can therefore be used either alone, when it is desirable to estimate the lysine and arginine content of a given protein sample, or in conjunction with other procedures when the complete amino acid composition of protein is in question, but an accurate assessment of the lysine level is important such as in animal experiments related to the study of lysine deficiency. The method may also find use in agricultural research laboratories devoted to the development of high lysine wheat and rice varieties.

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Light-Dependent Carotenoid Synthesis in the Tomato Fruit

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(1) Light was excluded from growing fruits of normal red, high-beta, apricot, and tangerine tomato genotypes while attached to the vine in order to study the effect of light on the biosynthesis of carotenoids. (2) Pigment formation in the immature fruit of the normal red, high-beta, and apricot genotypes was inhibited in darkness. The carotenoids of dark-ripened, dark-grown fruits of the three genotypes are qualitatively similar to those found in either the light-grown control fruits or the dark-grown fruit which was further ripened in light. (3) β-Carotene was not detected in the immature dark-grown tangerine tomato and only a small amount of the pigment was present in the ripe dark-grown fruit. Lycopene and neurosporene did not accumulate in the dark-grown, dark-ripened fruit but appeared when the fruit was exposed to light probably due to the photoconversion of the poly-cis carotenoids to their corresponding all-trans isomer. The carotenoid composition of dark-grown, light-ripened tangerine tomato is identical with that of the light-grown control fruit. (4) Biosynthetic autonomy of chloroplast and choromoplast carotenoids was suggested.

The carotenoid composition of the tomato fruit undergoes extensive modification during ripening, quantitatively as well as qualitatively. The predominantly cyclic nature of the carotene of the chloroplast changes to the more diverse constitution characteristic of the chromoplast.

The synthesis of chromoplast carotenoids in the tomato fruit is inhibited at high temperature. Light, on the other hand, has a more profound effect than temperature on the biosynthesis of carotenoids in the chloroplast.

Light is necessary in the induction of chloroplast replication and in the control of certain phases of plastid transformation in higher plants (Boasson et al., 1972). Etiolated plants accumulate protochlorophyll and small amounts of carotenoids (Valadon and Mummery, 1969). Synthesis of the carotenoid component and rapid conversion of protochlorophyll to chlorophyll are initiated on exposure to light (Smith and Benitez, 1954; Goodwin and Phagpolngarm, 1960; Virgin, 1967).

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In addition, many plant tissue cultures will synthesize chloroplast pigments when grown in light (Powell, 1925; Stobart et al., 1967). Roots, whether excised or attached to the plant, can be induced to form chloroplasts by exposure to light (Powell, 1925; Bjorn, 1963; Heltne and Bonnett, 1970; Bajaj and McAllan, 1969). However, light is reported to be not essential for the synthesis of carotenoids in ripening tomato fruit (Smith, 1936; Vogele, 1937). The effect of light on pigment biosynthesis in higher plants as well as in nonphotosynthetic, photochromogenic microorganisms has been reviewed by Kirk and Tilney-Bassett (1967).

In the present study, changes in the carotenoid composition of tomato fruits grown in the absence of light were investigated.

EXPERIMENTAL SECTION

Fruits. The tangerine tomato fruits were obtained from plants grown in the greenhouse from August to May as well as from plants grown in the field during the following summer. Summer Sunrise, apricot, and high-beta tomatoes were all field grown.

Flowers at anthesis or fruits less than 10 mm in diameter were wrapped with a black polyethylene bag or a bag made from carbon paper. Each bag was then enclosed in an

aluminum foil bag which reflected light and prevented temperature build-up. The temperature inside some of the bags was monitored at 2-hr intervals during the day and every 4 hr at night with a recording thermograph. The thermistor probe was held in place by taping the wire between two pieces of styrofoam rings which prevented contact with the sides of the bag. The control fruits were either wrapped with clear polyethylene bags or left unbagged.

