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Increased Lycopene and Flavor Volatile Production in Tomato Calyces and Fruit Cultured in Vitro and the Effect of 2-(4-Chlorophenylthio)triethylamine

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Earlier it was shown that tomato (*Lycopersicon esculentum* cv. VFNT Cherry) calyces develop into fruit tissue when cultured at 16−22 °C, producing flavor compounds and changes in color and sugar content characteristic of ripening fruit. Here are reported unusually high [580 μg (g of fresh weight)$^{-1}$] lycopene concentrations in these fruit compared to reports on field-grown tomatoes (~10 times higher). Addition of 2-(4-chlorophenylthio)triethylamine (CPTA) (75 mg L^{-1}) to the culture medium produced further increases in lycopene in fruit and calyces. Some carotenoid-derived flavor volatiles also increased, as well as some not related to carotenoids. The greatest increase in lycopene, however, resulted not from addition of CPTA but from a mechanism triggered by cool temperatures, which was previously shown to involve tomato AGAMOUS (*TAG1*) gene activation and which seems correlated to ripening. Concentrations of these compounds in fruit and calyces grown in vitro at 26 °C with and without CPTA are also given.

Keywords: *Calyx; CPTA; in-vitro culture; flavor; fruit; lycopene; Lycopersicon esculentum; ripening; tomato*

INTRODUCTION

Calyces (sepals) of tomato fruit (*Lycopersicon esculentum* cv. VFNT Cherry) change their developmental program to that of fruit when grown in vitro at temperatures between 16 and 22 °C. They lose their green color, swell, and become red and succulent. Coinciding with the change in color, biochemical and physiological processes that characterize fruit ripening occur, such as increased tissue levels of 1-aminocyclopropane-1-carboxylic acid (ACC, the immediate precursor of ethylene) followed by a dramatic increase in ethylene production, expression of polygalacturonase, subsequent cell wall degradation as indicated by ultrastructural analyses, and production of many flavor volatiles characteristic of tomato fruit. These changes occur even when calyces are detached from the tomato flower or fruit and cultured separately (Ishida, 1991; Ishida et al., 1993).

In our laboratory we are interested in increasing the lycopene content of tomatoes. Lycopene has long been valued for its attractive and appealing red color in fresh tomatoes and tomato products. Recently, epidemiological studies (Giovannucci et al., 1995) showed a strong correlation between consumption of tomato products and reduction in risk of prostate cancer. Lycopene was also shown to be more effective against proliferation of human endometrial, mammary, and lung cancer cells than either α- or β-carotene (Levy et al., 1995). It is believed to protect organs from damage due to free radicals because of its antioxidant properties (Di Mascio et al., 1989).

2-(4-Chlorophenylthio)triethylamine (CPTA) increases lycopene production and chromoplast differentiation in a variety of higher plants (Chang et al., 1977; Coggins et al., 1970; Hayman et al., 1977; Rabinowitch and Rudich, 1972; Simpson et al., 1974b, 1977; Yokoyama et al., 1972), in cultured plant cells (Radin, 1986; Robertson et al., 1995), and in fungi (Elahi et al., 1973; Hsu et al., 1972; Murillo, 1980). CPTA also inhibits β-carotene formation, presumably by blocking cyclization reactions leading to its formation (Elahi et al., 1973; Simpson et al., 1974b).

Studies on pigment evolution in ripening tomato fruit (Laval-Martin et al., 1975) show that pigment distribution and formation differ in the outer pericarp and inner locular pulp. Pigments that are characteristic of foliar tissue increase in both parts of the fruit and then remain constant, except for chlorophyll and neoxanthin, which decrease at the yellow-green stage of fruit development. Lycophyll and lycoxanthin increase gradually throughout development and maturation of the fruit. Lycopene and phytofluene, on the other hand, are detected only at the breaker stage of fruit ripening, primarily in the outer pericarp. Phytofluene, which is colorless, is detected only in the outer flesh, whereas lycopene appears in both pericarp and locular tissue. Lycopene increases from 0 nmol (g of dry weight)$^{-1}$ in yellow-green fruit to 2530 nmol (g of dry weight)$^{-1}$ (82.9% of the total pigment) in deep red fruit (Laval-Martin et al., 1975). β-Carotene, which is the second most abundant pigment, reaches its peak concentration before the fruit is fully ripe (Meredith and Purcell, 1966) and is more concentrated in locular tissue (Thompson et al., 1965).

