# THE COMPETITIVE EFFECT OF THE BETA- AND DELTA-CAROTENE GENES ON ALPHA- OR BETA-IONONE RING FORMATION IN THE TOMATO[1]

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IT has been proposed that, in the tomato, lycopene is converted to *beta*-carotene, via *gamma*-carotene (PORTER and LINCOLN 1950). A possible alternative would derive $\gamma$- and $\beta$-carotene from neurosporene via $\beta$-zeacarotene (PORTER and ANDERSON 1962). In either case, the cyclic carotenes would be derived by closing the ring at the end of the carotene molecule. Results from a number of studies (TOMES, QUACKENBUSH, NELSON, and NORTH 1953; TOMES, QUACKENBUSH, and MCQUISTAN 1954; TOMES, QUACKENBUSH, and KARGL 1956, 1958; TOMES 1963) are compatible with the idea that gene *B* ($\beta$-carotene) mediates this conversion. PORTER's hypothesis also suggests that $\delta$- and $\alpha$-carotene are derived by shifting the double bond in the $\beta$-ionone ring to form an $\alpha$-ionone ring (see Figure 1). The gene *Del* (*delta*-carotene) appears to mediate this conversion.

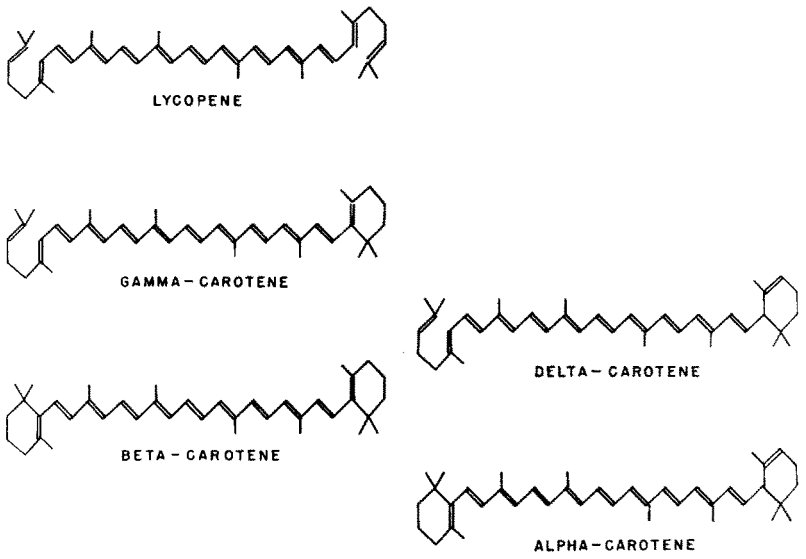


FIGURE 1.—Skeletal structures of lycopene, *gamma*-carotene, *beta*-carotene, *delta*-carotene, and *alpha*-carotene.

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A number of studies, on tomatoes and other organisms, which support the sequential synthesis proposed by PORTER and ANDERSON might be cited. Other authors, including GOODWIN and JAMIKORN (1952), JENKINS and MACKINNEY (1953), MACKINNEY, RICK and JENKINS (1956), SHNEOUR and ZABIN (1957), FRANCIS (1958), PURCELL, THOMPSON and BONNER (1959), and PURCELL (1964), studying pigment synthesis in the tomato, have favored the view that carotene pigments arise in a parallel synthesis rather than by the conversion of one pigment or polyene to another. None of these authors studied strains with *Del* which produce quantities of carotenes with an $\alpha$-ionone ring.

If the sequence is as proposed by PORTER, one would predict that genotypes which produce enhanced quantities of $\beta$-carotene because gene *B* is present would give rise to increased quantities of carotenes with an $\alpha$-ionone ring when both *B* and *Del* are present. That is, if $\beta$-ionone precedes $\alpha$-ionone, genes which increase $\beta$-ionone should provide increased opportunity for the formation of carotenes with an $\alpha$-ionone ring. Data related to this premise are reported in this paper.

## MATERIALS AND METHODS

Crosses between a standard *Del/Del* strain and three different *B/B* strains were made. The *B/B* strains were a high $\beta$-carotene line, an intermediate $\beta$-carotene line, and the variety Caro-Red, an intermediate *beta* type. The high $\beta$-carotene line differs from the intermediate types in the presence of a recessive modifier of *B* (TOMES, QUACKENBUSH, and MCQUISTAN 1954; TOMES, QUACKENBUSH, and KARGL 1956). The high and intermediate *beta* strains were standard parent lines we have used in other studies. The variety Caro-Red was described by TOMES and QUACKENBUSH (1958). Mature fruits of the $F_1$ and $F_2$ progenies were frozen and held at —25°C until analyzed. The pigments from 25g of ripe flesh were extracted, separated chromatographically, and quantities were determined spectrophotometrically, as reported by TOMES (1963). A small $F_3$ progeny from each of the first 20 $F_2$ plants from the cross high *beta* × *delta*, and small $F_4$ progenies from certain $F_3$ selections were grown. Fruit from appropriate progenies were analyzed as above.