To obtain light-ripened, dark-grown tangerine, apricot, and Summer Sunrise fruits, the ripe dark-grown fruits were harvested and subsequently exposed to diffuse laboratory light for 5 to 10 days at 25°C together with bagged fruits for dark-ripened control. The light-ripened, dark-grown high-beta fruits, on the other hand, were obtained by exposing ripe, aluminum foil wrapped fruits to light while attached to the plant together with the corresponding dark-grown, dark-ripened fruits for control. The fruits, on exposure to light, were wrapped with two layers of cheesecloth. The immature fruits were 28- to 30-days old; their locules were still firm in contrast to the mature green fruits which had soft, gelatinized locules.

Pigment Extraction. Individual fruits of Summer Sunrise, high-beta, and tangerine tomatoes were homogenized in a Waring Blendor. Two to four apricot tomato fruits were homogenized at one time and a 50-g aliquot of the homogenate was extracted with acetone–petroleum ether (petroleum ether, bp 35–50°C), except in the case of ripe control tangerine fruits where a 30-g aliquot was used instead. The extraction and chromatographic procedures were as described previously (Raymundo et al., 1967, 1970).

Pigment Purification. Neurosporene, lycopene, and γ-carotene were rechromatographed on short columns of neutral grade alumina III. The neurosporene column was developed with 1% diethyl ether (Et_2O) in petroleum ether and the pigment was eluted with 2% Et_2O. The lycopene column was developed with 2% Et_2O and eluted with 5% Et_2O from the adsorbent. The γ-carotene column was developed initially with 0.5% Et_2O. A 1% Et_2O–petroleum ether solution eluted the pigment from the column.

The main ζ-carotene band was eluted from the neutral grade alumina II column with 45% Et_2O in petroleum ether and purified further by TLC on precoated silica gel F_{254} plates. The chromatogram was developed with 4% benzene in petroleum ether. The β-zeacarotene band which was eluted ahead of the ζ-carotene band in the neutral grade alumina II column with 25–30% Et_2O was similarly purified except that the plate was developed with 5% benzene–petroleum ether.

Spectrophotometric Analysis. The pigments were identified by their absorption spectrum in petroleum ether and by their position on the MgO–Hyflo Super-Cel columns. The identity of the poly-cis carotenoids was further verified by their characteristic bathochromic shift after I_2 catalysis. The $E_{1\ cm}^{1\%}$ values for proneurosporene and prolycopene were 1560 at 430 nm and 1880 at 440 nm, respectively.

RESULTS

The immature dark-grown fruits of the normal red, high-beta, and apricot tomato genotypes are white; that of the tangerine is light orange. The green immature tangerine tomato fruit grown in light has an orange color confined to the center of the fruit. The color gradually progresses outward as the fruit matures.

Carotenoid synthesis in ripening tomato fruits does not require prior induction by light, although light is necessary for maximum carotenoid production in the red, high-beta, and tangerine genotypes (Tables I, II, and IV). Light inhibits carotenoid synthesis in the apricot tomato (Table III).

The green, immature fruits of the normal red, high-beta, and tangerine tomatoes contain essentially the same amount of β-carotene, i.e., 18.3 ± 2.5, 20.5 ± 0.5, and 15.3 ± 1.3 μg/g dry weight, respectively. The β-carotene content of immature apricot tomato fruit, on the other hand, is 36.5 + 0.4 μg/g dry weight. Thus, the *at* allele affects the carotenoid content of both the ripe and the unripe fruit.

Residual β-carotene was not detected in the immature fruit of the tangerine genotype grown in the dark while similar fruits of the normal red, high-beta, and apricot contain detectable levels of β-carotene (0.7 to 0.8 μg).

