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Comparative fruit colouration in watermelon and tomato

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Abstract

The characteristic red pigmentation of watermelon and tomato fruits is determined by accumulation of the carotenoid pigment lycopene and this phenotype is polyphyletic. Since several carotenoids are known to have health promoting activity, and watermelon can be a significant source of lycopene and other carotenoids, it is important to understand the genetic basis of watermelon fruit-specific carotenoid biosynthesis. Unlike tomato, very little is known about the regulation of carotenoid biosynthesis during fruit development in watermelon, a non-climacteric fruit. We have HPLC analyzed the carotenoids of red, yellow and orange watermelons and compared their carotenoid patterns with those of known fruit colour mutants of tomato. Interestingly, we could detect tomato mutant equivalents to most watermelon fruit colour phenotypes, including *r*, *og*, *B* and *t*.

Keywords: Watermelon; Tomato; Lycopene; Carotenoid; HPLC

1. Introduction

Carotenoids are tetraterpenoid pigments which are accumulated in the chloroplasts of leaves and in the chromoplasts of many flowers and fruits, where they contribute to the red, orange and yellow colour of many flowers and fruits. In addition to their roles in plants as photosynthetic accessory pigments and colourants, carotenoids have fundamental contributions to human health (Reviewed in Fraser & Bramley, 2004; Galili, Galili, Lewinsohn, & Tadmor, 2002). Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) and tomato (*Lycopersicon esculentum* Mill.) accumulate lycopene as their major mesocarp carotenoid, giving them their typical red colour. In recent years, lycopene has aroused considerable interest as a health promoting phytochemical. Lycopene intake has been particularly associated with protection from prostate cancer (Giovannucci, 2002; Giovannucci, Rimm, Liu, Stampfer, & Willett, 2002) as well as with a lowered risk of coronary heart disease and lung cancer (Fraser & Bramley, 2004).

Watermelon exhibits a wide range of fruit colour mutations, similar to tomato; however, data regarding watermelon carotenoid production is limited. Tomato and watermelon differ in the way lycopene is accumulated. The immature tomato fruit is green and thus accumulates xanthophylls, which is comparable to green leaf tissue (Bramley, 2002; Hirschberg, 2001), whereas the young watermelon fruit mesocarp is usually white and contains only trace amounts of carotenoids

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(unpublished data). Moreover, fruit development of tomato (including lycopene accumulation) is dependent on climacteric ethylene (climacteric fruit) whereas watermelon fruit ripening is non-climacteric. Although most wild *Lycopersicon* species have green fruits, the ancestor of the cultivated tomato has red fruit (Rick, 1995). Conversely, *C. lanatus* var. *citroides* (L.H. Bailey) Mansf., the putative wild progenitor of the cultivated edible watermelon (Navot & Zamir, 1987), has white fleshed fruits. Thus, the genetic changes that led to the development of red watermelon fruit probably occurred after watermelon had been domesticated. This indicates that red fleshed fruit developed independently in these two species, suggesting that this trait is polyphyletic.

The genetic basis of fruit colour variation in tomato and its association to carotenoid composition is well established (Hirschberg, 2001). Mutant fruit colours were assigned to specific carotenogenesis genes; the yellow fruit *r* results from a mutated *Psy*-1 (Fray & Grierson, 1993), the orange fruit *t* is due to a mutated *CrtISO* (Isaacson, Ronen, Zamir, & Hirschberg, 2002), the high δ-carotene in fruits of the mutant *Delta* (*Del*) is due to a mutation in the gene for lycopene epsilon-cyclase (*LCY-E*; Ronen, Cohen, Zamir, & Hirschberg, 1999), the high β-carotene fruits in the mutant *Beta* is determined by a dominant allele of *Cyc-b* and the *crimson* (*og*, og^c) is a null allele of *Cyc-B* (Ronen, Carmel-Goren, Zamir, & Hirschberg, 2000). In contrast to tomato, very little is known about carotenoid biosynthesis in watermelon fruits, although watermelon exhibits a similar range of fruit flesh colour mutations. We have analyzed fruit carotenoids in various watermelon mutants and compared their profiles with known tomato mutants, in order to infer relatedness. We present here data on the carotenoid composition of yellow, orange and red flesh watermelon accessions and compare these to known tomato fruit colour mutants.

