

Carotenoid Pigmentation Affects the Volatile Composition of Tomato and Watermelon Fruits, As Revealed by Comparative Genetic Analyses

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Tomato near-isogenic lines differing in fruit carotenogenesis genes accumulated different aroma volatiles, in a strikingly similar fashion as compared to watermelon cultivars differing in fruit color. The major volatile norisoprenoids present in lycopene-containing tomatoes and watermelons were noncyclic, such as geranial, neral, 6-methyl-5-hepten-2-one, 2,6-dimethylhept-5-1-al, 2,3-epoxygeranial, (*E,E*)-pseudoionone, geranyl acetone, and farnesyl acetone, seemingly derived from lycopene and other noncyclic tetraterpenoids. β -Ionone, dihydroactinodioidide, and β -cyclocitral were prominent in both tomato and watermelon fruits containing β -carotene. α -Ionone was detected only in an orange-fleshed tomato mutant that accumulates δ -carotene. A yellow flesh (*r*) mutant tomato bearing a nonfunctional *psy1* gene and the yellow-fleshed watermelon Early Moonbeam, almost devoid of carotenoid fruit pigments, also lacked norisoprenoid derivatives and geranial. This study provides evidence, based on comparative genetics, that carotenoid pigmentation patterns have profound effects on the norisoprene and monoterpene aroma volatile compositions of tomato and watermelon and that in these fruits geranial (*trans*-citral) is apparently derived from lycopene *in vivo*.

KEYWORDS: Tomato (*Lycopersicon esculentum* Mill.); watermelon (*Citrullus lanatus* [Thunb.] Matsum. and Nakai); aroma; norisoprenoids; carotenoids; lycopene; citral

INTRODUCTION

The aroma of fruits and vegetables is determined by unique combinations of volatile compounds. Although different fruits often share many aroma characteristics, each fruit has a distinctive aroma that is a function of the proportions of key volatiles and the presence or absence of unique components. The most important aroma compounds include, among others, mono- and sesquiterpenes, phenolic derivatives, lipid-derived compounds, and amino acid-derived compounds (1). Some aroma molecules, namely, the apocarotenals (norisoprenes), are derived from carotenoid (tetraterpene) breakdown (2, 3). Carotenoids and monoterpenes share a common biosynthetic origin: they are derived from the condensation of isopentenyl diphosphate (4) through a plastid-localized pathway and initiated by the condensation of pyruvate and glyceraldehyde 3-phosphate (4). Isopentenyl diphosphate is converted into geranyl diphosphate, farnesyl diphosphate, and geranylgeranyl diphosphate en

route to carotenoid biosynthesis. Geranyl diphosphate, however, is also deemed to be the universal precursor for monoterpenes, key constituents of the aromas of flowers, fruits, and spice plants (4). Among the latter compounds, citral, a mixture of the *cis* and *trans* noncyclic monoterpene aldehyde isomers (neral and geranial, respectively), possesses an agreeable scent, reminiscent of lemon. Citral is a major component of lemon basil (*Ocimum basilicum* L., Lamiaceae), lemongrass [*Cymbopogon citratus* (DC.) Stapf., Poaceae], litsea (*Litsea cubeba* Pers., Lauraceae), and other lemon-scented aromatic plants (5, 6). Citral has a major impact in the aroma of tomato (*Lycopersicon esculentum* Mill.) and watermelon (*Citrullus lanatus* [Thunb.] Matsum. and Nakai), two fruits that accumulate high levels of the tetraterpene red pigment lycopene. Recently, a geraniol synthase gene has been isolated from lemon basil. The enzyme encoded by this gene releases geraniol from geranyl diphosphate (7). In many plants, geraniol is readily oxidized to geranial by alcohol dehydrogenases (4, 8–10). It has, however, also been shown that geranial and other apocarotenoids are formed *in vitro* by oxidative cleavage of lycopene (11). It is presently not known whether the citral that accumulates in lycopene-rich fruits is

