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Dual Role of the Pigmentation Gene *B* in Affecting Carotenoid and Vitamin E Content in Squash (*Cucurbita pepo*) Mesocarp

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The yellow and orange colorations of the mesocarp of pumpkins and squash of *Cucurbita pepo* are due to the presence of carotenoid pigments that also greatly contribute to their nutritional value. Carotenoids, as well as tocopherols (vitamin E), are isoprenoid compounds formed by branches of a common biosynthetic pathway. Photodiode array HPLC analysis was used to simultaneously determine the content and composition of fruit-flesh carotenoids and tocopherols in five pairs of near-isogenic lines differing in the allelic state of genes previously identified as having profound effects on fruit color. The dominant *B* allele promoted carotenoid accumulation up to 5-fold and prevented tocopherol accumulation in all genetic backgrounds. The dominant *L-2* allele doubled carotenoid content and, in combination with the dominant *B* allele, increased carotenoid content by 10−15-fold as compared to the recessive *l-2* allele. The genes *D* and *L-1* had no significant effects on mesocarp tocopherols or carotenoids content. These results indicate that the *B* gene, which affects both carotenoids and tocopherols, may play a regulatory role in the flux of the isoprenoid pathway products.

KEYWORDS: Tocopherol; carotenoids; carotene; squash; pumpkin; gene *B*

INTRODUCTION

Cucurbita L. species (pumpkin, squash) rank collectively among the 10 leading vegetable crops worldwide with an annual production (2004) of almost 20 million metric tons (http://faostat.fao.org). *C. pepo* L. is the economically most important species of the genus and is grown extensively in temperate and subtropical regions of the world (*1*). This is one of the widely cultivated plant species in which variable characteristics of fruits are well-documented, and this species has one of the largest fruit diversity within the entire plant kingdom due to the great range of variation for shape, size, and color (*2*−*4*). Shape and size in pumpkin fruits are under polygenic control (*5*, *6*), whereas over a dozen major genes have been identified that affect fruit color (*7*).

Among pumpkin and squash cultivars, intense fruit-flesh coloration is a positive sensory attribute (*8*), and the intensity of fruit-flesh color has a positive association with total carotenoid content (*9*, *10*). Carotenoids are red, orange, and yellow molecules that can act as photoprotective agents and accessory light-harvesting pigments, but also add nutritional and ornamental value to many plants (*11*, *12*). Carotenoids are tetraterpenes and thus are products of the plastidial isoprenoid metabolic pathway (*13*). The 20-carbon diterpene molecule geranylgeranyldiphosphate (GGPP) is the precursor of the carotenoids, generated by head-to-head condensation of two GGPP molecules, a reaction catalyzed by the enzyme phytoene synthase (*14*). This step is considered to be the first committed step in carotenoid biosynthesis, and thus phytoene synthase is regarded as a key regulatory point in the pathway (*13*, *15*). Phytoene, a colorless compound, is converted into colored carotenoids through a series of desaturation, isomerization, cyclization, hydroxylation, epoxidation, and esterification reactions (*13*, *16*, *17*). Pumpkins synthesize both hydrocarbon and xanthophyll carotenoids. Some of these compounds, such as β-carotene, α-carotene, and β-cryptoxanthin, are referred to collectively as provitamin A (*18*, *19*).

Vitamin E is the generic descriptor for all tocopherols that qualitatively exhibit the biological activity of α-tocopherol (*20*, *21*). Vitamin E is produced only by plants, being most concentrated in plant oils (*20*). Tocopherols are antioxidants and prevent autoxidation of highly unsaturated fatty acids mediated by molecular oxygen. Thus, one of the roles of vitamin E in humans, as well as in plants, might be the preservation of membrane integrity from oxidative damage (*20*, *22*, *23*). Recent studies have shown that tocopherols have the potential to reduce the risk of cancer and cardiovascular diseases (*24*, *25*).

Tocopherols, similar to the chlorophylls, contain a phytol-derived chain and thus also are partially formed by the isoprenoid metabolic pathway from GGPP. Therefore, carotenoids, tocopherols, and chlorophylls share a common precursor, the 20-carbon diterpene molecule GGPP (*13*, *16*).

