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THE PHYTOENE CONTENT OF TOMATOES

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With an appropriate genetic makeup, the fruit of the tomato, *L. esculentum*, is capable of synthesizing substantial amounts of carotenoid pigments. The common red-fruited varieties may contain 70–150 μg. of all-*trans* lycopene per gram of fresh fruit. Beta-orange types, first obtained by Lincoln and Porter,[1] contain comparable concentrations of β-carotene. A single gene difference between the two at the *B* locus determines orange versus red phenotypes. The present study is an outcome of the discovery by O. Brauer and C. M. Rick—made in-

dependently also by Dr. A. E. Thompson, of the University of Illinois[2]—of a mutant, "ghost," an albino with an unstable chlorophyll deficiency which appeared spontaneously in tomato lines having normal red fruit. Ghost is the manifestation of a single completely recessive gene *gh*, and the inheritance is not complicated by cytoplasmic factors or low viability.

Ghost seedlings start their development with green cotyledons but rapidly lose chlorophyll in subsequent growth. Most tissue above the first true leaf is entirely white, except for purple zones due to anthocyanin. The grafting of normal scions on ghost stock frequently induces emergence of shoots with some green tissue from the *gh* stock. Such grafted plants and rare ungrafted seedlings can flower and set fruit.

The fruit is somewhat subnormal in size and milky white in color until maturity, when the skin develops the noncarotenoid alkali-soluble yellow pigment typical of the parent line and of normal tomatoes. Occasional immature fruits show faint green streaks or blotches. These areas will ultimately turn red. In such cases the mucilage surrounding the seeds may be green.

We have found high concentrations of phytoene in mature fruit of ghost, 160–220 μg/gm, levels comparable with those of lycopene in the red, or of β-carotene in the beta-orange, phenotypes. However, the effect of *gh gh* is radically different from that of other gene pairs examined (e.g., tangerine, *tt*, yellow, *rr*, etc.) because the development of chlorophyll is also inhibited.

Before the significance of phytoene accumulation in ghost can be assessed, its occurrence in fruit of our current lines had to be established. We have therefore made a detailed analysis of fruit from nine available genotypes at three stages of maturity, to determine whether phytoene accumulates. The leaves of the nine plants were also examined for carotenoid differences, and we report also on ghost and its hybrid with red, namely, the heterozygote *gh*[+] *gh*.

<center>EXPERIMENTAL</center>

1. *Fruit.*—Samples from nine different green-leaved genotypes and ghost were collected and analyzed at different stages of ripening (green, green-ripe, and ripe) for phytoene and β-carotene. Extractions were made as previously described,[3] the sample being blended with acetone and the carotenoid taken up in petroleum ether. Impurities were found in the acetone which were transferred to petroleum ether and which had measurable absorption *ca.* 260 mμ. The use of methanol is therefore to be preferred. With care, however, the effect of the impurity may be minimized by chromatography on a column of MgO-SiO$_2$. The bulk of the impurity is eluted immediately preceding the phytoene. The uncertainty is greatest where the phytoene content is least.

2. *Leaf Material.*—Leaf samples were collected somewhat late in the season, and, although only healthy tissue was selected, one cannot be certain that the samples were strictly comparable. Samples 2–5 gm. in size were weighed, blended in methanol, and treated as before, except for a saponification step to remove the chlorophyll.

3. *Calculations.*—The values of the absorption coefficients used for computing concentrations were as follows: for β-carotene, 250 at 450 mμ, and for phytoene, 85 at 285 mμ,[4] in liters per gram, centimeter, in petroleum ether solution. In the

case of two unidentified pigments (Table 1) the value arbitrarily selected for pigment I was 250 at 465 mμ, and for pigment II, 200 at 420 mμ.   Pigment I might be neurosporene, but pigment II is not ζ-carotene.