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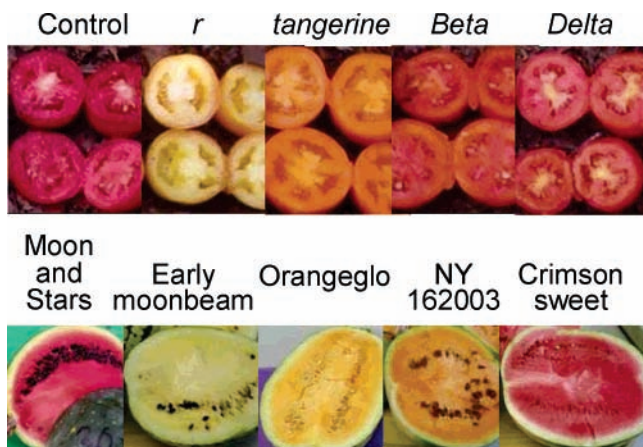


Figure 1. Tomato and watermelon fruit color mutants.

derived in vivo directly from geranyl diphosphate or whether it is produced by the oxidative degradation of lycopene.

The genetic variability of fruit carotenoid composition—and hence color—in tomato has been extensively studied, and pigment analyses as well the genetic basis for each mutant are available in the literature (12). A comparable range of fruit coloration is also known among cultivars of watermelon, a nonclimacteric cucurbitaceous fruit. Genetic studies have facilitated the identification of genes that control flesh color in watermelon fruit (13). The aim of this work was to apply a comparative biology approach to study the pigmentation and norisoprene aroma patterns of both tomato and watermelon fruit. Our findings indicate that there is a direct relationship between carotenoid and volatile norisoprene compositions and that the lemon-scented monoterpene aldehyde geranial is derived from lycopene in these fruits.

MATERIALS AND METHODS

Plant Material. The tomato color mutations *yellow flesh* (*r*), *tangerine* (*tg*, orange), *High Delta* (*Del*, red-orange), and *High Beta* (*B*, orange-red) were introduced, via backcross hybridizations, into a common genetic background to create near-isogenic lines (14). The watermelon cultivars included Moon and Stars (red), Crimson Sweet (red), Orangeglo (orange), NY162003 (orange-yellow), and Early Moonbeam (canary yellow). Tomato and watermelon plants were grown—with standard cultural practices—in the field at the Newe Ya'ar Research Center and at the 'Akko Experiment Station, both in northern Israel, during the summers of 2002 and 2003. Typical fruit colorations are shown in Figure 1.

Carotenoid Analyses. Samples for carotenoid extraction were taken from at least three fruits of each accession. Carotenoids were extracted by grinding 0.5 g of fresh fruit in hexane/acetone/ethanol [50:25:25 (v/v)], followed by 5 min of saponification in 8% (w/v) KOH. The saponified material was extracted twice with hexane, which was then evaporated under vacuum. The solid pellet was resuspended in 400 μ L of acetonitrile/methanol/dichloromethane [(45:5:50 (v/v))] and passed through a 0.2- μ m nylon filter. Forty microliters was injected into a 2996 Waters HPLC equipped with Waters PDA detector 996, C18 Nova-Pak (Waters, Milford, MA) column (250 \times 4.6 mm i.d.; 60 \AA ; 4 mm), and a Nova-Pak Sentry Guard cartridge (Waters) (15). Data were analyzed using Millenium software (15).

Determination of Volatiles. At least six fully ripe fresh tomato fruits or three slices of three watermelon fruits were cut into small (\sim 0.5 cm^3) pieces. Two replicates, each of 100 g of cubes, were vigorously shaken for 16 h with 100 mL of methyl *tert*-butyl ether (MTBE) containing 100 μ g of isobutylbenzene as the internal standard (16). The upper phase was dried with Na_2SO_4 and concentrated under a gentle stream of nitrogen to 0.5 mL. One microliter of each sample was analyzed by GC-MS. Identification of the main components was done

by comparison of mass spectra and retention time data with those of authentic standards and supplemented with a Wiley GC-MS library (16).

