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Technical paper

Relationship of Carotene and Xanthophyll Production in Tomato Strains and Their Progenies

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Gene *B* and gene *Del* involved in the biosynthesis of carotenoids, particularly xanthophylls such as tunaxanthin, lutein and zeaxanthin, which are contained in the fruits of various tomato strains and their hybrids were studied. It is known that gene *B* blocks the biosynthesis of lycopene and transforms lycopene to β-carotene via β-zeacarotene and γ-carotene. This study revealed that gene *B* is also involved in the biosynthesis of zeaxanthin, a xanthophyll of the β-ionone end group, and increases the production of lutein. Gene *Del* is also known to block the biosynthesis of lycopene and produce δ-carotene, α-carotene and ε-carotene. It was revealed by the present study that gene *Del* also markedly increases the biosynthesis of lutein, a xanthophyll of the ε-ionone end group. Further, tunaxanthin which was previously undetected in tomatoes was observed for the first time. This study has thus confirmed that gene *Del* induces its production.

Keywords: tomato, xanthophylls, tunaxanthin, lutein, zeaxanthin

There have been many reports on the biosynthesis of carotenoids in tomatoes in which hereditary factors are estimated by the contents of carotenoids in the phenotypes obtained by crossing various genotypic strains. Oxygenated derivatives, however, have been neglected. Using current distribution techniques, Tomes *et al.* (1963) were able to demonstrate the presence of a large number of xanthophylls, and Goodwin & Williams (1965) reported carotene-epoxides using TLC.

In this study, we examined the separation of tomato xanthophylls using HPLC and the biosynthetic differences between carotenes and xanthophylls, especially tunaxanthin, lutein and zeaxanthin, using tomato strains and their hybrids harvested under the same conditions.

Materials and Methods

Materials The strains used in this study are Red (red fruit of Marglobe), Yellow (yellow fruit without lycopene), Tangerine (yellow orange fruit with high prolycopene), Beta orange (High Beta) and Delta (High pigment Delta) strains. Table 1 shows the history and phenotype of these strains. To closely compare the relationship between the behavior of gene *B* and gene *Del* and those of other genes, the F_1 generation was also studied: the F_1 generation was obtained by crossing Red×Tangerine, Yellow×Tangerine, Yellow×Beta orange, Tangerine×Beta orange, Yellow×Delta, Tangerine×Delta and Beta orange×Delta. Tables 2 to 3 show the phenotype (color) and genotype of the F_1 generations used in this study. The strains (Mackinney, 1956) used in this study were generously supplied by the late Dr. G. Mackinney to the author in 1970 and cultivated between March and October annually up to 1992.

Methods Preparation, purification and extraction of the pigment were carried out by the method of Hirota *et al.* (1995).

The spectra of the pigment were measured by the method previously reported (Hirota *et al.*, 1993).

The HPLC system (Davies, 1976) used for the pigment analysis consisted of a high-pressure pump (Shimadzu LC-6AD), a UV-visible detector (Shimadzu SPD-6AV) and a Shimadzu C-R3A integrator. The column used for the hydrocarbon carotenoid analysis was Inertsil ODS 80A (GL science, 4.6×150 mm i.d.). The mobile phase was 5% ethanol-acetonitrile. The flow rate monitored at 286, 347 and 440 nm was 1.2 ml/min.

The column used for hydroxy carotenoid analysis was a Unisil Q CN (GL Science, 4.6×250 mm i.d. with 5 μm particle size). Stepwise gradient chromatography was employed. The mobile phase compositions were (A) *n*-hexane (5 min), (B) *n*-hexane-dichloromethane (88:12, 5.01–8 min), (C) *n*-hexane-dichloromethane (84:16, 8.01–29 min), (D) *n*-hexane-dichloromethane (75:25, 29.01–38 min) and (E) *n*-hexane-dichloromethane-ethanol (74:25:1, 38.01–60 min). The flow rate monitored at 440 nm was 1.0 ml/min.

The molecular extinction coefficients (Davies, 1976) shown in Table 4 were used for calculation of the carotenoid contents. Carotenoid concentrations in solution were obtained and the calibration curves in HPLC were prepared. The formula is shown below (Arinobu, 1993).

Concentration of the reference solution containing carotenoid (μg/ml)

$$= A \times M_{(\text{carotenoid})} \times \varepsilon^{-1} \qquad (1)$$

where M (xanthophyll) is molecular weight of the carotenoid.