<center>RESULTS</center>

In Figure 1 is shown the curve for optical density as a function of wave length, for a crude extract, in petroleum ether solution, of mature ghost fruit.   The same extract was used for both visible and ultraviolet portions of the curve, although for the latter a 1:10 dilution was necessary, correction for which has been made in the plot.
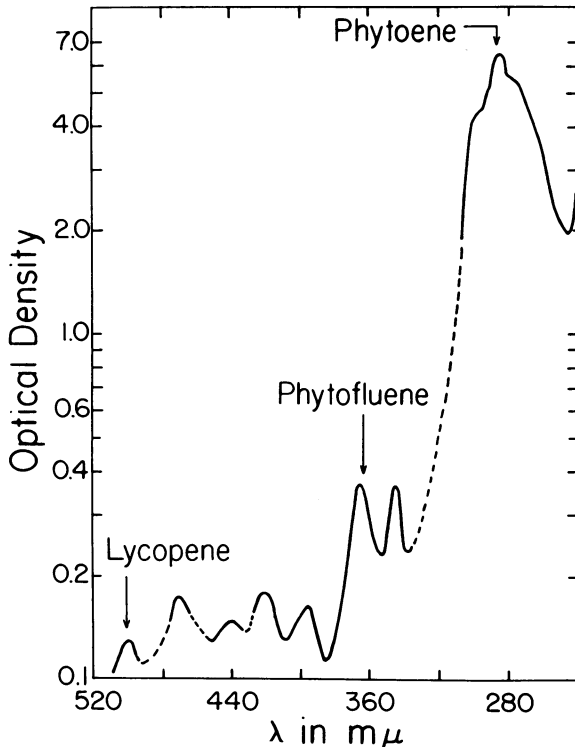

FIG. 1.—Absorption spectrum of crude extract, in petroleum ether solution, of mature ghost fruit.

In Table 1 are listed analyses for fruit from ghost, *gh gh*, and from the heterozygote *gh+ gh*, of normal red appearance.   The latter gave pigment yields characteristic of red phenotypes.

Results are shown in Table 2 for phytoene in the fruit of ten different genotypes at different stages of maturity.   The fruit of the first three types listed are all high in carotenoid when ripe.   Types represented by yellow, apricot,[5] and the various combinations of yellow, apricot, and tangerine all show restriction of carotenoid to varying degrees.   Thus yellow restricts the total amount formed and virtually eliminates lycopene.

The phytoene concentrations for the ripe fruit for types 3–9 in Table 2 are all low and show no major changes during maturation. Whatever the biochemical block which prevented the customary accumulation of colored carotenoid in these types, it did not cause accumulation of phytoene. Apart from the ghost, the highest phytoene concentrations are to be found in the red and tangerine types, both of which normally accumulate members of the lycopene set.   Furthermore, colored carotenoid and phytoene are formed concurrently, the color change from green-ripe to ripe coinciding with the increase in phytoene.

The β-carotene content of the green fruit (Table 3) showed little variation (1.50–3.02 μg/gm), except for that of the beta-orange type, which had 5.50 μg/gm.   As the fruit ripened, variation became progressively greater; the β-carotene

virtually disappeared from both yellow-apricot and yellow-tangerine-apricot, while it increased most in the beta-orange type.

TABLE 1
CAROTENOIDS OF GHOST AND HETEROZYGOTE (μG/GM)

	Ghost gh gh	Heterozygote gh^+ gh		Ghost gh gh	Heterozygote gh^+ gh
Lycopene	1.5	102	Pigment II max. 420	3.2	....
β-carotene	...	10.9	Phytofluene	6.3, 6.1	8.8
Pigment I max. 468	0.8	...	Phytoene	187, 208	12.1

TABLE 2
PHYTOENE CONTENT OF DIFFERENT TYPES AT THREE STAGES OF MATURITY (μG/GM)

	Green	Green-ripe	Ripe
Red	2.2, 7.4, 4.9	5.8	40.5, 53*
Tangerine	3.2, 6.0, 10.2	15.2	67, 84
Beta-orange	2.9, 1.9, 9.9	3.3	2.3
Yellow	3.1, 2.1	3.6	2.2
Apricot	0.7, 8.7	11.8	1.7
Yellow-tangerine	2.3, 4.8	6.3	10.2
Yellow-apricot	3.4, 4.5	5.5	0.7, 1.9
Tangerine-apricot	2.2, 2.1	6.0	11.9
Yellow-tangerine-apricot	2.3, 6.7	3.4	9.5 (max.)
Ghost	79, 85	..	164, 210, 189

* Concentrations in red lines may vary considerably. In a winter-ripened fruit, concentrations of lycopene and phytoene were 50 and 6.5 μg/gm, respectively.

