J. (2003). Dynamics of fruit growth and oil quality of sunflower (*Helianthus annuus* L.) exposed to brief intervals of high temperature during grain filling. *Field Crops Research, 83*, 79–90.

Savin, R., & Nicolas, M. (1996). Effects of short periods of drought and high temperature on grain growth and starch accumulation of two malting barley cultivars. *Australian Journal of Plant Physiology, 13*, 201–210.

Savin, R., & Nicolas, M. (1999). Effects of timing of heat stress and drought on growth and quality of barley grains. *Australian Journal of Plant Physiology, 50*, 357–364.

Savin, R., Stone, P., Nicolas, M., & Wardlaw, I. (1997). Grain growth and malting quality of barley. 1. Effects of heat stress and moderately high temperature. *Australian Journal of Plant Physiology, 48*, 615–624.

Sergi, B., Fetoni, A. R., Ferraresi, A., Troiani, D., Azzena, G. B., Paludetti, G., et al. (2004). The role of antioxidants in protection from ototoxic drugs. *Acta Oto-Laryngologica Supplement, 552*, 42–45.

Velasco, L., Fernández-Martínez, J. M., García-Ruíz, R., & Domínguez, J. (2002). Genetic and environmental variation for tocopherol content and composition in sunflower commercial hybrids. *Journal of Agricultural Science, 139*, 425–429.

Wardlaw, I., & Wrigley, C. (1994). Heat tolerance in temperate cereals: an overview. *Australian Journal of Plant Physiology, 21*, 695–703.

Wrigley, C., Blumenthal, C., Gras, P., & Barlow, E. (1994). Temperature variation during grain filling and changes in wheat–grain quality. *Australian Journal of Plant Physiology, 21*, 875–885.

Zimmerman, D. C., & Fick, G. N. (1973). Fatty acid composition of sunflower (*Helianthus annus* L.) oil as influenced by seed position. *Journal of the American Chemists' Society, 50*, 273–275.