Our experiments were aimed at examining the effects of CPTA on concentrations of the two major carotenoid pigments, lycopene and β-carotene in tomato calyces and fruit cultured in vitro. We were also interested in changes in volatile flavor compounds that are characteristic of tomato fruit, particularly those derived from carotenoids.

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Table 1. Lycopene and β-Carotene Concentrations[a] in VFNT Cherry Tomato Tissues Treated in Vitro with 75 mg L^{-1} CPTA

	calyx at 16 °C		calyx at 26 °C		fruit at 16 °C		fruit at 26 ° C		greenhouse		
compound	−CPTA	+CPTA	−CPTA[c]	+CPTA	−CPTA	+CPTA	−CPTA	+CPTA	green calyx	green fruit	red fruit
lycopene	330	600	1.5	5.9	580	660	340	640	27	1.1	200
SD[c]	82	93	0.14	1.6	70	49	51	72	3.5	0.19	2.5
β-carotene	31	nd[d]	18	nd	29	nd	25	nd	15	3.9	
SD	1.7		1.0		2.2				1.6	0.11	

[a] Concentrations are in micrograms per gram of fresh weight. One to three samples, minimum of three replicate analyses/sample. [b] Green calyx cultured in vitro for 3 days. [c] SD, standard deviation. [d] nd, not detected.

MATERIALS AND METHODS

Ovulary and Calyx Cultures. Ovularies and calyces of greenhouse-grown tomato plants *(Lycopersicon esculentum* cv. VFNT Cherry) were cultured in vitro (Nitsch, 1951), starting with postanthesis flowers or small, green fruit (<5 mm). Plant material was wrapped in several layers of cheesecloth, disinfested by immersing for 10 min in a saturated calcium hypochlorite solution containing Tween 20 (polyoxyethylene-sorbitan monolaurate, Sigma Chemical Co. St. Louis, MO) [2 drops $(100\ mL)^{-1}$], and then rinsed three times in autoclaved, deionized water. Ovulary cultures (for fruit samples) were prepared by removing corolla, stamen, and most of the pedicel from the flower and inserting the pedicel either into a hole made in the center of a filter paper platform (Heller, 1949) that was placed on the surface of a medium containing Murashige and Skoog (1962) salts, sucrose ($60\ g\ L^{-1}$), myo-inositol ($100\ mg\ L^{-1}$), and White's (1963) vitamins and glycine, at pH 5.7, or into the surface of the same medium solidified by the addition of 2% Phytagel (Sigma Chemical Co.). Calyces from flowers or small, green tomato fruit (<5 mm) were cultured separately and placed on solidified medium. Cultures were kept in a growth room at either 16−17 or 24−26 °C and illuminated by Grolux lamps (Sylvania, Inc., Danvers, MA) at an average photosynthetically active radiation of 27.7 μmol $m^{-2}\ s^{-1}$ (range = 21−31 μmol $m^{-2}\ s^{-1}$) for 16 h day^{-1}.

CPTA was synthesized according to the methods of Schuetz and Baldwin (1958). Aqueous stock solutions were prepared, filter-sterilized, and added to autoclaved culture medium, when indicated, to give a final concentration of $75\ mg\ L^{-1}$.