The normal red genotype has the highest concentration of lycopene in the ripe fruit among the four genotypes while the high-beta fruit has the highest level of β-carotene. The *t* allele promotes the accumulation of the more saturated carotenoid precursors phytoene, phytofluene, ζ-carotene, and neurosporene (Table IV). In addition *t* causes the accumulation of proneurosporene and prolycopene rather than their corresponding all-trans isomers. The *at* allele specifically inhibits lycopene synthesis (Table III). It appears from the data that the *at* allele promotes the synthesis of a metabolite in light which acts to suppress lycopene but not β-carotene synthesis in the ripening fruit. In the absence of light, production of the metabolite is either curtailed or it may be synthesized and remain in an inactive form, thus unable to perform the inhibitory function.

Qualitatively there is no difference between the carotenoid composition of dark-grown and light-grown control fruits of the red, high-beta, and apricot genotypes. β-Carotene is the only carotene detected in the immature fruits. Its synthesis is inhibited in darkness. The entire carotene complement was, however, synthesized in the dark during ripening. The percentage of lycopene in the red genotype increased on exposure of the dark-grown fruit to light. The increase was accompanied by a decrease in the level of the more saturated precursors. The carotenoid composition of dark-grown, high-beta fruit, on the other hand, was not altered by exposure to light.

The effect of light on the distribution of secondary carotenoids in the tomato fruit is most visible in the apricot genotype. The ripe light-grown control fruit is golden yellow, whereas the ripe, dark-grown fruit is red. The change in visual coloration from yellow to red when grown in the dark is due to the accumulation of large quantities of lycopene rather than β-carotene (Table III). The ratio between lycopene and β-carotene changed from approximately 1:6 in light-grown control fruit to about 5:1 when light was excluded. The total carotenoid content was also higher in the dark.

Neurosporene, lycopene, and β-carotene did not accumulate in immature, dark-grown tangerine tomato fruit (Table IV). β-Carotene is present in the immature control fruit which similarly did not accumulate either neurosporene or lycopene. However, proneurosporene, prolycopene, and the more saturated carotenoids phytoene, phytofluene, and ζ-carotene were present in the immature fruit. The poly-cis carotenoids do not accumulate in much younger fruits (Mackinney et al., 1956). Ripe, dark-grown fruits maintained in the dark synthesized small amounts of β-carotene. Neither neurosporene nor lycopene, however, could be detected. In similar fruits exposed to light, neurosporene and lycopene accumulated and the β-carotene content slightly increased.

Table I. Effect of Light Exclusion on the Carotenoid Composition of the Fruit of the Normal Red Tomato Genotype, Variety Summer Sunrise

	Dark grown						Light-grown control			
	Immature		Ripe				Immature		Ripe	
			Ripened in darkness		Ripened in light					
	μg/g dry[a] wt	%	μg/g dry wt	%	μg/g dry wt	%	μg/g dry wt	%	μg/g dry wt	%
Phytoene			109.8 ± 34.1	10.0 ± 2.2	64.6 ± 4.2	6.5 ± 0.3			157.1 ± 33.6	9.2 ± 1.1
Phytofluene			81.2 ± 5.9	7.6 ± 1.5	30.4 ± 6.4	3.1 ± 0.7			133.4 ± 25.4	7.8 ± 1.0
ζ-Carotene			7.0 ± 2.9	0.6 ± 0.1	3.5 ± 0.7	0.4 ± 0.1			29.4 ± 8.9	1.7 ± 0.5
Neurosporene			1.2[b]	0.1	1.2 ± 0.9	0.1 ± 0.1			2.4 ± 0.9	0.1 ± 0.1
Lycopene			869.9 ± 222.9	78.4 ± 2.7	862.7 ± 40.4	86.2 ± 1.0			1252.4 ± 120.2	73.7 ± 1.7
γ-Carotene			4.6 ± 1.6	0.4 ± 0.1	4.2 ± 1.5	0.4 ± 0.1			9.5 ± 3.3	0.5 ± 0.1
β-Carotene	0.7 ± 0.2	100	31.6 ± 4.5	3.0 ± 0.6	34.8 ± 8.4	3.5 ± 0.9	18.3 ± 2.5	100	117.6 ± 14.6	6.9 ± 0.4
Mean of total	0.7 ± 0.2		1104.6 ± 255.0		1001.3 ± 32.4		18.3 ± 2.5		1701.4 ± 194.0	

[a] Average of three replications. [b] Detected in one sample only.