RESULTS AND DISCUSSION

Carotenoid Pigmentation Patterns Influence Fruit Monoterpene and Norisoprenoid Volatile Composition. Tomato near-isogenic lines displayed marked differences in carotenoid compositions and in monoterpene and norisoprenoid volatiles. The mesocarp of wild-type (red) tomatoes contained high concentrations (\sim 85 wt % of total carotenoids) of the acyclic pigment lycopene and lower concentrations ($<$ 10 wt %) of the bicyclic β -carotene (Table 1). Wild-type tomatoes also accumulated noncyclic volatile norisoprenoids, such as 6-methyl-5-hepten-2-one, farnesyl acetone, (*E,E*)-pseudoionone, 2,3-epoxygeranial, 2,6-dimethylhept-5-1-al, geranyl acetone, and dihydro-*apo*-farnesal, as well as the monoterpene aldehydes geranial and neral (citral) and the cyclic norisoprenoid β -ionone (Table 1). The *yellow flesh* (*r*) mutation of tomato is caused by a nonfunctional phytoene synthase (*psy1*) gene resulting in the accumulation of very low levels of carotenoids in these yellow-fleshed fruits (17). Geranial and neral as well as all of the norisoprenoid volatiles present in the wild-type tomato, with the exception of farnesyl acetone, were for the most part absent in the *r* tomato (Table 1). The levels of other non-isoprenoid-derived compounds, such as phenylethyl alcohol, 4-vinylphenol, and *o*-guaiacol, were apparently unaffected by the *r* mutation (not shown). These findings indicate that a functional phytoene synthase is required for the synthesis of the precursors and, consequently, of aroma volatiles (including citral), revealing pleiotropic effects of fruit pigmentation genes on aroma traits. The *tangerine* (*tg*) mutant carries a dysfunctional carotenoid isomerase (*CRTISO*) gene and thus accumulates the orange pigment polycopene (tetra-*cis*-lycopene) and higher levels of tetraterpene precursors, including phytoene, phytofluene ζ -carotene, and neurosporene, than the wild-type tomato (18). Similar concentrations of noncyclic norisoprenes and citral were found in *tg* and wild-type red tomatoes. The levels of 6-methyl-5-hepten-2-one, geranyl acetone, dihydro-*apo*-farnesal, and farnesyl acetone were higher in the *tg* mutant line than in wild-type red tomato. The tomato *Delta* (*Del*) mutant was found to accumulate high levels of the monocyclic tetraterpene pigment δ -carotene (\sim 80% of the total carotenoids) due to a marked increase in the activity of lycopene ϵ -cyclase (*CrtL-e*) during fruit maturation (19). Our study showed α -ionone to be very prominent in the tomato *Del* mutant and citral and the noncyclic norisoprenes detected in the wild-type and *tg* tomatoes to also accumulate in the *Del* mutant (Table 1). The orange-colored *High Beta* (*B*) genotype accumulated high levels of β -carotene in addition to low levels of lycopene. This phenotype is caused by higher expression levels of lycopene β -cyclase (*LycB*) than in wild-type tomatoes, in which lycopene β -cyclase transcript levels are significantly diminished during fruit ripening (20). The dihydroactinodioidide, β -ionone, and β -cyclocitral accumulated in the *B* line (Table 1) seem to be derived from β -carotene.

In an earlier study, the volatile profiles of tomato lines differing in flesh color were found to be closely related to the fruit carotenoid composition (21). In the current study, the use of advanced genetic material with near-isogenic backgrounds enabled us to exclude linkage drag and varietal effects: we were therefore able to conclude that genes that affect carotenoid pigmentation have pleiotropic effects on aroma volatiles.

To corroborate these findings, we extended our studies to watermelon, a fruit that is botanically and physiologically distant

Table 1. Carotenoids, Monoterpenes, and Norisoprenes of Tomato and Watermelon Fruits^a