Isolation and Analysis of Volatile Flavor Compounds. Freshly harvested tomato tissues (~5 g) were homogenized as described previously (Ishida et al., 1993). Volatile components in homogenates were then isolated as described by Buttery et al. (1987). These compounds were analyzed by gas−liquid chromatography and their identities confirmed by capillary gas−liquid chromatography/mass spectrometry analysis. Normally three replicates were analyzed, and standard deviations were ±10%. Three internal standards [3-pentanone, 2-octanone, and anethole (4-propenylmethoxybenzene)], as well as the phenols, methyl salicylate and eugenol, were used (Buttery et al., 1988).

Lycopene and β-Carotene Analyses. Tomato tissues were harvested from culture tubes, quickly frozen in liquid N_2, and stored at −20 °C (−80 °C, if stored for >1 month). For each sample, tissues (12−15 g) were pooled, weighed, homogenized using an Omni-Mixer (Sorvall/DuPont Medical Products, Newtown, CT), and divided into three replicates. Each replicate was washed, using 25 mL of ice-cold absolute ethanol, and the mixture was homogenized again for 30 s. This suspension was vacuum-filtered through Whatman No. 1 filter paper (Whatman International, Ltd., Maidstone, Kent, U.K.) and the filtrate discarded. Particulate matter on the filter paper was extracted with 25 mL of CH_2Cl_2 (Omni-Solv, EM Science, Gibbstown, NJ) by blending for 30 s and filtering again. The filtrate was then evaporated in vacuo to ~5 mL and diluted to 10 mL with CH_2Cl_2. Five-microliter samples were analyzed by HPLC (Waters Corp., Milford, MA), using a 5-μm Microsorb C18 column (4.6 × 250 mm with a 50-mm guard column) (Rainin Instruments, Woburn, MA), a mobile phase of CH_3CN/CH_2Cl_2/hexane (16:4:1 by vol) at 2 mL min^{-1}, and detection at 450 nm. Under these conditions, lycopene and β-carotene retention times were 4.2 and 7.8 min, respectively. Lycopene and β-carotene concentrations were determined by comparison to standard curves obtained using lycopene purified from tomato (90−95% pure) and β-carotene from carrots (minimum purity = 95%) (Sigma Chemical Co.). Standard curves were corrected for impurities by examining absorption spectra and comparing values with extinction coefficients (Davies, 1965).

RESULTS AND DISCUSSION

Pigment Formation. Lycopene contents of standard, field-grown tomato fruit range from 43.6 to 59.6 μg (g of fresh weight)$^{-1}$ (Thompson et al., 1965; Tomes, 1963). Higher values have been reported: 75.0 and 112.5 $\mu g\ g^{-1}$ in variety Crimson and in a High Pigment strain, Illinois 1252, respectively (Thompson et al., 1965), 82.8 in Homestead (Meredith and Purcell, 1966), and 181.2 $\mu g\ g^{-1}$ in a cross of Fireball × Cornell (Davies and Hobson, 1981). In contrast, the lycopene concentration shown in Table 1 in fruit cultured in vitro at 16 °C is 580 μg (g of fresh weight)$^{-1}$. This concentration is 3.2 times that of the highest concentration reported and ~10 times that of standard tomato fruit. Lycopene concentrations in fruit cultured in vitro at 26 °C and calyces at 16 °C were also very high (~6 times higher than that of standard, field-grown fruit). Addition of $75\ mg\ L^{-1}$ CPTA to the medium resulted in further increases in lycopene: 1.14-fold in fruit and 1.82-fold in calyces at 16 °C. At 26 °C, CPTA induced a 1.88-fold increase in lycopene in fruit. Our analyses of VFNT Cherry tomato fruit grown in the greenhouse showed that this cultivar has unusually high lycopene concentrations [mean value = 200 μg of lycopene (g of fresh weight)$^{-1}$], which increased 2.9-fold when cultured in vitro at 16 °C. β-Carotene contents are very similar in both calyx and fruit tissues grown at 16−17 °C.