Table II. Effect of Light on the Carotenoid Content of the Fruit of the High-Beta Tomato Genotype

	Dark grown						Light-grown control					
	Immature		Ripe				Immature		Ripe[a]		Ripe[b]	
			Ripened in darkness		Ripened in light							
	μg/g dry wt	%	μg/g dry wt	%	μg/g dry wt	%	μg/g dry wt	%	μg/g dry wt	%	μg/g dry wt	%
Phytoene			139.4 ± 4.3	16.6 ± 2.4	147.1 ± 19.8	15.9 ± 2.0			160.9 ± 11.9	6.8 ± 0.4	86.7 ± 30.1	5.5 ± 1.1
Phytofluene			42.4 ± 0.7	5.0 ± 0.8	56.6 ± 12.4	6.0 ± 1.3			79.8 ± 3.2	3.4 ± 0.1	42.2 ± 11.9	2.8 ± 0.6
ζ-Carotene			1.0 ± 0.2	0.1 ± 0.0	1.2 ± 0.2	0.1 ± 0.0			3.8 ± 0.4	0.2 ± 0.1	2.3 ± 1.5	0.1 ± 0.1
Lycopene			13.2 ± 1.6	1.5 ± 0.1	10.2 ± 6.1	1.1 ± 0.7			1.3 ± 0.6	0.05 ± 0.02	1.2 ± 1.6	0.1 ± 0.1
β-Zeacarotene			1.4 ± 0.5	0.2 ± 0.1	1.5 ± 0.2	0.2 ± 0.1			4.5 ± 0.5	0.2 ± 0.0	2.4 ± 1.6	0.2 ± 0.0
γ-Carotene			13.1 ± 4.3	1.5 ± 0.3	12.1 ± 2.0	1.3 ± 0.2			36.7 ± 1.1	1.6 ± 0.1	26.1 ± 12.4	1.6 ± 0.1
β-Carotene	0.8 ± 0.2	100	643.8 ± 130.9	73.8 ± 3.3	696.9 ± 62.3	75.3 ± 4.1	20.5 ± 0.5	100.0	2077.7 ± 36.7	87.9 ± 0.5	1432.2 ± 570.7	89.8 ± 1.8
Mean of total	0.8 ± 0.2		854.3 ± 142.0		924.5 ± 50.0		20.5 ± 0.5		2364.8 ± 42.1		1593.1 ± 624.0	

[a] Unbagged control. [b] Wrapped with clear polyethylene bag.

Table III. Effect of Light on the Carotenoid Distribution in the Fruit of the Apricot Tomato Genotype

	Dark grown						Light-grown control			
	Immature		Ripe				Immature		Ripe	
			Ripened in darkness		Ripened in light					
	μg/g dry[a] wt	%	μg/g dry wt	%	μg/g dry wt	%	μg/g dry wt	%	μg/g dry wt	%
Phytoene										
Phytofluene					Trace	Trace			Trace	Trace
ζ-Carotene										
Neurosporene										
Lycopene			248.8 ± 85.1	81.0 ± 0.8	261.4 ± 77.2	76.1 ± 10.8			14.0 ± 1.8	13.1 ± 3.3
γ-Carotene			5.0 ± 1.6	1.7 ± 0.1	6.6 ± 0.8	2.0 ± 0.1			4.1 ± 1.3	3.8 ± 0.8
β-Carotene	0.7 ± 0.1	100	52.9 ± 15.6	17.4 ± 0.7	70.5 ± 25.3	21.6 ± 10.9	36.5 ± 0.4	100	90.2 ± 13.8	83.2 ± 2.5
Mean of total	0.7 ± 0.1		305.7 ± 102.2		338.5 ± 52.8		36.5 ± 0.4		108.2 ± 13.3	

[a] Average of three replications.