## RESULTS AND DISCUSSION

In the normal, red fleshed tomato the major pigment is lycopene. Some $\beta$-carotene is formed during maturation, and there is a small amount of $\gamma$-carotene. The polyenes phytoene and phytofluene are also present. For example, typical ripe fruit values for the red-fleshed variety Campbell 146 are (means in $\mu$g/g fresh weight): lycopene 64.7, $\gamma$-carotene 0.9, $\beta$-carotene 5.1, phytofluene 4.8, and phytoene 11.3. $\alpha$-Carotene, *zeta*-carotene, and neurosporene are often detected in red-fleshed varieties but, if found, are present only in trace quantities.

When gene *B* is inserted into the genotype, there is a tremendous increase in $\beta$-carotene at the expense of lycopene. There is also a shift in flesh color from red to orange. When the *delta* gene is inserted into the normal genotype, some $\delta$-carotene and small quantities of $\alpha$-carotene are formed. The flesh becomes reddish-orange. Typical values for the delta strain used are (means in $\mu$g/g fresh weight): lycopene 16.3, $\gamma$-carotene 4.8, $\delta$-carotene 31.5, $\beta$-carotene 5.0, $\alpha$-carotene 2.4, phytofluene 3.4 and phytoene 10.6. Traces of $\zeta$-carotene, neurosporene, and di-*alpha* or *epsilon*-carotene can be detected. As with *B*, *Del* reduces lycopene. Thus,

δ- and α-carotene are formed at the expense of lycopene. *Del* is a single gene, and qualitatively, at least, it is dominant. Either *Del/Del* or *Del/+* plants produce detectable quantities of δ- and α-carotene. As with β-carotene formed under the influence of *B* (TOMES *et al.* 1953; TOMES *et al.* 1958), *Del* requires dominant genes at the *r*, *t*, and *at* loci to produce δ- and α-carotene in quantity (TOMES, unpublished). In the normal red tomato, lycopene synthesis is inhibited by high temperatures, while normal β-carotene synthesis is not (GOODWIN and JAMIKORN 1952; TOMES *et al.* 1956). Temperatures which inhibit lycopene formation, however, also inhibit the production of enhanced quantities of β-carotene under the influence of *B* (TOMES *et al.* 1956; TOMES 1963). δ-Carotene formed under the influence of *Del* is also sensitive to high temperatures, although it appears to be less sensitive than the β-carotene fraction produced by *B* (TOMES 1963). Similarities between *B* and *Del* suggest that they operate at about the same place in the sequence and that they involve common substrate.

The segregation and recombination of these two genes can be illustrated by one small progeny. Table 1 presents the pigment fractions from an $F_3$ progeny segregating for both *B* and *Del*. Only 13 plants were grown. The phytoene and phytofluene fractions have been deleted in Table 1 since they are not pertinent.

The gene segregation is obvious. Plants 5 and 6 lacked α- and δ-carotene. These plants were +/+ for the *Del* factor, lacking *Del* in dominant form. The remaining plants were *Del*/–. The β-carotene column shows clearly the segregation of *B*. Plants 2, 4, 6, and 11 all had β-carotene values typical of plants which are +/+ at the *B* locus. Plants 1, 3, 5, 7, 8, 9, 10, 12, and 13 all had enhanced β-carotene values, indicating the presence of *B*. Thus, the genotype with respect to *B* and *Del* can be assigned for each plant. Plant 6, for example, is +/+ +/+. Plants 2, 4,

TABLE 1

*The pigment content (μg/g fresh weight) of ripe fruit from individual plants of an* $F_3$ *progeny from the cross, high* beta × delta

| Plant No. | α-carotene | β-carotene | δ-carotene | γ-carotene | lycopene |
|---|---|---|---|---|---|
| 1 | 6.1 | 31.7 | 2.0 | 3.1 | 9.8 |
| 2 | 1.5 | 6.8 | 16.7 | 3.6 | 16.0 |
| 3 | 5.1 | 28.9 | 1.6 | 2.5 | 7.8 |
| 4 | 1.0 | 5.0 | 11.2 | 3.0 | 15.0 |
| 5 | .. | 24.9 | .. | 2.0 | 20.6 |
| 6 | .. | 5.9 | .. | 0.6 | 30.5 |
| 7 | 8.6 | 45.0 | 1.5 | 4.3 | 1.5 |
| 8 | 9.6 | 44.4 | 4.7 | 5.0 | 2.7 |
| 9 | 7.8 | 30.9 | 2.2 | 2.7 | 2.2 |
| 10 | 4.1 | 27.5 | 1.7 | 3.3 | 8.9 |
| 11 | 1.1 | 4.4 | 17.3 | 3.0 | 16.2 |
| 12 | 7.6 | 28.6 | 3.8 | 4.3 | 2.4 |
| 13 | 4.9 | 29.5 | 2.2 | 3.3 | 10.2 |

and 11 are +/+ *Del*/–. Plant 5 is *B*/– +/+, and plants 1, 3, 7, 8, 9, 10, 12, and 13 are *B*/– *Del*/–.