TABLE 3
β-CAROTENE CONTENTS OF TYPES AT THREE STAGES OF MATURITY (μG/GM)

	Green	Green-ripe	Ripe*
Red	1.94	4.1	5 -10
Tangerine	2.39	2.04	3 -12
Beta-orange	5.50	13.5	80.0-130
Yellow	2.84	2.1	1 -3
Apricot	2.68	5.75	6 -10
Yellow-tangerine	1.96	1.60	0.5-1.0
Yellow-apricot	3.02	2.73	0.4-1.2
Tangerine-apricot	2.72	1.75	0.7-2.1
Yellow-tangerine-apricot	1.50	0.43	0.4-1.0

* Many analyses are available for the ripe fruit, and the range of values is indicated in this column.

The total leaf carotenoid for the nine green-leaved types varied from 65 to 124 μg per gram of leaf tissue. In all cases β-carotene comprised *ca.* 35 per cent of the total. This variation is due to unavoidable differences in the proportion of vascular tissue in the samples. The carotenoid spectra for all nine extracts were superimposable between 500 and 400 mμ. Slight differences were noticeable at 370 and 285 mμ, but the presence of phytoene could not be unequivocally established. The ghost leaves contained significant quantities of phytoene (51 μg/gm).

DISCUSSION

In the ripening of the normally pigmented tomato fruit, chlorophyll disappears and carotenoid is formed. Green fruit (Table 3) is presumably capable of photosynthesis, and no significant difference is observed between the types in pigmentation or in phytoene content (Table 2). In two of the nine cases there is an accumulation of phytoene which coincides with ripening.

The effects of genes hitherto studied in the tomato, namely, r, t, at, and B, apparently have not interfered in any way with leaf pigmentation or with that of the immature fruit. The effect of the ghost gene, on the other hand, is drastic. When homozygous, it inhibits chlorophyll and carotenoid syntheses and causes phytoene to accumulate. If phytoene is considered as a precursor to lycopene, then yellow (rr) or apricot ($atat$), which do not interfere with chlorophyll but which do restrict lycopene, should cause accumulation of phytoene. This is not borne out by the analyses. Lycopene formation in red and tangerine types coincides with appreciable phytoene formation, and lack of one is paralleled by lack of the other in all other lines except ghost. Formation of β-carotene in the beta-orange type is not accompanied by significant amounts of phytoene. Thus it is hard to reconcile the single gene difference between ghost (gh) and red (gh^+) with a biochemical pathway involving dehydrogenation of phytoene to lycopene, and even harder between red (B^+) and orange (B) to visualize lycopene as a precursor of β-carotene. At this juncture, no scheme is entirely free from objection. We hope clarification may be possible by introduction of the gene pair gh gh into the types currently under examination.

Two single gene differences at the B and gh loci[6] determine the beta-orange versus red and ghost phenotypes. We may consider the possibility of independent mutations at two loci, B to B^+ and gh^+ to gh. This permits four phenotypes which differ genotypically and biochemically as shown in the accompanying table. The

Phenotype	Genotype	Principal Carotenoid
Beta-orange	$B\ B\ gh^+\ gh^+$	β-carotene
Red	$B^+\ B^+\ gh^+\ gh^+$	All-*trans* lycopene
Ghost	$B^+\ B^+\ gh\ gh$	Phytoene
Ghost?	$B\ B\ gh\ gh$	Unknown

last-mentioned phenotype has yet to be isolated. The effect of ghost is almost certain to make it an albino, and it is not improbable that the fruit will also contain phytoene.

SUMMARY

The leaves and fruit of an albino mutant from a normal red-fruited tomato stock have been analyzed for carotenoid content. A phytoene concentration was found comparable to that of lycopene in normal red fruit. Analyses have been made for the phytoene contents of fruits of several genotypes. Difficulties in the way of accepting phytoene as a precursor in lycopene synthesis have been discussed.

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[5] The "apricot" gene is so named because of the apricot-like color of the fruit.

[6] The old system of designating gene pairs, e.g., B B versus b b, is being systematically replaced by standardizing the genotypic formula for a selected phenotype—the Marglobe variety in the case of tomatoes. This red-fruited phenotype has the formula $B^+\ B^+\ gh^+\ gh^+$. This would earlier have been designated $b\ b\ Gh\ Gh$. The beta-orange phenotype becomes $B\ B\ gh^+\ gh^+$, where B is dominant over B^+. The ghost is represented by $B^+\ B^+\ gh\ gh$, where gh^+ is dominant over gh. The plus sign indicates identity with the situation in Marglobe (see D. W. Barton, L. Butler, J. A. Jenkins, C. M. Rick, and P. A. Young, *J. Heredity*, **46**, 22, 1955).