	identification method ^b	tomato mutant					watermelon variety				
		<i>wt</i>	<i>r</i>	<i>tg</i>	<i>B</i>	<i>Del</i>	Moon and Stars	Early Moonbeam	Orangeglo	Crimson Sweet	NY162003
Carotenoids, Micrograms per Gram of Fresh Weight											
phytoene	t _R , UV-vis, AS	4.6 (1.0)	0	20.3 (4.9)	0.7 (0.1)	0.8 (0.01)	traces	0	5.4 (1.2)	traces	traces
phytofluene (tentative) ^c	t _R , UV-vis	0	0	15.4 (3.8)	0	0	0	0	traces	0	0
ζ-carotene (tentative)	t _R , UV-vis	1.9 (0.4)	0	17.5 (0.2)	0	0	traces	0	4.6 (0.5)	0	traces
neurosporene (tentative)	t _R , UV-vis	traces	0	3.1 (1.2)	traces	0	0	0	1.2 (0.9)	traces	traces
pro-lycopene (tentative)	t _R , UV-vis	0	0	16.3 (3)	0	0	0	0	8.2 (2.5)	0	0
lycopene	t _R , UV-vis, AS	63.6 (1.6)	0.04 (0.01)	0	9.0 (0.8)	14.6 (0.3)	45.8 (4)	0	0	42.6 (5.3)	traces
β-carotene	t _R , UV-vis, AS	5.5 (1.5)	1.1 (0.1)	0	35.2 (6.2)	1.5 (0.1)	2.7 (0.8)	traces	0.2 (0.1)	traces	10.8 (1.6)
δ-carotene	t _R , UV-vis, AS	0	0	0	traces	39.8 (1)	0	0	0	0	0
Monoterpenes and Norisoprenes, Nanograms per Gram of Fresh Weight											
farnesyl acetone	t _R , MS, AS	272 (47)	177 (72)	492 (96)	224 (44)	307 (4)	64 (3)	0	51 (8)	0	0
geranyl acetone	t _R , MS, AS	56 (7)	1 (1)	206 (33)	26 (1)	40 (0)	28 (5)	0	157 (15)	0	0
6-methyl-5-hepten-2-one	t _R , MS, AS	364 (47)	0	567 (45)	222 (9)	408 (23)	157 (15)	0	138 (8)	10 (4)	0
2,6-dimethylhept-5-1-al	t _R , MS, AS	60 (15)	0	18 (9)	25 (2)	26 (13)	18 (0)	0	7 (0)	5 (3)	0
2,3-epoxygeranial (tentative)	t _R , MS	62 (11)	1 (1)	47 (2)	43 (2)	50 (25)	32 (2)	0	6 (1)	6 (4)	0
neral (<i>cis</i> -citral)	t _R , MS, AS	36 (4)	0	34 (4)	39 (2)	31 (16)	5.5 (0.5)	0	3.9 (0.5)	1.7 (0.2)	0
geranial (<i>trans</i> -citral)	t _R , MS, AS	81 (13)	0	57 (4)	51 (2)	93 (3)	39 (2)	0	15 (2)	16 (6)	0
dihydro- <i>apo-trans</i> -farnesal (tentative)	t _R , MS	16 (5)	0	126 (34)	33 (1)	8 (5)	0	0	0	0	0
pseudoionone	t _R , MS, AS	78 (16)	0	58 (6)	35 (2)	90 (2)	39 (4)	0	11 (2)	14 (8)	0
α-ionone	t _R , MS, AS	1 (1)	0	0	0	278 (10)	0	0	0	0	0
dihydroactinodiolide	t _R , MS, AS	12 (1)	1.5 (0.2)	3.5 (3.5)	252 (0.15)	13.5 (0.17)	15 (2)	0	traces	0	23 (2)
β-cyclocitral (tentative)	t _R , MS	0	0	2 (1)	90 (4)	0	traces	0	traces	0	traces
β-ionone	t _R , MS, AS	27 (11)	6 (2)	6 (2)	185 (11)	22 (11)	38 (2)	0	11 (2)	0	23 (3)

^a Data represent the means of at least three extractions of six fully ripe fresh tomato fruits or of three slices of three watermelon fruits (standard errors of the means are in parentheses). ^b Identification was done according to t_R, known retention times or index (from the literature); UV-vis, known UV-visible spectrum (from the literature); MS, comparison to known mass spectrum (from the literature); AS, co-injection with an authentic standard (Sigma-Aldrich Chemical Co.). ^c Tentatively identified on the basis of only two methods.