Table IV. Effect of Light on the Carotenoid Composition of the Fruit of the Tangerine Tomato Genotype, Variety Golden Jubilee

	Dark grown						Light-grown control					
	Immature		Ripe				Immature		Mature green		Ripe[c]		Ripe[d]	
	μg/g dry[a] wt	%	Ripened in darkness μg/g dry wt	%	Ripened in light[b] μg/g dry wt	%	μg/g dry wt	%	μg/g dry wt	%	μg/g dry wt	%	μg/g dry wt	%
Phytoene	82.4 ± 8.3	43.6 ± 3.8	374.7 ± 8.3	50.4 ± 5.5	363.3 ± 48.6	53.4 ± 4.4	15.8 ± 5.8	25.7 ± 5.4	20.7 ± 8.5	21.7 ± 2.7	768.9 ± 185.9	34.8 ± 10.4	1075.0 ± 319.1	33.8 ± 2.9
Phytofluene	26.4 ± 0.7	13.7 ± 0.8	116.8 ± 24.3	15.6 ± 1.4	96.1 ± 3.2	15.1 ± 1.3	9.2 ± 1.8	15.2 ± 1.5	13.6 ± 5.2	14.3 ± 1.3	397.4 ± 91.5	17.7 ± 3.5	538.0 ± 150.1	17.0 ± 1.6
ζ-Carotene	23.8 ± 1.3	12.3 ± 0.3	122.9 ± 48.6	16.1 ± 3.9	96.7 ± 2.7	15.2 ± 1.2	7.6 ± 1.2	12.8 ± 2.5	13.8 ± 5.1	14.6 ± 1.5	318.0 ± 30.4	14.3 ± 1.7	659.8 ± 216.2	20.6 ± 2.4
Neuro-sporene					5.2 ± 1.8	0.9 ± 0.4					57.8 ± 43.9	2.5 ± 1.7	96.2 ± 11.5	3.1 ± 0.4
Lycopene					1.8 ± 0.5	0.3 ± 0.05					108.5 ± 112.7	4.5 ± 4.2	54.9 ± 5.9	1.8 ± 0.2
β-Carotene			1.0 ± 0.4	0.1 ± 0.05	1.9 ± 0.7	0.3 ± 0.1	15.3 ± 1.3	26.1 ± 5.9	16.0 ± 3.0	17.4 ± 1.6	22.1 ± 20.5	0.9 ± 0.7	27.7 ± 6.2	0.9 ± 0.2
Proneuro-sporene	19.3 ± 1.7	10.0 ± 1.2	68.1 ± 20.2	8.9 ± 1.1	31.7 ± 0.4	4.7 ± 0.5	3.7 ± 0.8	6.1 ± 0.8	5.5 ± 1.4	6.4 ± 3.0	96.4 ± 13.2	4.4 ± 0.8	150.7 ± 48.7	4.7 ± 0.9
Prolycopene	41.2 ± 5.0	21.3 ± 2.3	68.4 ± 15.4	9.1 ± 0.8	44.1 ± 5.9	10.0 ± 1.3	8.6 ± 2.6	14.1 ± 2.4	23.9 ± 5.9	25.7 ± 1.0	476.3 ± 213.2	21.0 ± 8.1	549.9 ± 98.0	18.2 ± 5.6
Mean of total	193.1 ± 9.3		751.9 ± 145.2		928.1 ± 498.5		60.3 ± 1.3		93.6 ± 26.7		2245.4 ± 265.6		3152.1 ± 702.0	

[a] Average of three replications. [b] Average of two replications only. [c] Unbagged control. [d] Wrapped with clear polyethylene bag.