Calyces cultured at the lower temperature developed into fruit tissue; at the higher temperature, a change in developmental program does not occur. Therefore, no ripening occurred, and lycopene concentrations were extremely low at 26 °C, much lower than in green calyx from fruit picked from greenhouse-grown plants but slightly higher than those in green fruit picked from the same plants. Low lycopene concentrations in calyces cultured at the higher temperature show that, during in-vitro culture at 26 °C, lycopene already present in the calyx is degraded, as is chlorophyll, so that the tissue becomes pale yellow.

CPTA induced an increase in lycopene synthesis [3.9-fold to a concentration of 5.9 μg (g of fresh weight)$^{-1}$] in calyces cultured at 26 °C, even though the tissue had not changed its developmental program to that of fruit. This phenomenon is similar to that observed in tomato cell suspension cultures (Fosket and Radin, 1983; Radin, 1986; Robertson et al., 1995), which did not differentiate into fruit cells but could be induced to produce lycopene by CPTA. Lycopene production was presumed to result

Table 2. Carotenoid-Derived Volatile Flavor Compounds[a] in VFNT Cherry Tomato Tissues Treated in Vitro with 75 mg L^{-1} CPTA

	calyx at 16 °C		calyx at 26 °C		fruit at 16 °C		fruit at 26 °C	leaves
compound	−CPTA	+CPTA	−CPTA[b]	+CPTA	−CPTA	+CPTA	+CPTA	−CPTA
6-methyl-5-hepten-2-one	540	1000−1600	<5	99	210	294	134	<1
6-methyl-5-hepten-2-ol	41	15	<5	0	8	0	19	<1
β-cyclocitral	<1	26	nd	24	5	16	<1	35
β-damascenone	<1	0	<5	0	<5	14	36	<1
geranylacetone	nd	18−590	nd	11	330	74	0	<1
β-ionone	<1	0	<1	38	18	0	0	<1
pseudoionone	nd	57	nd	61	6	43	51	<1

[a] Concentrations are in micrograms per liter. [b] Green calyx cultured in vitro for 3 days.

from enhanced synthesis of proteins required for carotenoid synthesis and chromoplast differentiation.

β-Carotene concentrations in calyx and fruit tissue cultured in vitro at 16 °C are similar (31 and 29 $\mu g\ g^{-1}$, respectively); they are both higher than those in green calyces grown in the greenhouse and in vitro at the higher temperature (15 and 18 $\mu g\ g^{-1}$, respectively). All of these values are higher than β-carotene concentrations in green tomato fruit from greenhouse-grown plants [3.9 μg (g of fresh weight)$^{-1}$]. Inhibition of β-carotene accumulation by CPTA has been observed and has been attributed to blocking of lycopene cyclization to β-carotene (Sandemann and Boger, 1989; Simpson et al., 1974b). The absence of β-carotene in tissues treated with CPTA in our experiments (Table 1) is consistent with inhibition of β-carotene synthesis.

Seyama and Splittstoesser (1975), however, showed that CPTA induces formation of carotenoids, particularly lycopene, that are not normally accumulated in pumpkin cotyledons and suggested that CPTA also stimulates enzymes involved in lycopene synthesis, in addition to inhibiting cyclases involved in α- and β-carotene syntheses. Addition of cycloheximide along with CPTA reduced the level of pigment synthesis compared to CPTA alone. In tomato cell suspension cultures (Fosket and Radin, 1983), cycloheximide, at concentrations that inhibit protein synthesis, prevented induction of lycopene synthesis. De novo protein was inhibited by 80%. This seemed to support the hypothesis that stimulation of lycopene formation is due in part to synthesis of new enzymes in the lycopene pathway. These results are also consistent with findings that cycloheximide blocks the CPTA-induced increase in carotenoids in fungi (Hsu et al., 1972; Murillo, 1980). In *Capsicum annum* fruit, CPTA caused an increase in phytoene synthetase, which was inhibited by cycloheximide but not by chloramphenicol or lincomycin (Camara, 1984). These studies also indicate that CPTA stimulates synthesis of enzymes involved in carotenoid formation.