Three known genes that have a major effect on fruit rind color intensity are *D* (*Dark*), *l-1* (*light coloration-1*), and *l-2* (*light*

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Table 1. Genotypes, Phenotypes, and Tocopherol and Carotenoid Contents (Micrograms per Gram of Fresh Weight) of the Mesocarp of the Fruits of Five Pairs of Near-Isogenic Lines of *C. pepo*[a]

genotype				mature fruit exocarp color	mature fruit mesocarp color	α-tocopherol	γ-tocopherol	vitamin E	xantho-phylls	hydro-carbons	total carotenoids
b/b	*d/d*	*l-1/l-1*	*l-2/l-2*	light yellow	light yellow	0.3 (0.08)	1.0 (0.20)	1.2 (0.26)	1.0 (0.17)	0.5 (0.19)	1.6 (0.26)
B/B	*d/d*	*l-1/l-1*	*l-2/l-2*	light yellow	light yellow	0.0	0.0	0.0	1.2 (0.23)	0.5 (0.10)	1.7 (0.28)
b/b	*D/D*	*l-1/l-1*	*l-2/l-2*	intense orange	light yellow-orange	0.3 (0.11)	0.9 (0.28)	1.2 (0.28)	1.0 (0.28)	0.5 (0.32)	1.5 (0.57)
B/B	*D/D*	*l-1/l-1*	*l-2/l-2*	intense orange	light yellow-orange	0.0	0.0	0.0	1.5 (0.40)	0.8 (0.33)	2.3 (0.62)
b/b	*d/d*	*L-1/L-1*	*l-2/l-2*	light yellow	light yellow	0.3 (0.10)	1.0 (0.21)	1.3 (0.29)	0.8 (0.19)	0.5 (0.20)	1.3 (0.36)
B/B	*d/d*	*L-1/L-1*	*l-2/l-2*	light yellow	light yellow	0.0	0.0	0.0	1.6 (0.45)	0.9 (0.39)	2.4 (0.73)
b/b	*d/d*	*l-1/l-1*	*L-2/L-2*	light orange	light green-yellow-orange	0.3 (0.17)	1.2 (0.34)	1.5 (0.46)	2.8 (0.68)	1.2 (0.44)	4.0 (1.07)
B/B	*d/d*	*l-1/l-1*	*L-2/L-2*	light orange	intense orange	0.0	0. 0	0.0	17.9 (3.51)	5.6 (2.93)	23.4 (5.19)
b/b	*d/d*	*L-1/L-1*	*L-2/L-2*	intense green	light yellow-orange	0.3 (0.09)	1.0 (0.37)	1.3 (0.36)	1.8 (0.68)	0.6 (0.24)	2.4 (0.85)
B/B	*d/d*	*L-1/L-1*	*L-2/L-2*	intense orange	intense orange	0.0	0. 0	0.0	10.9 (3.56)	3.0 (1.16)	13.8 (4.43)

[a] Numbers are means of at least three independent extractions and analyses of three fruits (standard deviations appear in parentheses). Compounds were identified by their retention times and UV–visible spectra in comparison to authentic standards.

coloration-2) (*26*). When these three loci are homozygous recessive, the fruits are light-colored throughout their development. The two dominant alleles, *L-1* and *L-2*, have a complementary interaction that results in intense coloration of the fruits throughout their development. The dominant *D* allele does not affect the color of the fruits early in their development but results in intense coloration in the maturing fruit.

A fourth fruit-color gene, designated *B* (*Bicolor*) (*27*), does not noticeably affect fruit exterior color intensity but rather fruit hue. In *b/b* plants, which is characteristic of practically all of the natural variations of *C. pepo*, the ovaries and young fruits begin development green. Depending on the genotype with regard to other fruit coloration loci, especially *D*, *l-1*, and *l-2*, the fruits either remain green or turn orange or yellow later in their development. The dominant *B* locus results in "precocious fruit pigmentation" (*27*); the ovaries and young fruits are cream-yellow from the earliest point of development, never accumulating chlorophyll and unable to develop normal chloroplasts (*28*). The fruits either remain yellow or turn orange later in their development, depending on the genotype (*26*).

Fruit-flesh (mesocarp) color is also affected by these genes. Most of the genotype combinations result in light yellow to light orange fruit-flesh color. However, there is a complementary interaction of the dominant *B* allele with the dominant *L-2* allele that results in an intense orange fruit-flesh color (*29*). Most pumpkin cultivars possess the *b/b L-2/L-2* genotype, and thus when the *B* allele was introduced, by backcrossing, into such cultivars, the fruit-flesh color was observed to be an intense orange (*30*).

The primary objective of this work was to observe the effects of the genes leading to carotenoid accumulation on the tocopherol and specific carotenoid content of squash fruit flesh. This was accomplished by analyzing the content, compositions, and genetic interaction effects of carotenoids and tocopherols of mature fruit mesocarps of five pairs *(B/B* and *b/b)* of near-isogenic lines of *C. pepo* differing in the allelic state at the other pigmentation loci, *D*, *l-1*, and *l-2*.

