Changes of Fatty Acids and Fatty Acid-Derived Flavor Compounds by Expressing the Yeast Δ-9 Desaturase Gene in Tomato

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Expression of the yeast Δ-9 desaturase gene in tomato (*Lycopersicon esculentum* Mill.) led to changes in the levels of unsaturated as well as saturated fatty acids in tomato fruits. Increases in palmitoleic acid, 9,12-hexadienoic acid, and linoleic acid were observed. At the same time there was a reduction in palmitic acid and stearic acid. Changes in the profile of fatty acids were associated with changes in certain flavor compounds derived from fatty acids, most notably *cis*-3-hexenol, 1-hexanol, hexanal, and *cis*-3-hexenal. Certain flavor compounds not derived from fatty acids, viz. 6-methyl-5-hepten-2-one and 2-isobutylthiazole, also showed increases in transgenic fruits. These results show that changes in the levels of fatty acids in a plant could lead to changes in its profile of flavor compounds.

Keywords: *Tomato; fatty acids; lipids; flavor compounds; transgenic plant*

INTRODUCTION

The tomato is one of the most popular vegetable crops in the world. Its popularity is mainly derived from its unique flavor and aroma, which consist of a mixture of volatile and semivolatile compounds. Fresh tomato flavor has been extensively studied. So far, over 400 such compounds have been identified in tomato, and some of them, such as *cis*-3-hexenal, *trans*-2-hexenal, 1-hexanal, *cis*-3-hexan-1-ol, hexanol, 2-isobutylthiazole, 6-methyl-5-hepten-2-one, are considered as important flavor-contributing agents (Kazeniac and Hall, 1970; Petro-Turza, 1987; Buttery *et al.*, 1987, 1988). Many of these flavor compounds are derived from peroxidation of unsaturated fatty acids during fruit ripening through the lipoxygenase/lyase enzyme pathway (Stone *et al.*, 1975; Galliard *et al.*, 1977; Vick and Zimmerman, 1986). Thus, the availability of appropriate unsaturated fatty acids is an important factor affecting fruit flavor and aroma development.

Plant fatty acid unsaturation starts with the conversion of 16:0 acyl carrier protein (ACP) and 18:0-ACP into 16:1-ACP and 18:1-ACP, respectively, by a soluble plastid Δ-9 desaturase (McKeon and Stumpf, 1982; Shanklin and Sommerville, 1991). Further unsaturation of 18:1-ACP takes place in the chloroplast inner membrane or endoplasmic reticulum (Browse and Sommerville, 1991) and is accomplished by two kinds of desaturases: the Δ-12 desaturase adding the second double bond at the Δ-12 position of 18:1 from the methyl end to form 18:2 and the Δ-15 desaturase adding the third double bond at Δ-15 position to form 18:3. Because the plant Δ-9 desaturase favors 18:0-ACP but not 16:0-ACP as substrate, only a small amount of 16:1 is present in so-called 18:3 higher plants like tomato. In yeast, the microsomal Δ-9 desaturase catalyzes the formation of the first double bond in the Δ-9 positions of both 16:0- and 18:0-CoA (coenzyme A) to make 16:1- and 18:1-CoA (Baker and Lynen, 1971). The unsaturated fatty acids along with saturated fatty acids are incorporated into phospholipids, triacylglyceride, and glycolipids.

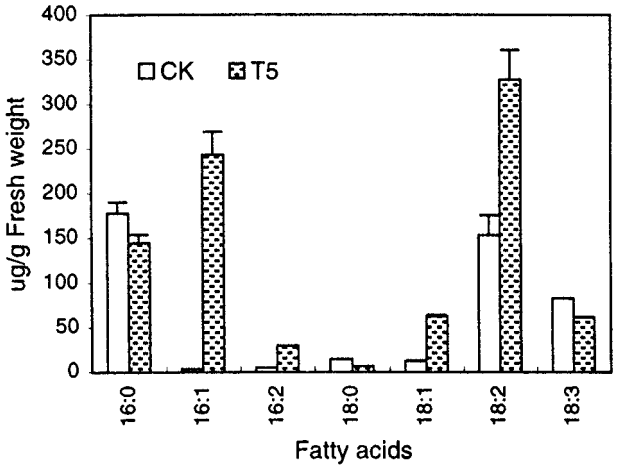

Figure 1. Fatty acids of control (CK) and transgenic (T5) fruits.