Our results (Table 1) show a far greater increase in lycopene resulting from culture in vitro at the lower temperature than with CPTA addition. Although the further increase in lycopene with added CPTA is greater than the decrease in β-carotene, it is difficult to exclude this increase as resulting from the inhibition of other processes.

Volatile Flavor Compounds. Concentrations of volatile compounds were measured in both VFNT Cherry calyx and fruit tissues cultured in vitro at the two temperatures indicated, and the effect of CPTA was observed. Results are given in Tables 2−6, along with values obtained from green leaves of greenhouse-grown plants.

Carotenoid-Related Flavor Compounds. A number of volatile flavor compounds of tomato are related to carotenoids (Buttery et al., 1988, 1990; Buttery and Ling, 1993; Marlatt et al., 1992; Stevens, 1970). Concentrations of carotenoid-derived volatile flavor compounds found in VFNT Cherry tomato calyx and fruit tissues cultured in vitro at 16 and 26 °C with and without the addition of 75 mg L^{-1} CPTA are given in Table 2, along with concentrations in leaf tissue. 6-Methyl-5-hepten-2-one and 6-methyl-5-hepten-2-ol are lycopene degradation products (Buttery et al., 1988; Stevens, 1970) and were shown to increase in calyces ripening in vitro (Ishida et al., 1993). Both compounds were present in green calyx at levels <5 $\mu g\ L^{-1}$ and increased to concentrations shown in Table 2. In both calyx and fruit tissues cultured at the lower temperature, CPTA induced an increase in 6-methyl-5-hepten-2-one and a decrease in 6-methyl-5-hepten-2-ol. (The concentration of 6-methyl-5-hepten-2-one in the analysis of calyx cultured at 26 °C in the presence of CPTA was hard to measure accurately because of interfering peaks, so the actual value may be considerably lower than 99 ppb.) Buttery et al. (1988) did not find significantly higher concentrations of these two compounds in the high-lycopene tomato line (V80007) compared to a common FM785 line. β-Cyclocitral, probably resulting from oxidation of β-carotene and considered a moderately potent odorant (Buttery et al., 1989), and pseudoionone concentrations, which are barely detectable in ripe calyx cultured in vitro, increased in response to CPTA in both calyx and fruit tissue at both temperatures, except for β-cyclocitral in fruit cultured at 26 °C. β-Cyclocitral increased in calyx cultured at the higher temperature in the presence of CPTA, as did β-ionone, probably resulting from oxidative degradation of β-carotene (Buttery et al., 1989) (see also absence of β-carotene in Table 1). In tomato fruit tissue, β-damescenone, a major contributor to fresh tomato aroma (Buttery et al., 1989), increased and β-ionone decreased in response to CPTA at both temperatures. No changes in β-damascenone and β-ionone concentrations were observed in ripening calyx tissue (16 °C). Concentration changes of carotenoid-derived flavor volatiles in calyx tissues cultured at the higher temperature were in the same direction as those grown at the lower culture temperature but generally of smaller magnitude. Again, one should keep in mind that calyx tissue cultured at the higher temperature does not undergo developmental changes resulting from the conversion of calyx to fruit tissue. The amounts of all of the compounds listed in Table 2 were very low (<1 mg L^{-1}) in leaf tissue, except for β-cyclocitral.