The carotenoid composition of dark-grown tangerine fruit subsequently exposed to light (light-ripened, Table IV) is qualitatively the same as in the control fruit wrapped with clear polyethylene, or the unbagged fruit (Table IV). Dark-grown light-ripened fruit had the lowest total carotenoid content while the fruit in clear polyethylene bags had the highest pigment level. The latter group also had the highest mean temperature during the entire growing period (unpublished data, 1971). The temperature recorded inside the aluminum foil bag of the dark-grown fruits, on the other hand, did not differ significantly from that obtained for fruits which were not bagged. Thus, the aluminum foil cover effectively prevented a drastic increase in temperature inside the bag.

Phytoene accumulated in the dark-grown fruits, which suggests that light is necessary for the desaturation steps. Subbarayan et al. (1970) reported that the conversion of phytoene to phytofluene and lycopene by an enzyme preparation from spinach leaves is reduced in the absence of light.

The prolycopene values in Table IV represent only the amount of the pigment in the main prolycopene band. Three other minor bands were observed between the main prolycopene band and lycopene on the MgO–Super-Cel column. These were identified as poly-cis isomers of lycopene (Zechmeister and Pinckard, 1947; LeRosen and Zechmeister, 1942). Other pigments detected on the column include a poly-cis γ-carotene isomer which cochromatographed with a γ-carotene isomer between ζ-carotene and proneurosporene. In all dark-grown fruits examined, however, the poly-cis γ-carotene band was adsorbed on top of the MgO–Super-Cel column above lycopene. The absorption spectrum of the two pigments in light petroleum before (λ_{max} 454, 432 nm) and after (λ_{max} 483, 455, 432 nm) iodine catalysis is similar to the published spectrum (Zechmeister, 1963).

Obviously light, or the absence of it, brings about certain structural modifications causing this change in adsorptivity. Further work is necessary in order to establish the chemical structure of the pigment.

DISCUSSION

The requirement for light for the maximum synthesis of β-carotene, the major plastid carotene of the green tomato fruit, is apparent in all four tomato genotypes studied. The presence of a light-dependent biosynthetic pathway for carotenoids in the green tomato fruit similar to that found in other photosynthetic tissue is thus confirmed. The accumulation of the normal carotenoid complement during ripening in darkness, on the other hand, indicates that carotenogenesis in the chromoplast is independent of light; the pathway may be stimulated by light, but light is not required for induction. Since the chromoplasts of the ripe fruit develop from plastids of the green fruit (Rosso, 1968; Harris and Spurr, 1969a,b), it is possible that there are two independent enzyme systems for carotenoid synthesis including β-carotene in the organelle. One would be initially repressed while the other is fully functional at the initiation of chlorophyll synthesis. Derepression is probably induced by some stimulus other than light. Thus, it would appear from the data that there are two pathways for the synthesis of β-carotene in the tomato fruit. One pathway is light dependent, while the other is independent of light. The light-dependent pathway for β-carotene synthesis is probably the one associated with the chlorophyll-forming system of the green tissue of the immature fruit. Essentially the β-carotene is a structural carotenoid found in the grana of the chloroplast in contrast to the chromoplastic β-carotene

which exists in oil droplets in the mature plastid (Rosso, 1968; Harris and Spurr, 1969a,b) as secondary carotenoids.

The residual β-carotene in the normal red, apricot, and tangerine genotypes that is formed in the dark is probably not a carry-over from the pathway in the etioplast since the latter pathway is nonfunctional as the data for the immature fruits show. There is evidence (Harris and Spurr, 1969a) that in the tomato fruit, the structural β-carotene is degraded during the transition from chloroplast to chromoplast. The β-carotene in dark-ripened fruits (Tables I–IV) probably results from the nonspecificity of the cyclizing enzyme for its substrate. Thus, when the grana structure (where the carotenogenic enzyme system is presumably confined in the chloroplast) disintegrates, the cyclizing enzyme is released into a medium where the proper substrates abound, i.e. lycopene, neurosporene, and γ-carotene. The suggestion could explain the differences in the results obtained with in vitro labeling experiments with plastid preparations (Hill et al., 1971; Decker and Uehleke, 1961; Kushwaha et al., 1969) and the data obtained with various inhibitors (Goodwin and Jamikorn, 1952; Tomes, 1963; Raymundo et al., 1970).