Table 1. Carotene contents of strains (μg/g).

Strains	Carotenoid contents: Gene marks	Coloring	Phytoene	Phytofluene	α-Carotene	β-Carotene	γ-Carotene
Red	r^+	Red	21.6	3.9	0.0	4.6	0.8
Yellow	r	Yellow	0.0	0.0	0.0	1.4	0.0
Tangerine	t	Yellow orange	35.7	5.1	0.0	2.9	0.1
Beta orange	B	Orange	15.5	3.5	0.0	54.8	2.3
Delta	Del	Brick reddish orange	12.5	3.3	1.3	3.8	0.5

δ-Carotene	ε-Carotene	ζ-Carotene	Proneurosporene	Neurosporene	Prolycopene	Lycopene	Xanthophylls
0.0	0.00	0.3	0.0	0.0	0.0	54.3	6.4
0.0	0.00	0.0	0.0	0.0	0.0	0.0	2.7
0.0	0.00	36.0	9.8	3.1	32.2	2.1	4.9
0.0	0.00	0.1	0.0	0.0	0.0	4.4	5.6
23.8	0.07	0.1	0.0	0.0	0.0	22.9	9.3

Red: known in America before Columbus. Marglobe plant marketed, generally; Yellow: known in America before Columbus. Yellow group plant. Used as curiosity, occasionally; Tangerine: known in America before Columbus. Golden Jubilee plant, Cultivated in areas; Beta orange: discovered by Tomes and Porter at Purdue, 1953 (obtained by hybridization). High-β-carotene plant; Delta: discovered by Tomes and Porter at Purdue, 1954 (obtained by hybridization).

Table 2. Carotene contents of hybrids (μg/g).

Hybrids	Carotenoid contents: Coloring	Phytoene	Phytofluene	α-Carotene	β-Carotene	γ-Carotene	δ-Carotene
Yellow×Delta (F_1)	Brick red	17.9	3.5	1.0	3.6	0.4	9.4
Tangerine×Delta (F_1)	Brick red	12.7	3.3	1.0	3.7	0.6	10.7
Beta orange×Delta (F_1)	Brick orange red	17.3	3.4	2.2	8.0	0.5	12.6

ε-Carotene	ζ-Carotene	Proneurosporene	Neurosporene	Prolycopene	Lycopene	Xanthophylls	Probable genotypes
0.01	0.1	0.0	0.0	0.0	25.2	4.9	$r^+r^+t^+t^+B^+B^+Del^+Del$
0.01	0.2	0.0	0.0	0.0	23.2	5.4	$r^+r^+t^+t^+B^+B^+Del^+Del$
0.02	0.1	0.0	0.0	0.0	14.3	9.4	$r^+r^+t^+t^+B^+B\ Del^+Del$

The plus sign indicates the condition of the gene in the red-fruited variety as Marglobe. Thus r^+, t^+, B^+, Del^+ are the situation where lycopene predominates in a red-fruited species and r, t, B, Del do not exist under this condition in Marglobe (Mackinney, 1956; Tomes *et al.*, 1963).

Table 3. Lutein, tunaxanthin and zeaxanthin contents of various strains and hybrid strain.

Strain & Hybrids	Coloring	Lutein μg/g	Tunaxanthin μg/g	Zeaxanthin μg/g	Probable genotypes
Red	Red	1.07	n.t.	0.02	$r^+r^+t^+t^+B^+B^+Del^+Del^+$
Yellow	Yellow	0.65	n.t.	0.01	$r\ r\ t^+t^+B^+B^+Del^+Del^+$
Tangerine	Yellow orange	0.95	n.t.	0.02	$r^+r^+t\ t\ B^+B^+Del^+Del^+$
Beta orange	Orange	1.03	n.t.	0.27	$r^+r^+t^+t^+B\ B\ Del^+Del^+$
Delta	Brick reddish orange	8.61	0.32	0.06	$r^+r^+t^+t^+B^+B^+Del\ Del$
Yellow×Delta (F_1)	Brick red	4.24	0.12	0.04	$r^+r^+t^+t^+B^+B^+Del^+Del$
Tangerine×Delta (F_1)	Brick red	4.15	0.13	0.04	$r^+r^+t^+t^+B^+B^+Del^+Del$
Beta orange×Delta (F_1)	Brick orange red	4.19	0.15	0.05	$r^+r^+t^+t^+B^+B\ Del^+Del$

Lutein, tunaxanthin and zeaxanthin: all *trans* form; n.t.: not detected.

$$C=\frac{\text{Peak area}_{(\text{sample})}}{S}\times\frac{V_{(\text{final})}}{W_{(\text{sample})}} \quad (2)$$

where, A=absorbance, M=molecular weight, ε=extinction coefficient, C=concentration (μg/g fresh fruits), S=area×(μg/ml)$^{-1}$, V=solvent volume, W=weight of fresh fruits.