MATERIALS AND METHODS

Plant Material. The *B/B* breeding line Precocious Fordhook Zucchini (*27*), a derivate of Fordhook Zucchini (*C. pepo* subsp. *pepo* Zucchini Group), has intensely colored fruits and genotype *B/B D/D L-1/L-1 L-2/L-2* (*26*). It was crossed with the cultivar Vegetable Spaghetti (*C. pepo* subsp. *pepo* Vegetable Marrow Group), which has light-colored fruits and genotype *b/b d/d l-1/l-1 l-2/l-2*. This was followed by six generations of backcrossing to the latter with selection for *B/b D/d L-1/l-1 L-2/l-2* in each generation. Sixth-backcross-generation plants were self-pollinated for several generations and phenotypically selected for various allelic combinations according to previous results (*26*, *29*). The genotypes together with their mature fruit external and internal colors are listed in **Table 1**.

Seeds of these 10 genotypes were sown in the field in late July at Newe Ya'ar (northern Israel). Standard methods of field preparation, irrigation, and fertilization were employed. Row centers were covered with silver-on-brown plastic mulch. Plants were spaced 100 cm apart within the rows, with a distance of 200 cm between row centers. Fruits were harvested late the following October, when they were fully mature (at least 40 days past anthesis), and stored in a refrigerator for up to 2 weeks. Each genotype was represented by at least three fruits, and at least three samples were taken from each fruit.

Analysis of Carotenoids and Tocopherols. All chemicals and solvents were purchased from Sigma Chemical Co., St. Louis, MO.

Mature fruit flesh, after removal of seeds and rind, was cut into small ($<1 \times 1$ cm) cubes that were weighed, frozen in liquid nitrogen, and freeze-dried. To calculate fresh-to-dry-weight ratio, freeze-dried samples were reweighed.

Four hundred milligrams of finely ground samples was extracted using a slight modification of the method described by Tadmor et al. (*31*). δ-Tocopherol (2 μg/mL) and ~400 mg of fine freeze-dried material were extracted for 5 min at 85 °C in 4 mL of ethanol containing 1 mg/mL butylated hydroxytoluene (BHT). All steps were carried out in darkness or under gold fluorescent light to prevent possible photodegradation of products, mainly tocopherols. No δ-tocopherol was present in any of the analyzed squash extracts, and thus we used it as an internal standard.

To hydrolyze esterified carotenoids that might complicate the chromatographic determinations, we saponified the samples (*32*). Eighty microliters of an 80% w/v KOH methanolic solution was added to each tube, and the samples were saponified for 10 min at 85 °C. The samples were cooled in an ice bath, and 2 mL of ice-cold water was added. The suspensions were extracted twice with 2 mL of hexane by vigorous vortexing followed by a 2000*g* centrifugation for 10 min at room temperature. The upper hexane layers were pooled and evaporated to dryness in a Savant SpeedVac apparatus and resuspended in 400 μL of an acetonitrile/methanol/dichloromethane (45:20:35 v/v/v) solution. Samples were filtered through a poly(tetrafluoroethylene) Syrasep syringe filter, pore size = 0.2 μm (Intersep, Berkshire, U.K.), and kept at room temperature in darkness for not more than 24 h before analysis by HPLC.

HPLC analyses were carried out using the method described by Tadmor et al. (*31*). An HPLC apparatus model 2690 equipped with a 996 photodiode array detector (Waters Co., Milford, MA), a 4.6 × 250 mm C18 60 Å 4 μm Nova-Pak column, and a Nova-Pak Sentry guard cartridge (Waters Co.) was used for the analyses.

The initial mobile phase consisted of acetonitrile/methanol (97:3, v/v/v) containing 0.05% (v/v) triethylamine. We used a linear gradient of dichloromethane from 0 to 10% in 15 min at the expense of acetonitrile, and then the dichloromethane was kept constant at 10% until the completion of the runs. Methanol and triethylamine contents were kept constant at 3 and 0.05%, respectively. The flow rate was

1.5 mL/min while the column temperature was 30 °C. A photodiode array detector was used to detect colored carotenoids at 450 nm and tocopherol at 295 nm. The detector was set to monitor spectra from 265 to 500 nm, at a sampling rate of 1 spectrum/s and utilizing an optical resolution of 2.4 nm. An injection volume of 40 μL was used. Compounds were identified by comparison of retention times, co-injection with known standards, and comparison of their UV−visible spectra with authentic standards. Calculation of carotenoid quantities was based on the internal standard, δ-tocopherol (*31*). Percentage of dry weight was calculated for each fruit and results were calculated on a fresh weight basis.

Statistical Analysis. Statistical analysis was conducted using the JMP version 3.2.6 software package for PC (SAS Institute Inc.).

RESULTS

Vitamin E Content and Composition. Tocopherols were not detected in the fruit mesocarp of any of the lines carrying the dominant *B* allele (**Table 1**). In contrast, all *b/b* lines possessed measurable amounts of tocopherols and ~3-fold more α-tocopherol than γ-tocopherol. Differences in total vitamin E content between the five *b/b* genotypes were small and nonsignificant (**Table 1**).