Some of the important fruit flavor compounds are derived from unsaturated fatty acids during fruit ripening. Usually the formation of the fatty acid-derived flavors follows the release of free fatty acids from lipids by lipases and then peroxidation of specific double bonds of the unsaturated fatty acids by lipoxygenase to produce hydroperoxides. The cleavage of 13-hydroperoxide from linoleic and linolenic acids will produce hexanal and *cis*-3-hexenal, respectively. These aldehydes can be reduced by alcohol dehydrogenase to form hexanol and *cis*-3-hexenol (Vick and Zimmerman, 1986).

The yeast Δ-9 desaturase has previously been shown to function in tobacco, leading to increases in the level of monounsaturated fatty acids (16:1 and 18:1) (Polashock *et al.*, 1992). In this study, we found that the overexpression of the yeast Δ-9 desaturase gene increased not only monoenoic fatty acids but also polyunsaturated fatty acids in tomato fruits. Since these fatty acids are precursors of certain important flavor

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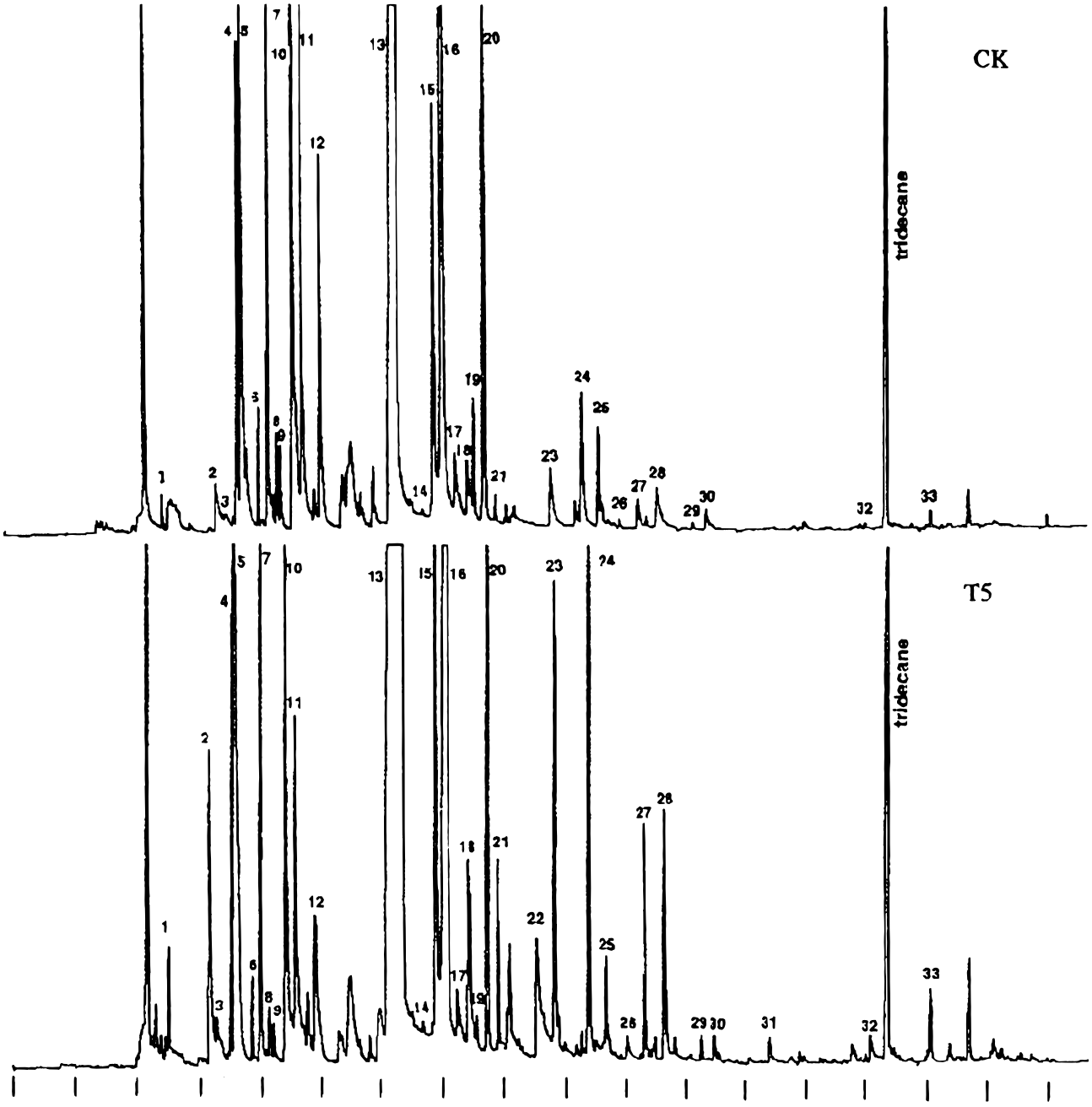