Results and Discussion

HPLC chromatograms of tomato carotenoids are shown in Figs. 1 and 2. UV and visible absorption spectra of the materials, which were extracted and purified from some tomatoes, showed peaks in the spectra at 468, 438 and 415 nm. After the addition of iodine, the peaks were shifted to 465, 435 and 413 nm respectively, and a *cis*-peak appeared at 327 nm. Based on these results, this was confirmed to be tunaxanthin (Davies, 1976).

In ^{1}H-NMR spectra of the samples, which were extracted and purified from tomatoes, the signals of methyl and methylene protons were observed at 0.85, 0.99, 1.25, 1.63, 1.91 and 1.97 ppm of δ values. The main peaks of olefin protons were at 6.09–6.66 ppm and the sub-peaks were at 4.25, 4.41, 5.43 and 5.54 ppm. These results were almost the same as the values for tunaxanthin measured by Englert (1982).

The mass spectrum of the purified samples showed fragment peaks appearing at $m/2$=93, 119, 145, 197, 223, 237, 263, 320, 369, 412, 458, 532 and 558 m/e. The molecular ion peak appeared at m/e=568. The data were almost the same as those repeated for tunaxanthin by Isler (1971).

The carotene content in each strain and its hybrid was calculated using standard curves (Hirota *et al.*, 1993). As shown in Tables 1 and 3, the genotype of each strain was estimated by comparing their colors and the analytical data for carotenes with those reported by Mackinney (1956) and Hirota *et al.* (1993).

The composition and contents of carotenes were characteristically different from strain to strain and from hybrid to hybrid. These biosynthetic differences between carotenes and xanthophylls in the strains and hybrids were compared as

Table 4. Molar extinction coefficients (ε) used for calculation.

Carotenoids	ε	Solvent	Wavelength (nm)
Phytoene	41.2	petroleum ether	286
Phytofluene	81.4	petroleum ether	347
ε-Carotene	167.0	petroleum ether	440
ζ-Carotene	135.2	petroleum ether	399
α-Carotene	150.3	petroleum ether	444
β-Carotene	139.2	petroleum ether	448
δ-Carotene	174.0	petroleum ether	456
Proneurosporene	83.9	petroleum ether	430
Neurosporene	134.5	petroleum ether	435
γ-Carotene	171.0	petroleum ether	459
Prolycopene	102.9	petroleum ether	434
Lycopene	185.2	petroleum ether	472
Tunaxanthin	142.0	acetone	440
Lutein	145.1	ethanol	445
Zeaxanthin	144.5	ethanol	450
β-Cryptoxanthin	131.0	petroleum ether	452

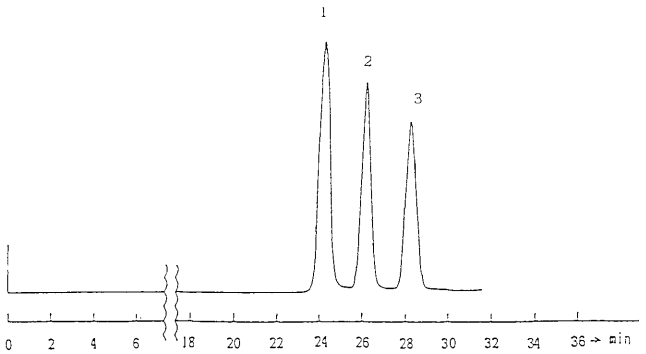

Fig. 1. HPLC of xanthophylls extracted from tomato. 1. Tunaxanthin, 2. lutein, 3. zeaxanthin. Column: Unisil Q CN 250×4.6 mm i.d. Flow rate: 1.0 ml/min, Temp.: 35°C, Detector: VIS 440 nm. Eluent: (A) *n*-hexane for 5 min, (B) *n*-hexane-dichloromethane (92:8) for 5.01 to 8 min, (C) *n*-hexane-dichloromethane (84:16) for 8.01 to 29 min, (D) *n*-hexane-dichloromethane (75:25) for 29.01 to 38 min, (E) *n*-hexane-dichloromethane-ethanol (74:25:1) for 38.01 to 60 min.

shown in Table 3.