2. Materials and methods

2.1. Plant material

The tomato color mutations, *yellow flesh* (*r*), *tangerine* (*t*, orange) and *Beta* (*B*, orange-red) were introduced, via backcross hybridizations, into a common *high pigment* (*hp*; Thompson, Hepler, & Kerr, 1962) genetic background to create near isogenic lines (Liu et al., 2003). Watermelon material included thirty two open pollinated varieties, covering a wide range of flesh colours; red, orange, yellow and white.

Plants were grown in the fields of Newe Ya'ar Research Center and in the Acco farm (Northern Israel) in the summer of 2003 under commercial conditions. Samples for carotenoid extraction were taken from at least three fruits of each variety.

2.2. Carotenoids analysis

Watermelon and tomato carotenoids were extracted and fractionated according to Tadmor et al. (2000) with slight modifications. Carotenoids were extracted by grinding 0.5 g fresh fruit in hexane:acetone:ethanol (50:25:25), followed by 5 min saponification in 8% (w/v in methanol) KOH. The saponified material was extracted twice with hexane which was then evaporated. The solid pellet was resuspended in 400 μl of acetonitrile:methanol:dichloromethane (45:5:50), passed through a 0.2 μm nylon filter and kept at room temperature in darkness for no more than 24 h before analysis by HPLC. Forty μl were injected to a 2996 Waters HPLC equipped with Waters PDA detector 996, C18 Nova-Pak (Waters, Milford, MA, USA) column (250 × 4.6 mm i.d.; 60 Å; 4 mm), and a Nova-Pak Sentry Guard cartridge (Waters, Milford, MA, USA). Compounds were identified by comparison of retention times, co-injection spiking, and by comparing their UV–Vis spectra with authentic standards. Quantification was performed by integrating the peak areas of the HPLC results using Millennium chromatography software (Waters, Milford, MA).

3. Results and discussion

Tomato near-isogenic lines displayed marked differences in carotenoid compositions. The mesocarp of wild type (red) tomatoes contained high concentrations (~85% of total carotenoids) of the acyclic pigment lycopene and lower concentrations (<10%) of the bicyclic β-carotene (Table 1). The *yellow flesh* (*r*) mutation of tomato is caused by a non-functional phytoene synthase (*Psy1*) gene resulting in the accumulation of very low levels of carotenoids in these yellow-fleshed fruits (Camara, 1993; Table 1). The *tangerine* (*t*) mutant carries a dysfunctional carotenoid isomerase (*CrtISO*) gene and thus accumulates the orange pigment pro-lycopene (tetra-*cis*-lycopene) and higher levels of other carotenoids, including phytoene, phytofluene and ζ-carotene, than the wild-type tomato (Isaacson et al., 2002; Table 1). The orange-coloured *Beta* (*B*) tomato genotype accumulated high levels of β-carotene in addition to low levels of lycopene (Table 1). 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 (Ronen et al., 2000).

Major carotenoids of representative samples of watermelon fruits are presented in Table 2. Our results are in accordance with a previous study of commercial cultivars in which lycopene levels of red watermelons were found to range from 36.5 to over 71 μg g^{-1}, depending on the variety (Perkins-Veazie, Collins, Pair,

Table 1
Major carotenoids of tomato wild-type (*wt*; red), *yellow flesh* (*r*), *tangerine* (*t*; orange), *old-gold* (*og*) and *Beta* (*B*; orange) fruits

	Tomato mutant				
	wt	*r*	*t*	*og*	*B*
Carotenoid μg g^{-1} FW					
Phytoene	4.6 (1.0)	0	20.3 (4.9)	1.4 (0.2)	0.7 (0.1)
Phytofluene	0	0	15.4 (3.8)	0	0
ζ-Carotene	1.9 (0.4)	0	17.5 (0.2)	1 (0.2)	0
Pro-lycopene	0	0	16.3 (3)	0	0
Lycopene	63.6 (1.6)	0.04 (0.01)	0	68.9 (1)	9.0 (0.8)
β-Carotene	5.5 (1.5)	1.1 (0.1)	0	2.1 (0.6)	35.2 (6.2)

Data represent the means of at least three extractions of fully ripe fresh tomato fruits (standard errors of the means are in parentheses).