In the apricot tomato (Table III), lycopene and β-carotene respond to the light stimulus independently rather than as precursor and product, respectively. Light enhances the synthesis of β-carotene. It inhibits lycopene formation and, consequently, the total carotenoid content of the ripe fruit is reduced.

Light, in conjunction with the *at* allele, is able to selectively suppress the lycopene-forming system. It does not affect the cyclase(s) activity. Otherwise, a concomitant stoichiometric increase in the concentration of the cyclic carotenoids should result if the rate of cyclization is increased in response to light. If light promotes the cyclization of lycopene → γ-carotene → β-carotene, it will have to preferentially block lycopene synthesis in a manner that does not diminish or impede the formation of β-carotene to account for the results in Table III. A light-dependent synthesis of cyclic carotenoids has been reported by Claes (1957) in *Chlorella vulgaris*, but its mode of action is not fully understood. Neither does the genetic scheme (Kirk and Tilney-Bassett, 1967; Khudairi, 1972) proposed for tomato account for the seemingly discordant response of the two pigment systems in the *atat* fruit to light.

The results of genetic studies on the inheritance of genes affecting tomato fruit color suggest biosynthetic autonomy between the carotenogenic systems of the chloroplast and the chromoplast. The genes which determine tomato fruit color, with the exception of the high pigment (hp^+/hp) and the ghost (gh^+/gh) genes (Baker and Tomes, 1964; Mackinney et al., 1956) do not affect the carotenoid composition of the leaves and green fruits. The genes that control carotenogenesis in the photosynthetic tissue and in the ripe fruit appear to be inherited independently. Furthermore, mutations in the chloroplasts do not necessarily affect the pigment composition of the chromoplasts, and vice versa (Kirk and Tilney-Bassett, 1967). The leaves and the very young fruit of the tangerine tomato (*tt*), for example, do not contain the characteristic poly-cis carotenoids that accumulate in the ripe fruit (Mackinney et al., 1956). The low total pigment gene, r^+/r, does not affect the carotenoid content of the green leaves and green fruit even though in the low pigment tomato (*rr*) the ripe fruit contains only about 5% of the total carotenoids of the r^+r^+ fruit (LeRosen et al., 1941; Mackinney and Jenkins, 1952; Jenkins and Mackinney, 1955). On the other hand, the *hp* allele increases the carotenoid and chlorophyll contents of both leaves and fruits (Baker and Tomes, 1964). In the ghost mutant only a small fraction of the chlorophyll accumulates in the tissue. While a large amount of phytoene is present in both leaves and fruit (Mackinney et al., 1956), the colored carotenoids are found only in areas containing chlorophyll. The effect of the *hp* and *gh* alleles is probably similar to that observed in maize (Robertson et al., 1966) where the modifiers of the albino mutant gene limit the amount of the chlorophyll produced to that level which can be protected by the carotenoid.

There is very little variation in the carotenoid content of the green fruit among various tomato fruit color mutants. In fact, the β-carotene contents of the green fruit of the normal red (B^+B^+,r^+r^+) and the low pigment (B^+B,rr) mutants are essentially the same, i.e. 29 ± 6, 25 ± 5, and 26 ± 6 μg/g dry weight (Harris and Spurr, 1969a), respectively. On ripening, more β-carotene is synthesized in the normal red (67 ± 7 μg/g) and in the high-beta mutant (675 ± 315 μg/g), while in the low pigment mutant (7 ± 1 μg/g) further synthesis of β-carotene is terminated.