Figure 2. GC profile of volatile compounds from control (CK) and transgenic (T5) fruits.

compounds, we decided to determine the connection between fatty acids unsaturation and flavor profile in both control and transgenic tomato fruits.

MATERIALS AND METHODS

Production of Transgenic Plants. Open-pollinated tomato line PSR55809 from the New Jersey Agricultural Experimental Station was used in this study. The transformation procedure described by McCormick *et al.* (1986) was employed to introduce Δ-9 desaturase gene into tomato. Briefly, *Agrobacterium tumefaciens* LBA 4404 containing the yeast Δ-9 desaturase and NPTII gene sequence driven by CaMV 35S promoters (Polashock *et al.*, 1992) was used to infect tomato cotylendon segments. Kanamycin-resistant plantlets were regenerated from the infected tissues on MS medium (Murashige and Skoog, 1962) containing 30 g/L sucrose, 0.05 mg/L naphthaleneacetic acid, 2.5 mg/L 6-benzylaminopurine, 50 mg/L kanamycin sulfate, and 300 mg/L augmentin (Beecham Chemicals). Transgenic plantlets were multiplied *in vitro* and transplanted to growing mix (vermiculite/peat, 1:1) and grown in a greenhouse. Routine greenhouse plant maintenance was implemented.

The kanamycin-resistant plants were subject to Southern blotting (Southern, 1975) and fatty acid analysis to confirm the status of genetic transformation of these regenerants. Plants regenerated from uninoculated cotyledons were also grown in the greenhouse as control plants.

Fatty Acid Analysis. Fatty acid analysis was carried out with fruits at the "red" stage with a protocol according to Browse *et al.* (1986). The gas chromatograph HP5890A was equipped with flame ionization detector and Shimadzu CR501 electronic integrator. Capillary column (Supelcowax TM 10, 30 m × 1.5 mm, 1 μm thick film) was used. Injector temperature was 240 °C, and detector temperature was 250 °C. A column temperature program was set initially at 170 °C and ramped at 3 °C/min to 200 °C 3 min after injection. The fatty acid methyl ester (FAME) peaks were identified with the authentic standards of FAME from Sigma Chemical.

Flavor Analysis. Freshly picked fruits at the red stage were cooled to 4 °C in refrigerator. Fruits were sliced and blended for 2 min in a prechilled blender. One hundred grams

of tomato slurry spiked with 10 μL of 1000 ppm tridecane as an internal standard was poured into a Wheaton purge and trap apparatus. Volatile compounds were trapped onto a Tenax−Carbotrap tube with a nitrogen gas flow at 40 mL/min at 50 °C for 30 min. The trapped tube was dried at room temperature with nitrogen at a flow rate of 40 mL/min for 1 h. The trap was thermally desorbed at 250 °C and injected into the GC with the short-path thermal desorption system.

Flavor analysis was carried out according to the method of Karmas *et al.* (1993). A Varian 3400 GC equipped with a DB-1 capillary column (60 m × 0.32 mm, 1 μm film thickness, J&W Scientific Co.) and FID detector was used. A short-path thermal desorption system TD-1 (Scientific Instrument Services, Inc.) was connected to the GC for desorbing trapped compounds into the GC column. The injector temperature was set at 270 °C and the detector temperature at 300 °C. A split ratio of 10:1 was used. Column temperature was programmed linearly from −20 to 40 °C at 3 °C/min and from 40 to 280 °C at 2 °C/min. GC−MS was carried out under the same GC conditions, except that the GC column was directly inserted into the ion source of the mass spectrometer through a heated (280 °C) transfer line. A Finnigan MAT 8230 high-resolution double-focusing magnetic sector instrument and a Finnigan MAT SS300 data system were used. The mass spectra were searched against the National Institute of Standards and Technology mass spectral reference collection. Some of the peaks were also identified with authentic standards.