Table 2
Major carotenoids of 'Calsweet' (red), 'Early Moonbeam' (yellow), 'Orangelo' (orange), 'Crimson Sweet' (red), 'Malali' (red-orange), and 'NY162003' (orange) watermelon fruits

	Watermelon mutant					
	Calsweet	Early moonbeam	Orangelo	Crimson sweet	Malali	NY162003
Carotenoid μg g^{-1} FW						
Phytoene	Traces	0	5.4 (1.2)	Traces	Traces	Traces
Phytofluene	0	0	Traces	0	0	0
ζ-Carotene	Traces	0	4.6 (0.5)	0	2.3 (0.6)	Traces
Pro-lycopene	0	0	8.2 (2.5)	0	0	0
Lycopene	48.8 (4)	0	0	42.6 (5.3)	38.7 (2.3)	Traces
β-Carotene	1.7 (0.5)	Traces	0.2 (0.1)	Traces	5.2 (1.5)	10.8 (1.6)

Data represent the means of at least three extractions of three slices of three watermelon fruits (standard errors of the means are in parentheses).

& Roberts, 2001). These results indicate that watermelon is a comparable source for lycopene when compared to tomato. What is more, it seems that lycopene from non-heat treated watermelon is just as bio-available as lycopene from heat-treated tomato juice (Edwards et al., 2003). Our HPLC analysis indicated that a typical red watermelon, represented by 'Calsweet' (Table 2; Fig. 1, I), an heirloom watermelon cultivar, resembled that of the wild-type tomato, consisting of mostly lycopene (95%) and β-carotene (~5%). Several other red watermelon cultivars, such as "Sugar Baby", "Bush Snakeskin", "Verona" and "Dixielee" displayed similar patterns of carotenoids (data not shown). In contrast, the yellow-fleshed cultivar "Early Moonbeam", lacked lycopene and accumulated only trace levels of lutein and β-carotene, (Table 2; Fig. 1, IV). These carotenoid patterns resembled those observed in the tomato *r* mutant (Table 1; Fray & Grierson, 1993). Other yellow watermelon cultivars such as "PI482318-1" and "Desert King", also displayed an analogous low-carotenoid-containing profile (data not shown). White-fleshed watermelon did not have any detectable amount of carotenoids (data not shown). The difference between the white and the yellow phenotypes could be at the plastid level; the yellow fruit has carotenoid producing plastids (chloroplasts or chromoplasts) while the white fruit lacks them. "Malali", a red-orange variety, showed increased levels of β-carotene at the expense of lycopene (Table 2; Fig. 1, III), a carotenoid profile similar to the tomato *B* mutant (Table 2), where increased expression of the chromoplast-specific lycopene β-cyclase (CYC-B) occurs during fruit maturation (Ronen et al., 2000). Carotenoid analyses of 'NY162003', an experimental orange-fleshed watermelon cultivar, 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 2; Fig. 1, V). This phenotype looks like an extreme case of the tomato *B* mutation where all lycopene had been converted to β-carotene. To the best of our knowledge this is the first report regarding such a phenotype in watermelon. The red variety 'Crimson Sweet' seems to contain lycopene with only trace amounts of β-carotene (Table 2; Fig. 1, VI), unlike 'Calsweet' that contains both lycopene (90% of total carotenoids) and β-carotene (Table 2; Fig. 1, I) and thus we assume it carries a mutation similar to the tomato *og* which is a null allele of the *B* gene (Ronen et al., 2000). Other varieties with similar carotenoid patterns are 'Desert Storm', 'Jubilant' and 'Big Crimson'. A *tangerine*-type watermelon was represented by 'Orangelo' (Table 2; Fig. 1, II), which has intense orange fruit flesh with poly-*cis*-lycopene (pro-lycopene) as its major pigment. In general, the carotenoid profile of 'Orangelo' is similar to the tomato *t* mutant (Table 1) that carries a mutated *CrtISO* (Isaacson et al., 2002). Another orange watermelon variety, 'Orange Flesh Tendersweet', displayed a very similar *t*-like carotenoid pattern (not shown). Salmon-yellow fleshed watermelon,