Terpenoids. Terpenoids do not generally occur at high concentrations in tomato fruit (Buttery et al., 1988,

Table 3. Volatile Terpenoids[a] in VFNT Cherry Tomato Tissues Treated in Vitro with 75 mg L^{-1} CPTA

	calyx at 16 °C		calyx at 26 °C		fruit at 16 °C		fruit at 26 °C	leaves
compound	−CPTA	+CPTA	−CPTA[b]	+CPTA	−CPTA	+CPTA	+CPTA	−CPTA
linalool + 2-phenylethanol	nd, <10	13	nd, <10	23	2	20	70	<10
neral	nd	36−62	nd	24	2	0	35	<5
geranial	nd, <5	93−510	<5	27	12	25	18	<5

[a] Concentrations of volatile compounds are in micrograms per liter. [b] Green calyx cultured in vitro for 3 days.

Table 4. Amino Acid-Related Volatile Flavor Compounds[a] in VFNT Cherry Tomato Tissues Treated in Vitro with 75 mg L^{-1} CPTA

	calyx at 16 °C		calyx at 26 °C		fruit at 16 °C		fruit at 26 °C	leaves
compound	−CPTA	+CPTA	−CPTA[b]	+CPTA	−CPTA	+CPTA	+CPTA	−CPTA
3-methylbutanal	<5	65	<5	33	27−65	95	56	<5
isobutyl cyanide	<1	5	<5	15	13−42	16	13	<1
3-methylbutanol	140	117	<5	14	150−380	115	201	<1
(*E*)-2-pentenal + 2-methylbutanol	<5	286	<5	0	100	342	123	<1
1-nitro-3-methylbutane	<1	24	<5	0	59−300	26	11	<1
phenylacetaldehyde	<10	153	<5	71	15−18	22	36	<1
2-isobutylthiazole	<10	154	<5	0	36−110	40	36	<1
phenylacetonitrile	<5	0	<5	0	3−8	0	10	<1
1-nitro-2-phenylethane	<5	0	<5	0	17−54	0	10	<1

[a] Concentrations of volatile compounds are in micrograms per liter. [b] Green calyx cultured in vitro for 3 days.

1989; Ishida et al., 1993). They are found, however, in leaves (Lundgren et al., 1985). Changes in terpenoid concentrations in response to CPTA are given in Table 3. Large increases in geranial were found in calyx tissues cultured at 16 °C in response to CPTA. Geranial also increases in fruit tissues but to a smaller extent than in calyx. Buttery et al. (1988) reported a significantly higher concentration (21 ppb) of geranial, which can be considered a degradation product of lycopene, in the high-lycopene line V80007 compared to the common FM785 line (12 ppb), although, as mentioned earlier, no significant difference was found between these two lines in the concentrations of the lycopene degradation products 6-methyl-5-hepten-2-one and 6-methyl-5-hepten-2-ol. In addition, in our experiments, neral and linalool concentrations in cultured calyces responded to CPTA. In fruit, no difference in neral was seen at 16 °C, but its concentration did increase at 26 °C. A large increase in linalool concentration is indicated at both temperatures. Stevens (1970) stated that the relationship between polyene-carotene and volatile compound contents suggests that oxidation of carotenoids results in the compounds 6-methyl-5-hepten-2-one, geranial, neral, geranylacetone, α-ionone, *β*-ionone, farnesal, and farnesyl acetone. Therefore, it is reasonable to assume that changes in pigment will affect tomato fruit flavor.

Amino Acid-Related Volatile Compounds. Also found in tomato fruit tissue, but not in leaves, are compounds derived from amino acids (Petro-Turza, 1986; Lundgren et al., 1985). 3-Methylbutanal, derived from leucine (Yu et al., 1968) and a major contributor to fresh tomato odor (Buttery et al., 1989), was barely detectable in tomato calyx ripened in vitro (Table 4). However, with the addition of CPTA, this aldehyde increased to levels comparable to that found in fruit grown in vitro, which was approximately twice as high as values reported in fresh field-grown tomatoes (Buttery et al., 1988, 1989). This compound also increased in fruit grown in vitro in response to CPTA, whereas 3-methylbutanol concentrations decreased. Dalal et al. (1965) reported that, in field-grown tomato fruit, the alcohol increases during ripening but the aldehyde, which contributes more to tomato odor, increases up to the breaker stage and then gradually decreases. CPTA also induced a significant increase in phenylacetaldehyde and 2-isobutylthiazole (another major contributor to tomato odor) in ripening calyx tissue, but little or no change in fruit tissue. Although not major contributors to fresh tomato odor, (*E*)-2-pentenal increased in both ripening tissues and 1-nitro-2-phenylethane decreased in fruit tissue, in response to CPTA. All of the compounds in Table 4, except for phenylacetonitrile, (*E*)-2-pentenal, and 1-nitro-3-methylbutane, are considered major volatile components of fresh tomatoes and contribute to fresh tomato odor (Buttery et al., 1989).