from tomato but that also accumulates lycopene. Although there is a clear difference in flavor and aroma between watermelon and tomato, these two fruits share many of the aroma chemicals that give rise to their respective unique aromas, particularly the noncyclic norisoprenoids apparently derived from lycopene (**Table 1**). Moreover, the volatile norisoprenes found in watermelon cultivars that differ in their carotenoid composition displayed a striking similarity to the norisoprenoid compositions found in the fruits of the near-isogenic tomato lines analyzed. The carotenoid composition of red watermelons, represented here by Moon and Stars (**Figure 1**), an heirloom watermelon cultivar, resembled that of the wild-type tomato, consisting of mostly lycopene (95%) and β-carotene (~5%). Similarly to the findings for the wild-type red tomato, Moon and Stars watermelons accumulated relatively high levels of farnesyl acetone, geranial, geranyl acetone, pseudoionone, epoxygeranial, and 6-methyl-5-hepten-2-one as well as minute levels of neral (**Table 1**). Several other red watermelon cultivars, such as Malali, Sugar Baby, Calsweet, and Dixielee, displayed similar patterns of carotenoids and norisoprene volatiles (data not shown). In contrast, in the yellow-fleshed cultivar Early Moonbeam (**Figure 1**), which lacked lycopene and accumulated only trace levels of β-carotene, no norisoprenes were detected (**Table 1**). These carotenoid and norisoprenoid patterns resembled those observed in the tomato *r* mutant. Other low-carotenoid-containing watermelon cultivars such as PI482318-1 also displayed an analogous absence of norisoprenoid volatiles (data not shown). Orangeglo (**Figure 1**) is an intensely orange-fleshed watermelon cultivar, the carotenoid profile of which resembled that of the *tg* tomato, having prolycopene as the major carotenoid, accompanied by relatively high levels of lycopene precursors such as phytoene and ζ-carotene (**Table 1**). The total carotenoid levels of Orangeglo were ~50% less than those found in Moon and Stars. The Orangeglo watermelons contained enhanced levels of geranyl acetone and lower levels of all other norisoprenoid volatiles than Moon and Stars, similar to the tomato *tg* mutant

line (**Table 1**) and other “*tg*-like” watermelon cultivars, such as Tastigold, Golden Honey, and Gold Strike (data not shown). Carotenoid analyses of NY162003, an experimental orange-fleshed watermelon cultivar (**Figure 1**), indicated that this cultivar contains mostly (>99%) β-carotene and only traces of lycopene, possibly due to efficient conversion of lycopene to β-carotene (**Table 1**). NY162003 watermelons lack noncyclic norisoprenoid volatiles and citral, but contain β-ionone and dihydroactinodiolide (**Table 1**). In a complementary way, the intensely red-fleshed Crimson Sweet watermelon (**Figure 1**), the mesocarp of which contained only lycopene and was essentially devoid of β-carotene, also lacked the cyclic norisoprenes β-ionone and dihydroactinodiolide (**Table 1**). Farnesyl and geranyl acetone were also absent in Crimson Sweet, a finding that reiterates the possible origin of the latter volatiles from noncyclic tetraterpenoids that are less saturated than lycopene. Other watermelon cultivars that mostly accumulated lycopene with undetectable levels of β-carotene, such as Big Crimson, Bush Snakeskin, and Verona, also lacked cyclic norisoprenoid volatiles (data not shown).

Our results for tomato and watermelon support the hypothesis that lycopene, prolycopene, δ-carotene, and neurosporene may give rise in vivo to the noncyclic volatiles neral and geranial, as well as 6-methyl-5-hepten-2-one, 2,6-dimethylhept-5-1-al, 2,3-epoxygeranial, and (*E,E*)-pseudoionone (**Figure 2**). β-Ionone, β-cyclocitral, and dihydroactinodiolide are apparently oxidative breakdown products of β-carotene, the predominant carotenoid in the *B* tomato mutant and NY162003 watermelon cultivar. δ-Carotene apparently gives rise to α-ionone, probably by the cleavage of the ε-ionone ring of δ-carotene (**Figure 2**). In vitro studies have indicated that many of the noncyclic norisoprenoids found as well as geranial may be derived from lycopene breakdown (11), whereas β-ionone, β-cyclocitral, and dihydroactinodiolide are products of the oxidative degradation of β-carotene (22). Farnesyl acetone, dihydro-*apo*-farnesal, geranyl acetone, and 6-methylhept-5-en-3-one are probably