RESULTS AND DISCUSSION

Fatty acids of fruits from three control and three individually transformed plants were analyzed. Fruits from the three control plants produced similar results with no significant difference among the three plants. In contrast, fruits from transgenic plants showed elevated levels of certain unsaturated fatty acids but the levels differed among the individual plants. These variations are not unexpected as differences in the degree of expression of foreign genes in transgenic plants as these are well-known (Polashock *et al.*, 1992). In this study fruits from one of the transgenic plants, transformed plant 5 (T5), were used.

Expression of the yeast Δ-9 desaturase led to changes in fatty acids in tomato fruits; increases in most of the unsaturated fatty acids and decreases in the saturated fatty acids indicate that the yeast Δ-9 desaturase functions efficiently in tomato plants, supplementing the native Δ-9 desaturase to convert 16:0-ACP to 16:1-ACP and 18:0-ACP to 18:1-ACP. The most dramatic increases were found with the palmitoleic acid (16:1), 9,12-hexadienioc acid (16:2), oleic acid (18:1), and linoleic acid (18:2) (as shown in Figure 1). The increase in unsaturated fatty acids exceeded the decline in saturated fatty acids leading to an overall increase of approximately 1-fold in total fatty acids.

The GC profiles of volatile compounds from control and transgenic fruits are shown in Figure 2. The major peaks have been identified, and the concentrations are shown in Table 1. The fatty acid precursors of some of these compounds (Frankel, 1985; Gardner, 1989) are also indicated. The linolenic acid peroxidation products, mostly *cis*-3-hexenal, reflected by *cis*-2-hexenal and *trans*-2-hexenal, which are most likely the artifacts of GC thermal conversion of *cis*-3-hexenal (Karmas *et al.*, 1993), and *cis*-3-hexen-1-ol, derived from *cis*-3-hexenal by alcohol dehydrogenase (ADH) were increased in transgenic fruits. The steady-state level of linolenic acid in transgenic ripe fruits was slightly lower than that in the control fruits. This may be due to the higher linolenic acid peroxidation in transgenic fruits. This suggestion is supported by the observation that other minor autoxidation products such as *trans*-2,4-heptadienal (Chan, 1987) were also elevated in transgenic fruits.

Table 1. Volatile Compounds Identified and Their Concentrations (Parts per Billion) in the Control (CK) and Transgenic (T5) Tomato Fruits[a]

peak no.	compound	CK	T5	T5/CK (%)	fatty acid precursor
1	pentane	0.8	2.0	250	18:2
2	butanal	2.7	5.5	204	
3	2,2-dimethylbutane	1.4	0.8	57	
4	2-methylfuran	9.6	6.4	67	
5	2,3-dimethyl-1-butene	31.8	34.3	108	
6	methylcyclopentane	1.3	0.9	69	
7	3-methylbutanal	11.7	6.8	58	
8	thiophene	0.9	0.8	89	
9	2-methylbutanal	2.1	2.4	114	
10	2,3-dihydro-4-methylfuran	43.6	22.9	52	
11	pentanal	18.6	14.3	77	
12	2-ethylfuran	10.4	9.8	94	
13	hexanal	1604.6	4279.6	267	16:1, 18:2
14	4-penten-1-ol	0.5	0.4	80	
15	*cis*-2-hexenal	21.9	21.2	97	18:3
16	*trans*-2-hexenal	232.6	473.1	203	18:3
17	*cis*-3-hexen-1-ol	2.5	9.6	384	18:3
18	1-hexanol	2.7	11.6	407	16:1, 18:2
19	3-ethylpentane	1.9	1.7	89	
20	heptanal	12.1	26.7	221	16:1
21	*trans*-2,4-heptadienal	2.0	5.3	265	18:3
22	5-ethyl-2(5H)-furanone	0.0	5.3		
23	2,3,3-trimethyl-1-butene	2.9	10.5	362	
24	6-methyl-5-hepten-2-one	5.8	23.0	396	
25	2-pentylfuran	3.3	4.7	142	18:2
26	hexyl acetate	0.4	1.8	450	
27	2-isobutylthiazole	2.0	6.3	315	
28	*trans*-2-octenal	3.0	7.8	260	16:1, 18:2
29	3,3-dimethylhexanal	0.9	0.8	89	
30	nonanal	1.0	0.9	90	18:1
31	2-nonenal	0.1	0.8	800	16:1
32	*trans*-2,4-decadienal	0.4	1.5	375	
33	geranylacetone	1.3	1.5	115	

[a] Data represent the means of three replicates. The peak numbers are given in Figure 2.