Lipid-Derived Volatiles. The compounds listed in Table 5 are C5, C6, and C7 lipid-derived, volatile flavor compounds. These compounds are present in all plant parts but at highest concentrations in leaves. Concentrations in calyces cultured at 26 °C showed no change from those in green calyces [see Ishida et al. (1993)]; however, this was expected because the calyx does not change its developmental program at this temperature. Interestingly, however, with CPTA addition, although calyces did not show any apparent physiological changes, most of the compounds in Table 5 decreased; only (*E*)-2-hexenal, hexanol, and (*E*)-2-heptenal increased. At 16 °C, the addition of CPTA also resulted in similar changes except that (*Z*)-3-hexenol increased. Extremely high concentrations of (*E*)-2-hexenal in calyx tissues cultured at 16 °C probably result from damage occurring before analysis during the harvesting of the soft, ripe tissue.

In fruit tissue cultured at 16 °C, on the other hand, CPTA addition resulted in small increases in concentrations of pentanol and (*E*)-2-heptenal, greater increases of 1-penten-3-ol, (*E*)-2-hexenal, (*Z*)-3-hexanol, and hexanol, and decreases in 1-penten-3-one, (*Z*)-3-hexenal, and hexanal. At 26 °C, CPTA caused larger decreases in concentrations of 1-penten-3-one, (*Z*)-3-hexenal, and hexanal and a larger increase in pentanol than at 16 °C. Concentrations of 1-penten-3-ol, (*E*)-2-hexenal, (*Z*)-3-hexenol, and (*E*)-2-heptenal decreased at 26 °C instead of increasing as they did at 16 °C. Concentrations of these volatile compounds in ripe tomato fruit cultured in vitro at 26 °C in the presence of CPTA resemble more those found in green fruit than those in the ripe fruit (Buttery et al., 1987). They are significantly lower than those reported in the common FM785 tomato line by

Table 5. Lipid-Derived Volatile Flavor Compounds[a] in VFNT Cherry Tomato Tissues Treated in Vitro with 75 mg L^{-1} CPTA

	calyx at 16 °C		calyx at 26 °C		fruit at 16 °C		fruit at 26 °C	leaves
compound	−CPTA	+CPTA	−CPTA[b]	+CPTA	−CPTA	+CPTA	+CPTA	−CPTA
1-penten-3-one	1500	498	560	101	450	312	24	2000
1-penten-3-ol	1100	51	370	250	100	414	45	2400
pentanol	130	85	150	17	30	41	93	430
(*Z*)-3-hexenal	15000	5820	43000	1233	15000	7925	45	220000
hexanal	7200	4184	1700	310	2000	1822	100	3900
(*E*)-2-hexenal	22000	12784	1100	1244	470	1399	39	17000
(*Z*)-3-hexenol	160	298	23000	258	120	229	95	10000
hexanol	<5	750	97	235	4	88	7	500
(*E*)-2-heptenal	<5	62	nd	6	40	43	13	<5

[a] Concentrations are in micrograms per liter. [b] Green calyx cultured in vitro for 3 days.