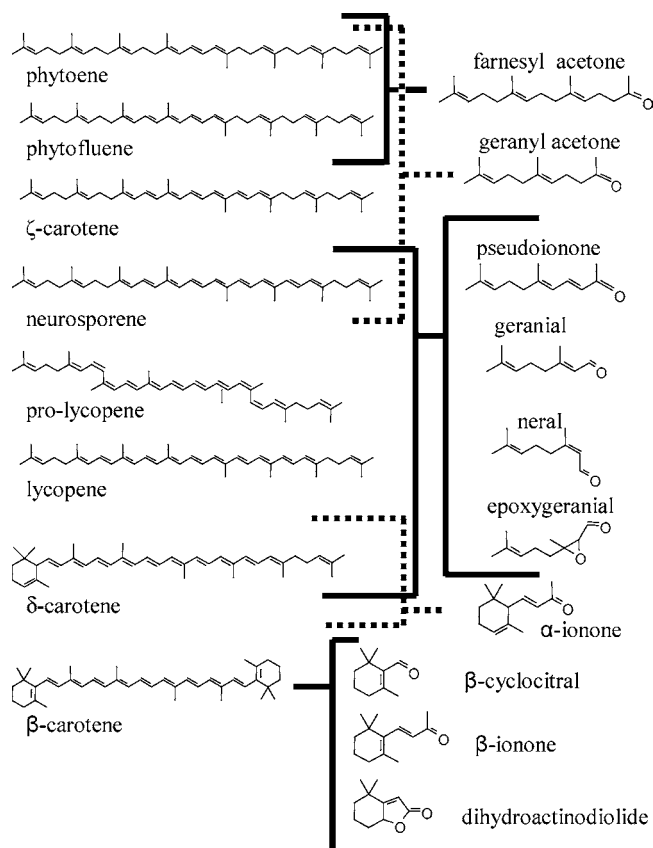


Figure 2. Tetraterpene carotenoid pigments and their precursors. Carotenoids (left) are apparently degraded into norisoprene and monoterpene aroma volatiles (right).

derived from phytoene or phytofluene (**Figure 2**), but the presence of farnesyl acetone in the *r* mutant indicates possible additional biosynthetic routes in tomato. In essence, color and aroma are highly associated in tomato and watermelon fruits, and this relationship is probably a function of the degradation of carotenoids into aroma volatiles. In conclusion, the availability of defined tomato mutations that influence the mesocarp color in near-isogenic backgrounds enabled us to reveal these pleiotropic effects of carotenoid biogenetic genes. Similar effects of pigmentation genes on the composition of aroma volatiles were observed in cultivars of watermelon, a nonclimacteric fruit of a distantly related plant family, indicating that in watermelon, too, carotenogenesis genes exhibit pleiotropic effects on aroma.

Citral Is Apparently Biosynthesized from Carotenoids in Tomato and Watermelon. Citral was present only in tomato and watermelon fruits that contained high levels of lycopene and its tetraterpene precursors (**Table 1**). Several lines of evidence, gathered from feeding experiments with radiolabeled isopentenyl diphosphate as well as enzymatic cell-free extracts, indicate that in many plants citral is biosynthesized from geranyl diphosphate to geraniol, which is, in turn, converted into geranial and neral [**Figure 3**, bold arrows (8, 9, 23)]. Specific geraniol dehydrogenases that catalyze the oxidation of geraniol to release geranial have been found in citrus fruits and lemongrass leaves (**Figure 3**) (8–10). Alcohol dehydrogenases have been isolated from tomato fruit and characterized (24, 25). Unlike alcohol dehydrogenases from other sources, the tomato enzyme seems to efficiently accept geraniol as a substrate (26). Nevertheless, transgenic tomato fruit ectopically expressing or repressed in alcohol dehydrogenase had altered levels of several volatile alcohols and aldehydes, but the ratios of geranial to geraniol remained unchanged (27). Recently, a gene coding for geraniol