In the case of xanthophylls, the lutein content was highest in all strains and hybrids. The strains and their hybrids which increased the xanthophylls of carotenes containing α-ionone (δ-carotene, α-carotene and ε-carotene) also increased biosynthesis of xanthophylls containing β-ionone (zeaxanthin).

Thus, the Delta strain containing the gene *DelDel* and hybrids containing gene *Del*$^+$*Del* had a tendency to promote the biosynthesis of carotenes and xanthophylls containing α-ionone, and the Beta orange strain containing the gene *BB* had a tendency to promote the biosynthesis of carotenes and xanthophylls containing β-ionone. The results were analyzed in the light of heredity.

Mackinney (1956) and Hirota *et al.* (1993) showed that gene *r* regulated the total biosynthesis of carotenes and that *t* participated in the biosynthesis of stereo isomers such as prolycopene and proneurosporene. It was previously reported that gene *B* blocked the biosynthesis of lycopene and transformed lycopene to γ-carotene and β-carotene via β-zeacarotene. Gene *Del* also blocked the biosynthesis of lycopene and transformed lycopene to δ-carotene via α-zeacarotene.

Based on the present results, it appears that gene *B* induced

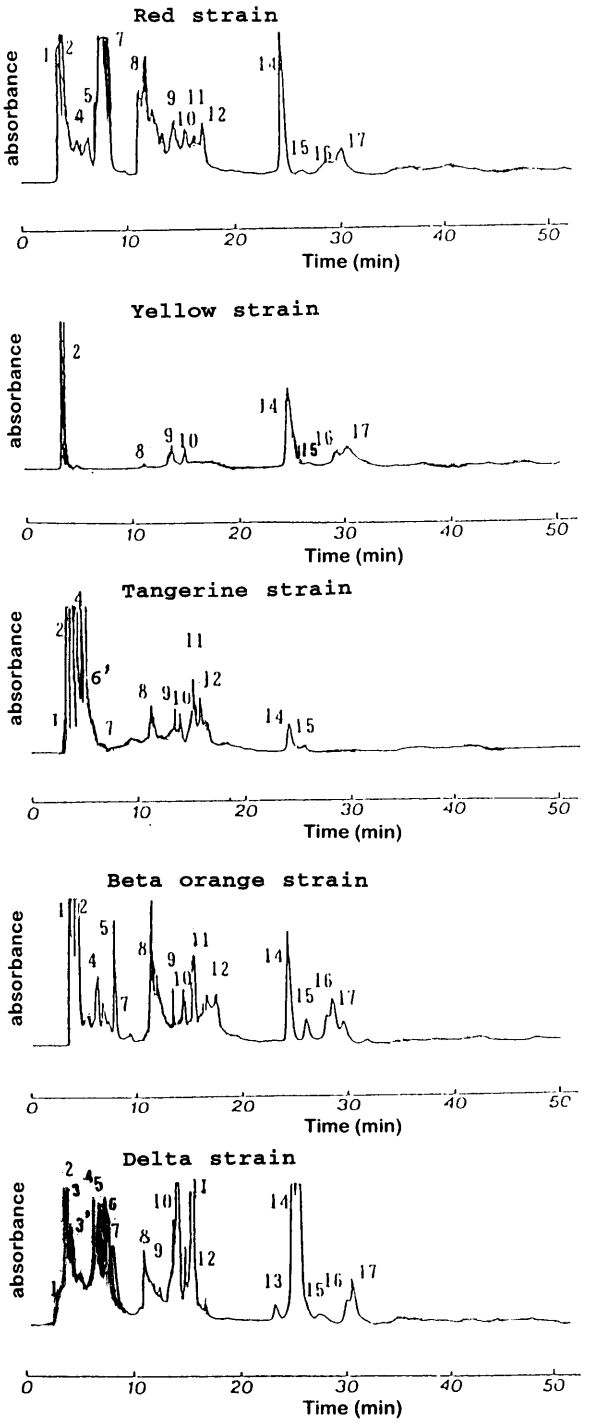

Fig. 2. HPLC (CN) chromatograms of each tomato strain. Column: Unisil Q CN 250×4.6 mm i.d. Eluent: (A) *n*-hexane for 5 min, (B) *n*-hexane-dichloromethane (92:8) for 5.01 to 8 min, (C) *n*-hexane-dichloromethane (84:16) for 8.01 to 29 