Table 6. Lignin-Related Volatile Flavor Compounds[a] in VFNT Cherry Tomato Tissues Treated in Vitro with 75 mg L^{-1} CPTA

	calyx at 16 °C		calyx at 26 °C		fruit at 16 °C		fruit at 26 °C	leaves
compound	−CPTA	+CPTA	−CPTA[b]	+CPTA	−CPTA	+CPTA	+CPTA	−CPTA
benzaldehyde	45	22	110	33	31	23	19	30
methyl salicylate	64	79	430	48	48	142	98	100−600

[a] Concentrations are in micrograms per liter. [b] Green calyx cultured in vitro for 3 days.

Buttery et al. (1988), except for pentanol and hexanol, which were similar.

Lignin-Related Volatile Compounds. In our earlier study (Ishida et al., 1993), we found that benzaldehyde concentrations in tomato calyx cultured in vitro decreased with ripening to levels more like that in ripe fruit. CPTA addition to the medium resulted in a further drop in benzaldehyde concentrations in both calyx and fruit tissues at both temperatures (Table 6). Methyl salicylate concentration, which also decreased with ripening of calyx tissue, increased somewhat with the addition of CPTA in calyx cultured at 16 °C and fruit cultured at both temperatures but decreased in nonripening calyx cultured at 26 °C. Concentrations of methyl salicylate in all ripe tomato tissues measured in the present study were within the concentration range found in other tomato fruit (10−230 ppb) (Buttery et al., 1988).

The results of the experiments described in this paper were unexpected. We began by investigating the mechanism by which CPTA enhances lycopene production. Its ability to block β-carotene formation is believed to result from inhibition of cyclization reactions leading to its formation (Elahi et al., 1973; Simpson et al., 1974b). Hsu et al. (1972) suggested that CPTA's action on carotenoid biosynthesis might be attributed to inhibition of cyclase(s) and derepression of a gene regulating the synthesis of specific enzyme(s) in the lycopene pathway. This hypothesis is supported by the results of Fosket and Radin (1983). The absence of β-carotene formation in our experiments supports the findings that cyclization reaction(s) may be blocked but the magnitude of increase in lycopene production in both in-vitro-cultured tomato calyces and fruit is much greater than can be accounted for by the decrease in β-carotene formation. In addition to the increase in lycopene content, concentrations of several carotenoid-derived, volatile flavor compounds increased significantly, namely, 6-methyl-5-hepten-2-one, β-cyclocitral, geranylacetone, and pseudoionone. Concentrations of other volatile flavor compounds also increased, but no evidence for the mechanism of this stimulation is available at this time. With regard to the mechanism of action of CPTA on lycopene accumulation, we cannot exclude inhibition of other processes that were not measured that might lead to increased lycopene accumulation.

The major finding of interest that resulted from these experiments is the high lycopene content of our tomato fruit cultured in vitro. This change is brought about by cold induction, as shown by comparing greenhouse-grown fruit and fruit cultured in vitro at the two temperatures. In an earlier publication (Ishida et al., 1998), we proposed that the induction of elevated *TAG1* gene expression plays a key role in developmental changes that result in ripening and that *TAG1* expression is correlated with ripening—the higher the *TAG1* expression, the riper the tissue, that is, the more lycopene produced. We also propose that cold induction of *TAG1* expression leads to elevated lycopene production by both calyces and fruit cultured in vitro and may, in part, explain the increase in lycopene production by tomato fruit cultured in vitro at 16 °C compared with greenhouse-grown fruit. CPTA addition to the medium increases lycopene levels even further. However, the larger increases in lycopene production [from 27 to 330 in calyces, from 1.1 to 580 in fruit cultured at 16 °C, and from 1.1 to 340 μg of lycopene (g of fresh weight)$^{-1}$ at 26 °C, respectively], as well as production of volatile flavor constituents, can be attributed to fruit ripening. CPTA has a much smaller effect. Studies are currently in progress to investigate mechanisms involved in these cool-temperature-activated processes.

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