Control plant fruits were found to possess a high level of hexanal. In transgenic fruits the hexanal level was increased further, over 2.5 times. Biochemically, hexanal is derived from 9-hydroperoxylinoleate produced by tomato lipoxygenase (Matthew *et al.*, 1977; Ferrie *et al.*, 1994), as shown in Figure 3A. Hexanal is then converted into 1-hexanol by ADH. Hexanal may also be converted from palmitoleic acid, which showed the most dramatic increase in transgenic fruits. Palmitoleic acid might also be autoxidized in plants following the same pattern of autoxidation of oleate (Frankel *et al.*, 1977), producing hexanal, 2-octenal, 2-nonenal, and heptanal (Figure 3B). This is supported by the observation that *trans*-2-octenal, 2-nonenal, and heptanal were all increased in transgenic fruits. However, since the amounts of these three compound were so much lower than that of hexanal, it is doubtful that autoxidation of palmitoleic acid was the main route for hexanal production. It is believed that the increases in hexanal, 1-hexanol, and autoxidation products including pentane, 2-pentylfuran, and *trans*-2,4-decadienal were due to the elevated level of their common precursor linoleic acid in transgenic fruits.

It was noted that two other flavor compounds, 6-methyl-5-hepten-2-one, a lycopene-derived fruit-like flavor element, and 2-isobutylthiazole, a leucine-derived tomato characteristic flavor compound, also increased in transgenic fruits. In addition, 5-ethyl-2(5*H*)-furanone, a volatile compound identified in tomato (Karmas *et al.*,

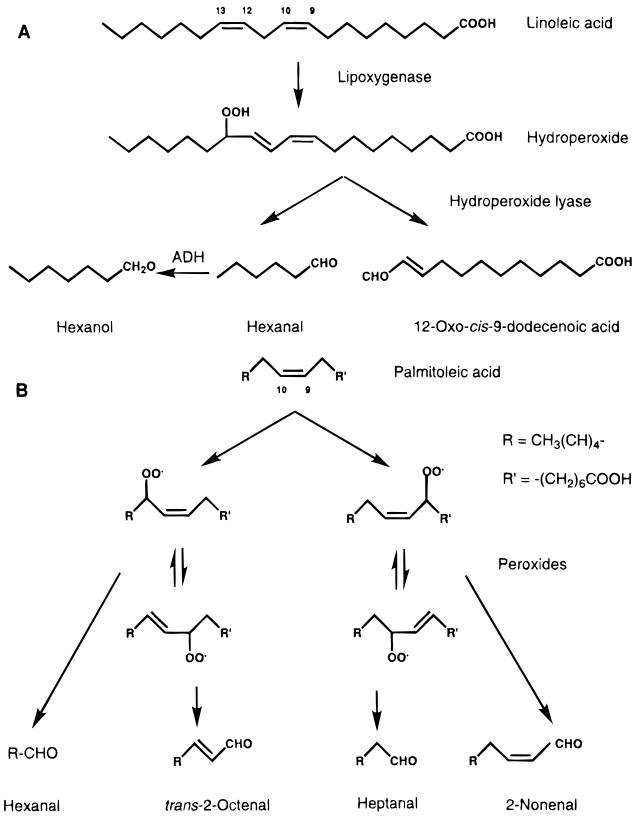

Figure 3. (A) Hexanal production from linoleic acid through lipoxygenase/hydroperoxide lyase pathway and (B) autoxidation of palmitoleic acid.

1993) was not found in the control fruits but was found in the transgenic fruits of tomato PSR55809. The reasons for these changes are not known.

This study shows that fatty acids of tomato fruits can be altered by the expression of the yeast Δ-9 desaturase gene. It also shows that changes in fatty acids would lead to changes in the flavor profile.

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