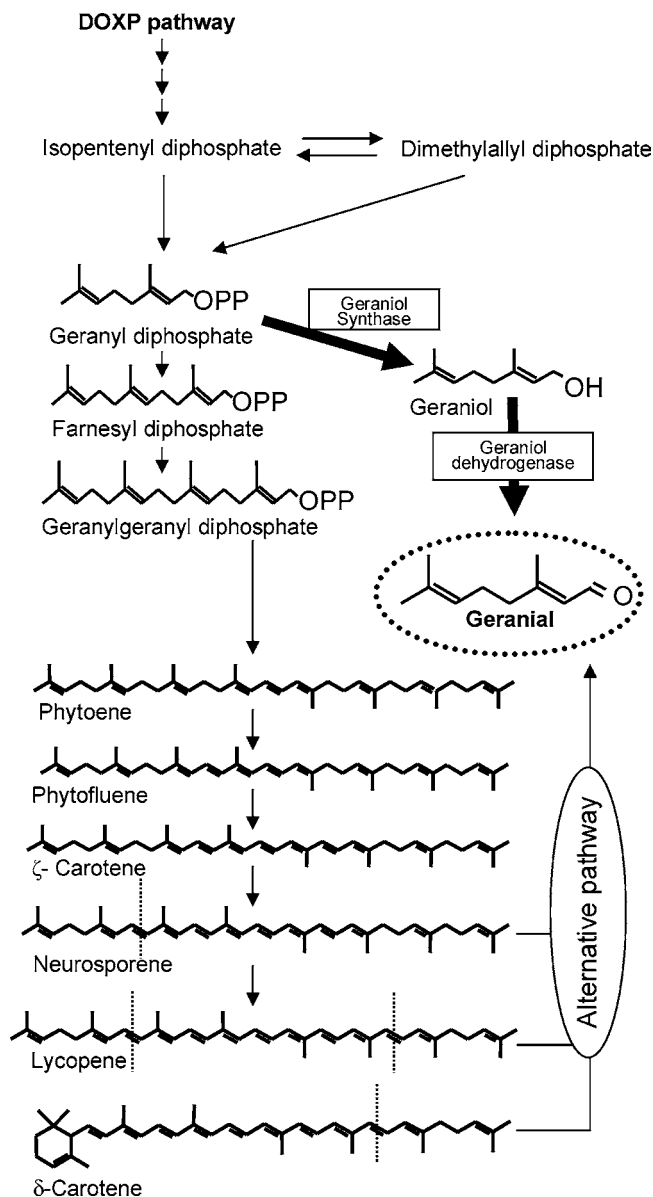


Figure 3. Alternative routes to geranial formation. Geranial (*trans*-citral) is formed from geranyl diphosphate and geraniol in lemon basil and other plant species (top bold arrows). Combined analyses of carotenoids and volatiles in tomato advanced genetic material indicate that lycopene is degraded *in vivo* to geranial in tomato and watermelon fruits (alternative route). Dotted lines on double bonds represent possible cleavage sites.

synthase, an enzyme that catalyzes the conversion of geranyl diphosphate to geraniol, has been identified and isolated in lemon basil (7) (**Figure 3**). The gene was isolated from mRNA expressed in glandular trichomes, a highly specialized tissue that accumulates copious levels of citral (7). Geraniol synthase directly cleaves the diphosphate moiety, as was shown by enzymatic ^{18}O incorporation experiments. Nevertheless, although it is widely accepted that citral is biosynthesized directly from geranyl diphosphate, our evidence—based on a comparative analysis of advanced genetic material from tomato and watermelon—indicates that this might not be the case in tomato and watermelon fruits. Rather, the monoterpenes geranial and neral are probably derived from carotenoids (mainly lycopene and its tetraterpene precursors) in these tissues (**Table 1**; **Figure 3**). Citral was consistently found in tomatoes and watermelons in a pattern that followed the noncyclic norisoprenoid present (**Table 1**). Most convincingly, no citral was found in *r* tomatoes

that carry a nonfunctional *psy1* gene and are devoid of lycopene (Table 1). The blockage of the metabolic flow to carotenoids should not have affected geraniol levels in the *r* mutant as compared with wild-type tomatoes, as would have been expected if geranyl diphosphate were the immediate precursor of geraniol and geraniol in tomato. This finding suggests, therefore, that lycopene might serve in vivo as a precursor for the formation of geraniol and neral in wild-type tomato fruit. Similarly, no geraniol or neral was detected in Early Moonbeam watermelon fruit, which does not accumulate lycopene (Table 1), a finding that further implies that, in accordance with the profiles found in tomato, the citral accumulated in the red watermelon is seemingly derived from the degradation of lycopene. Our results showed that neurosporene, prolycopene, lycopene, and δ -carotene may also give rise to citral, both in tomato and in watermelon (Figures 2 and 3).