Plant 6 is the only red-fleshed plant in the progeny. It produced the most lycopene, 30.5 μg. The substitution of either *B* or *Del* reduced the lycopene, e.g. plant 5 (*B*/– +/+) produced only 20.6 μg of lycopene, plant 2 (+/+ *Del*/–) only 16.0 μg.

Now, plants 2, 4, and 11 which contained the *Del* factor, but not *B*, produced 16.7, 11.2, and 17.3 μg of δ-carotene. When *B* and *Del* were both present (plants 1, 3, 7, 8, 9, 10, 12, and 13) there was a gross reduction in δ-carotene. The δ-carotene values ranged from 1.5 to 4.7 μg. This reduction in δ-carotene was accompanied by an increase in α-carotene. Plants 2, 4, and 11 produced 1.5, 1.0, and 1.1 μg of α-carotene. Those that were *B*/– *Del*/– ranged from 4.1 to 9.6 μg.

One might argue that *B* simply promotes rings, but *Del* or its allelic alternative determines whether the ring is *alpha* or *beta*. Then *B*/– *Del*/– plants might produce pigment complexes with a greatly enhanced δ-carotene fraction. This is unlikely, for *B* appears capable of cyclizing either end of the molecule, whereas *Del* apparently is efficient in converting only one end. It would appear more likely that *B* causes cyclization to form a *beta*-ionone on both ends, and *Del* shifts the double bond on one end. One would thus predict enhancement of the α-carotene fraction in *B*/– *Del*/– plants. In any case, if *B* and *Del* function as proposed, carotenes containing the α-ionone structure should increase.

These increases in the α-carotene fraction are less than expected. If one adds the two fractions containing the α-ionone ring, it is significant that when *B* and *Del* are in the same genotype, the plant produces less carotene containing an α-ionone ring than when *Del* alone is present. The insertion of *B* actually reduces the quantity of α-ionone. There is competition between the two factors.

This is a strong conclusion to draw from 13 plants in a small $F_3$ progeny, but 85 plants from the $F_2$ of high *beta* × *delta*, 59 plants from the $F_2$ of intermediate *beta* × *delta*, and 148 from the $F_2$ of *delta* × Caro-Red were analyzed. In these $F_2$ progenies the interaction of *B* and *Del* was clear. The plants were classified as illustrated by the small $F_3$ progeny above.

Table 2 summarizes the α- and δ-carotene contents of plants which were +/+

TABLE 2

*The mean* alpha *and* delta- *carotene contents (μg/g fresh weight ± SD) of ripe fruit from plants which are* +/+ Del/–, *and* B/– Del/–, *in the* $F_2$ *progenies of three crosses between* B *and* Del

| | +/+ *Del*/– | | | | *B*/– *Del*/– | | | |
|---|---|---|---|---|---|---|---|---|
| $F^2$ | No. of plants | α-carotene | δ-carotene | *alpha* + *delta* | No. of plants | α-carotene | δ-carotene | *alpha* + *delta* |
| High *beta* × *delta* | 14 | 2.2±0.8 | 15.3±10.9 | 17.5 | 45 | 7.3±2.7 | 1.9±1.9 | 9.2 |
| Intermediate *beta* × *delta* | 15 | 1.4±1.8 | 15.5± 8.4 | 16.9 | 27 | 3.9±1.4 | 4.2±3.2 | 8.1 |
| *Delta* × Caro-Red | 31 | 1.2±0.5 | 15.6± 9.6 | 16.8 | 78 | 5.1±1.7 | 3.4±2.6 | 8.5 |

*Del*/– and *B*/– *Del*/– from these three $F_2$ progenies. Other pigment and polyene fractions are deleted from Table 2.

If only a few plants had been analyzed, one might argue that other genetic modifiers are responsible for this result, since the stocks were not isogenic. But the result is the same in three separate crosses, from a number of plants in each $F_2$ progeny. Unless closely linked, other modifiers by which the parents differed should be segregating at random. Modification is an unlikely explanation.

Table 2 shows that almost twice as much carotene containing the α-ionone is present when *Del* alone is in the genotype. The introduction of *B* impairs α-ionone production.