min, (D) *n*-hexane-dichloromethane (75:25) for 29.01 to 38 min, (E) *n*-hexane-dichloromethane-methanol (74:25:1) for 38.01 to 60 min. Detector: 440 nm; Flow rate: 1.0 ml/min; Temp.: 35°C. 1: β-carotene *cis*-isomers; 2: β-carotene; 3: α-carotene; 3′: ε-carotene 4: ζ-carotene; 5: γ-carotene; 6: δ-carotene; 6′: prolycopene; 7: lycopene; 8: β-cryptoxanthin *cis*-isomer; 9: β-cryptoxanthin *cis*-isomer; 10: β-cryptoxanthin; 11: α-cryptoxanthin *cis*-isomer; 12: α-cryptoxanthin; 13: tunaxanthin; 14: lutein; 15: zeaxanthin; 16: violaxanthin; 17: neoxanthin.

the biosynthesis of zeaxanthin, a xanthophyll of the β-end group, and that gene *Del* induced the biosynthesis of ε-carotene and tunaxanthin, a xanthophyll of the ε-end

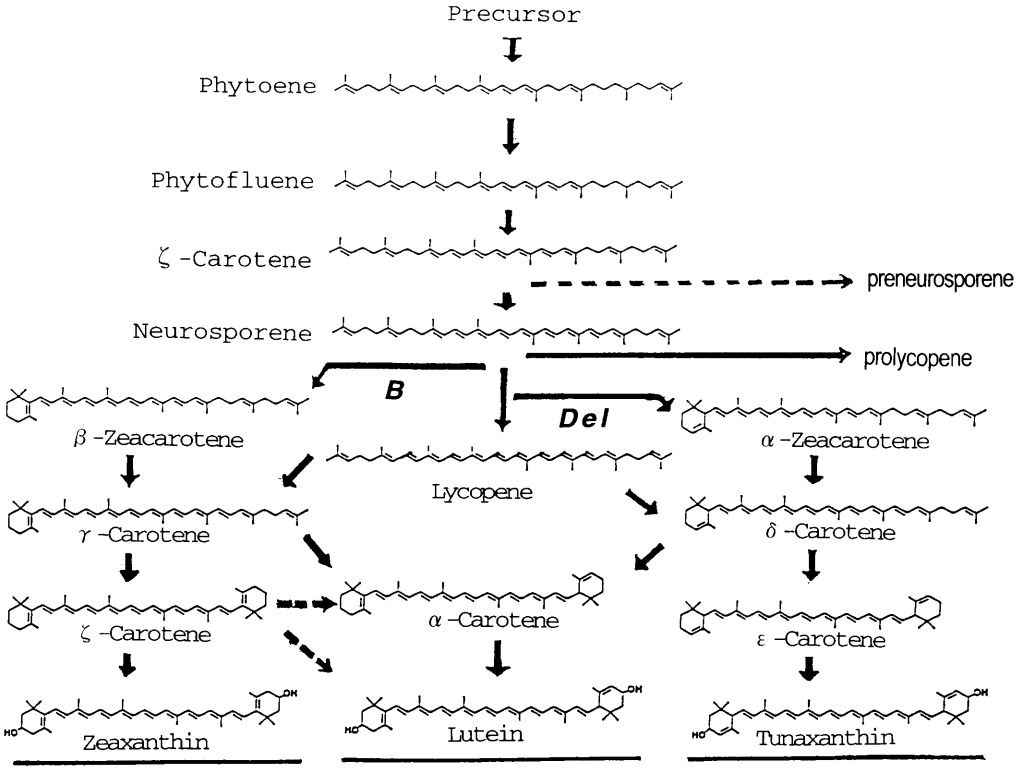

Fig. 3. Pathway scheme of carotenoid biosynthesis. ⟶ sufficient evidence; ⇢ imperfect testimony.

group, and also increased the biosynthesis of lutein containing α-ionone.

Figure 3 shows the scheme of carotenoid biosynthesis which was based on the results of this study and previous studies.

We examined the carotene content and biosynthetic differences of xanthophylls in various tomato strains and hybrids and found that gene *B* and gene *Del* controlled the biosynthesis of xanthophylls as well as that of carotenes. These results are significant in studying the biosynthesis of carotenes and xanthophylls.

We also confirmed the existence of tunaxanthin in tomato fruits for the first time.

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