Biosynthesis of Aroma Volatiles from Carotenoids. The carotenoid degradation pathway is considered to be a key route for the formation of aroma compounds in many plants and plant products. In this pathway, carotenoids serve as substrates, but the nature of the biochemical mechanisms (whether enzymatic or nonenzymatic) mediating these oxidative degradations is still to be elucidated in each particular case. It is, however, known that the final reactions are efficiently catalyzed in vitro by peroxidases, lipoxygenases, or dioxygenases (3, 28, 31) or may be nonenzymatic (11, 22). It has been found that carotenoids degrade into aroma compounds during curing of tobacco and fermentation of tea leaves (32, 33) and during the long-term storage of carrots (34). Some of these reactions are catalyzed by microorganisms and fungi (30, 35), but plant enzymes also catalyze these types of conversions. In star fruit (*Averrhoa carambola* L., Oxalidaceae) and quince (*Cydonia oblonga* P. Mill., Rosaceae), enzymatic activities capable of converting β -carotene to β -ionone have been demonstrated and partially characterized (36, 37). In addition, genes that code for the enzymes involved in the specific cleavage of carotenoids into aroma compounds have been described and isolated from *Arabidopsis* (29), *Crocus sativus* L. (31), and petunia (38). The enzymes that catalyze these reactions in tomato and watermelon have not yet been fully identified. Still, correlations between lycopene and aroma volatile content have been reported in cherry tomato grown under different conditions that affect lycopene content and under the influence of 2-(4-chlorophenylthio)triethylamine (CPTA), an inhibitor of β -carotene cyclase (39). A tomato 9-*cis*-epoxycarotenoid dioxygenase gene involved in abscisic acid formation is known (40, 41). Recently, a tomato dioxygenase able to cleave carotenoids at position 9, and inferred in the formation of geranyl acetone, pseudoionone, and β -ionone, has been described (42). It is not clear whether this, or similar, genes and enzymes have active roles in the formation of other aroma metabolites from carotenoids (such as geraniol) in tomato fruits. It could be that the apparent carotenoid degradative processes observed are chemical or enzymatic in vivo. However, a close association between carotenoid pigmentation and volatile terpene content is clearly noticeable. The above notwithstanding, the use of advanced genetic tools and comparative biology in this study has unveiled an alternative pathway to geraniol that apparently operates in vivo in ripening tomato and watermelon fruits.

Dual Role of Carotenoids as Quality Attributes. More than 400 volatile compounds have been identified in tomato fruits and their products, including monoterpenes, phenolics, norisoprenes, and lipid-derived products (43). Much less is known about the factors that contribute to the unique flavor of

watermelon. Among the most important aroma components, in both tomato and watermelon, are short- and medium-chain alcohols and aldehydes as well as norisoprenes (43–46).

The events that lead to the regulation of carotenoid formation and degradation into aroma compounds affecting fruit color, nutritional value, and the full flavor of our food have important economic repercussions. In this context, we report here that carotenoids are not only key pigments in fruits and vegetables but that they also have a profound impact on the composition of the aroma volatiles of the fruits. Fruit color and aroma are important quality and marketability attributes, selected for during domestication and crop improvement (47). These traits have apparently evolved from their primordial roles as ecological cues for attracting organisms engaged in seed dispersal in the crops' wild ancestors (48). Carotenoids are regarded as key semiochemicals in these processes due to their visual effects. Our results indicate that carotenoids might have additional important roles in plant communication with other organisms by serving as precursors for olfactory cues. The marked taste differences among fruits of different colors were easily detected by a panel of untrained tasters. It is known that preconceptions of taste perception based on the color of our food are common (49), especially for red colors, which often show consistent associations with taste. Colors certainly influence our sensory evaluation of overall taste and thus our food preferences (50). We have shown here that different pigmentation patterns, in both tomato and watermelon, are correlated with different sets of volatiles, directly influencing the aroma properties of the fruits.

The understanding of the key chemical, enzymatic, and molecular mechanisms that control the formation of aroma volatiles in crop plants is still in its infancy (51, 52). Using comparative biology we have shown pleiotropic effects of carotenogenesis genes on aroma volatile profiles and a new operational pathway to citral in tomato and watermelon. The incorporation of this knowledge into existing breeding programs will undoubtedly contribute to the production of novel fruit and vegetable varieties with superior aromas, colors, nutritional value, and shelf-life properties without impairing other overall agronomical and quality traits.

ACKNOWLEDGMENT

We thank Joseph Hirschberg for helpful suggestions and Fabian Baumkoler and Gaby Gera for excellent field work assistance. We especially thank Erica Clair Renaud from Seeds of Change, Santa Fe, NM, for providing the watermelon seeds. We also thank Ari Schaffer and Inez Mureinik for critical review and editing of the manuscript.

LITERATURE CITED

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Received for review December 9, 2004. Revised manuscript received February 22, 2005. Accepted February 27, 2005. Publication 114/2004 of the Agricultural Research Organization, Bet Dagan Israel. Note: This work was partially supported by a US–Israel Binational Agricultural Research and Development Fund (BARD) grant IS-3332-02C, by the Center for the Improvement of Cucurbit Fruit Quality, ARO and by the Israel Ministry of Science Grant.

JF047927T