These data are difficult to explain if α-ionone is being formed from β-ionone as postulated. One would expect that a genotype which produces more β-ionone by virtue of the presence of *B* would yield increased α-ionone, if the *Del* gene performs this conversion. An alternative is that there is no direct conversion, that *B* and *Del* are each specific for the *beta* or the α-ionone configuration and that they compete for common substrate. This suggests parallel derivation.

If *B* and *Del* do act sequentially, it is even less logical to postulate that *Del* functions before *B* to form an α-ionone ring at one end of the molecule. From the acyclic carotenes this would require both cyclization and rearrangement of the double bond. *B* would then close the ring at the other end of the molecule. Unless *B* converts some α-ionone back to the *beta* form, this would still result in at least as much α-ionone when both *B* and *Del* are present, as with *Del* alone.

The final determination of the derivation of the β- and α-ionone rings in tomato carotenoids will have to await critical enzyme studies. Meanwhile, these data cast doubt on the proposed derivation of the α-ionone ring by conversion of the β-ionone structure. These data suggest a parallel derivation of the two types of rings with competition for a limited common precursor.

## SUMMARY

Progenies from crosses between *B*/*B* (β-carotene) and *Del*/*Del* (δ-carotene) strains were analyzed chromatographically to determine the effect of *B* and *Del* on ionone ring formation in the carotenoids produced during maturation in the tomato. *B* alone causes a tremendous increase in carotenes with the β-ionone structure, at the expense of the acyclic carotene lycopene. *Del* alone results in the production of carotenes with an α-ionone ring, especially δ-carotene, again at the expense of lycopene. In *B*/– *Del*/– plants less total α-ionone is formed than in +/+ *Del*/– plants. This is contrary to expectation if the α-ionone ring is derived from the β-ionone ring by shifting the double bond, as has been postulated. The data suggest parallel derivation of the two types of ionone rings with competition for a common precursor.

LITERATURE CITED

FRANCIS, F. J., 1958 Some observations on carotenoid biosynthesis in tomatoes. Proc. Am. Soc. Hort. Sci. **71**: 349–355.

GOODWIN, T. W., and M. JAMIKORN, 1952 Biosynthesis of carotenes in ripening tomatoes. Nature **170**: 104–105.

JENKINS, J. A., and G. MACKINNEY, 1953 Inheritance of carotenoid differences in the tomato hybrid yellow × tangerine. Genetics **38**: 107–116.

LINCOLN, R. E., and J. W. PORTER, 1950 Inheritance of $\beta$-carotene in tomatoes. Genetics **35**: 206–211.

MACKINNEY, G., C. M. RICK, and J. A. JENKINS, 1956 The phytoene content of tomatoes. Proc. Natl. Acad. Sci. U.S. **42**: 404–408.

PORTER, J. W., and D. G. ANDERSON, 1962 The biosynthesis of carotenes. Arch. Biochem. Biophys. **97**: 520–528.

PORTER, J. W., and R. E. LINCOLN, 1950 I. Lycopersicon selections containing a high content of carotenes and colorless polyenes. II. The mechanism of carotene biosynthesis. Arch. Biochem. **27**: 390–403.

PURCELL, A. E., 1964 Biosynthetic relationships between carotenes. Arch. Biochem. Biophys. **105**: 606–611.

PURCELL, A. E., G. A. THOMPSON, JR., and J. BONNER, 1959 The incorporation of mevalonic acid into tomato carotenoids. J. Biol. Chem. **234**: 1081–1084.

SHNEOUR, E. A., and I. ZABIN, 1957 On the biosynthetic relationship between lycopene and colorless polyenes in tomatoes. J. Biol. Chem. **226**: 861–866.

TOMES, M. L., 1963 Temperature inhibition of carotene synthesis in tomato. Botan. Gaz. **124**: 180–185.

TOMES, M. L., and F. W. QUACKENBUSH, 1958 Caro-Red, a new provitamin A rich tomato. Economic Botany **12**: 256–260.

TOMES, M. L., F. W. QUACKENBUSH, and M. MCQUISTAN, 1954 Modification and dominance of the gene governing formation of high concentrations of *beta*-carotene in the tomato. Genetics **39**: 810–817.

TOMES, M. L., F. W. QUACKENBUSH, and T. E. KARGL, 1956 Action of the gene *B* in biosynthesis of carotenes in the tomato. Botan. Gaz. **117**: 248–253. —— 1958 Synthesis of B-carotene in the tomato fruit. Botan. Gaz. **119**: 250–253.

TOMES, M. L., F. W. QUACKENBUSH, O. E. NELSON, JR., and B. NORTH, 1953 The inheritance of carotenoid pigment systems in the tomato. Genetics **38**: 117–127.