In addition, the apricot gene in the homozygous recessive form, *atat*, specifically inhibits lycopene formation. The *atat* and at^+at^+ fruits, however, have the same amount of β-carotene (Jenkins and Mackinney, 1955; Tomes et al., 1958). The total carotenoid content of the apricot (*atat*) fruit is only about 10% of the carotenoid content of the red (at^+at^+) genotype, suggesting that the pigment lost is mainly lycopene and the associated polyene precursors. A logical explanation to this observation is that β-carotene and lycopene are formed independently and that the recessive allele preferentially inhibits the lycopene pathway in the apricot (*atat*) fruit. The *B* allele analogously has no effect on the β-carotene fraction of the tangerine tomato fruit (Tomes et al., 1956).

The β-carotene fraction which is not affected by the *at* and *B* alleles could be the structural carotenoid of the green tissue. This structural β-carotene may be the pigment required for the formation of the normal lamellar system of maturing chloroplasts as well as for the protection of chlorophyll (Anderson and Robertson, 1960) and the grana structure against photodynamic action (Walles, 1967; Blass et al., 1959; Yamamoto et al., 1962; Burns et al., 1971). This β-carotene could be that fraction in the normal red (B^+B^+), intermediate beta (*BB*), high-beta (*BB*), delta (*DelDel*), tangerine (*tt*), and high pigment (*hphp*) tomatoes which is not affected by ripening at 32°C (Goodwin and Jamikorn, 1952; Tomes, 1963; Tomes et al., 1958), by Me_2SO treatment (Raymundo et al., 1967, 1970), and γ-irradiation (Burns and Desroisier, 1957; Villegas et al., 1972).

The light-induced accumulation of neurosporene and lycopene in the tangerine tomato is probably the result of a photochemical conversion of proneurosporene and prolycopene to their corresponding all-trans isomer rather than due to an enzyme-catalyzed transformation. Similar photoconversions have been demonstrated both in vivo and in vitro in tangerine tomato fruit pulp (Ulrich and Mackinney, 1968) and in *Chlorella* (Claes and Nakayama, 1959). In the latter carotenogenic system, the process is catalyzed by blue light; the transformation is sensitized by chlorophyll in red light and is inhibited by oxygen.

The β-carotene that accumulates in the dark, on the other hand, is probably the pigment associated with the etioplast and does not originate by isomerization of a poly-cis β-carotene since the latter is not found in the tangerine fruit.

The data reported herein are consistent with separate pathways for the formation of the structural carotenoids in the chloroplast and the secondary carotenoids in the

chromoplast of the tomato fruit.

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Distribution of Protein within Sweet Potato Roots (*Ipomea batatas* L.)

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Distribution of protein within roots of three sweet potato cultivars was studied. End-to-end gradients of protein concentration were small but significant in Jewel and Centennial, with higher concentration toward the stem end. Circumferential protein gradients in Jewel and Centennial were consistent year to year but were not statistically significant. Cultivar 213×228-1 had no significant gradients. There was no evidence of radial gradients in any cultivar. All gradients were too small to suggest modified processing to obtain high protein products.

Sweet potato could be a significant source of protein with some varieties containing up to 9% protein (Purcell et al., 1972). Protein contents differ between cultivars and possibly from year to year (Purcell et al., 1976). Some of the reported variation might be due to sampling error caused by uneven distribution of protein in roots. Uneven distribution of starch and carotene in roots apparently has been recognized, since it was standard practice to cut a longitudinal section from the root as a sample (Anderson, 1956).

If protein were unevenly but consistently distributed within roots, sampling might be improved and processing modified to increase protein content of products from sweet potatoes. We have studied sweet potatoes to determine whether protein distribution does vary and whether variation is influenced by cultivar, root size, or

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