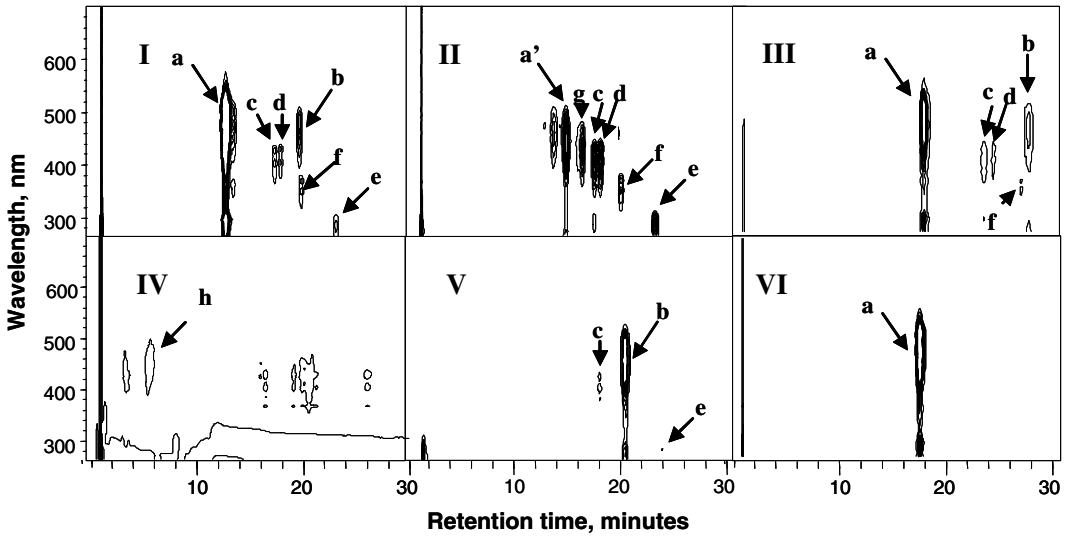

Fig. 1. HPLC 3D chromatograms of carotenoids extracted from Red ('Calsweet'; **I**), Orange ('Orangelo'; **II**), orange-red ('Malali'; **III**), Light yellow ('Early Moonbeam'; **IV**), Orange-yellow ('NY162003'; **V**) and Red ('Crimson Sweet'; **VI**) watermelons. Carotenoids are: a = lycopene; a′ = tetra-*cis*-lycopene (pro-lycopene); b = β-carotene; c = ζ-carotene; d = di-*cis*-lycopene; e = phytoene; f = phytofluene; g = neurosporene; h = lutein.

such as 'Yellow Crimson', 'Whillhite's Tendergold', 'Tastigold', 'Yellow Crimson', 'Yellow Flesh Black Diamond', 'Golden Honey' and 'Gold Strike' also had pro-lycopene as their major carotenoid, but in low amounts (not shown).

Henderson, Scott, and Wehner (1998) described five fruit colour phenotypes: Canary yellow, Salmon yellow, Orange, Red, and white, and conjectured the genetic relationships among these colours based on segregating populations. Our identification of the watermelon flesh colour mutants is in accordance with the results presented therein. It seems that the tomato fruit colour mutations *r*, *t*, *og* and *B* have equivalents in the watermelon genome, with similar genetic relationships as has been observed in tomato. Thus far, we have not detected a watermelon fruit flesh that is containing high levels of δ-carotene, similar to the *Del* tomato mutation. Tomato *Del* mutants accumulate high levels of δ-carotene due to increased expression of lycopene epsilon cyclase (*LCY-E*) during fruit maturation (Ronen et al., 1999).

4. Conclusions

L. esculentum and *C. lanatus* accumulate lycopene as their major fruit carotenoid and this trait is polyphyletic. Nevertheless, we have detected mutations in watermelon that seem similar to the *r*, *t*, *og* and *B* tomato mutations. Red watermelons might have any of the three following carotenoid patterns: (1) High levels of lycopene and small amounts (less than 5% of the total carotenoids) of β-carotene, similar to wild type tomato; (2) Accumulation of mostly lycopene and significant (>10%) amounts of β-carotene, similar to the *B* fruit mutation of tomato (Ronen et al., 2000); and (3) Lycopene exclusively, with no detectable amounts of β-carotene, similar to the *og* fruit mutation of tomato. Yellow watermelon is a result of two different carotenoid patterns: (1) Accumulation of trace amounts of lutein and β-carotene, similar to the tomato *r* mutation; and (2) Accumulation of small amounts of pro-lycopene. The first one was designated as 'canary yellow' while the second one was designated as 'salmon yellow' (Henderson et al., 1998). Orange flesh watermelon, results from either the accumulation of the orange pigment pro-lycopene, an equivalent mutation to the tomato *t* mutation (Isaacson et al., 2002), or due to the accumulation of β-carotene, as was observed in the experimental line 'NY162003'.

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