Carotenoid Content and Composition. The major carotenoids present in the mesocarp of all of the lines were the xanthophyll lutein and the hydrocarbons β- and α-carotenes. Lower levels of α- and β-cryptoxanthins and an unidentified compound with a spectrum identical to that of lutein were also present (not shown).

Phenotypically, the fruits were easily distinguishable into two categories, one having light-colored, orange to yellow mesocarp and the other having intense orange mesocarp. Light-colored flesh contained carotenoid levels ranging from 1 to 4 μg/g of fresh weight (**Table 1**). The intense-orange flesh contained 14−23 μg/g of fresh weight. There were eight near-isogenic lines that possessed light-colored mesocarp and two that possessed intense-colored mesocarp. The intense-colored fruit flesh was exclusive to the two lines that possessed two dominant alleles, *B* and *L-2*. The other eight lines, which lacked either or both of these dominant alleles, had light-colored fruit flesh (**Table 1**). The intense-colored mesocarp had a higher proportion of xanthophylls (3.48 ± 0.25) than did the light-colored mesocarp (2.05 ± 0.43). The intense-colored fruit mesocarps contained on average 11-fold more xanthophylls, whereas the increase in hydrocarbon levels was 7-fold as compared to the mesocarp of light-flesh fruits.

The effect of *B* on carotenoid content was strongly dependent on the genetic background. In the triple recessive (*d/d l-1/l-1 l-2/l-2*) genotype, *B* had no effect on mature fruit flesh carotenoid content. In the two backgrounds that contained either the dominant *D* or dominant *L-1*, the *B/B* genotype had ~50% greater total carotenoid content than did the *b/b* genotype. Generally, *B* increased the xanthophylls content slightly more than it increased the carotene content, leading to a higher proportion of xanthophylls than in *b/b* fruits.

The *b/b L-2* lines had a slightly higher carotenoid content than the respective *b/b l-2* lines, indicating an independent effect of L-2 on fruit pigmentation intensity. The lines that had dominant *L-2* also had a significantly ($P < 0.05$) higher ratio of xanthophylls to hydrocarbons. Most significant, however, was that the *B* allele greatly increased (5−15-fold) the carotenoid content of the fruit flesh only in the presence of the dominant *L-2* allele

The effect of *L-1* and *D* seems to be limited to the exocarp. No significant effect of the allelic state in these loci on the mesocarp carotenoids content and composition has been observed in the analysis of the 10 NILs (**Table 1**).

DISCUSSION

The most striking result of our study was the effect of the *B* allele on tocopherol content, effectively preventing its accumulation in all of the genetic backgrounds studied. Until this study the *B* allele was reported to affect chlorophyll, carotenoid, and starch contents (*33*, *34*). The major effect on tocopherol content contributes an additional piece of information that sheds light on the molecular function of this unique gene.

Squash fruit flesh is not a major nutritional source of vitamin E. Even among the *b/b* genotypes, levels are not high (1.32 ± 0.10 μg/g of fresh weight), with a fairly constant ratio of α- to γ-tocopherol (3.55 ± 0.50), and little variation for vitamin E content. However, the *B* allele, concomitant with inhibiting chlorophyll accumulation, similarly affects accumulation of tocopherol, together with increases in carotenoid content and decreases in flesh starch content.

The common denominator of all four of these components, carotenoids, tocopherol, chlorophyll, and starch, is the plastidic component of the cell. Three of the components, carotenoids, tocopherol, and the chlorophyll phytol chain, are synthesized via the plastidic isoprenoid pathway sharing common biosynthetic intermediates (*13*). Both tocopherols and chlorophylls share a similar phytol chain derived from GGPP (*16*, *35*). The classical phenotypic effect of gene *B* is "precocious pigmentation", the prevention of chlorophyll accumulation in the young ovary (*27*), and in light of our results the primary cause may be the lack of phytol needed for both chlorophyll and tocopherol biosynthesis (*35*). One of the genotypes classified as having a light-colored mesocarp, *b/b d/d l-1/l-1 L-2/L-2*, also retained chlorophyll in the mature flesh, leading to a light greenish hue (**Table 1**), and when the *b/b* of this genotype was substituted with *B/B*, the mesocarp was intensely orange. Thus, it may be that the dominant *B* allele stimulates the flow through the pathway to tetraterpenes (carotenoids) and at the same time greatly reduces the levels of the diterpene-derived metabolites in the flesh (tocopherols and chlorophylls). The recessive *b* would accordingly allow the formation of the diterpene-derived tocopherols and chlorophylls, perhaps at the expense of the tetraterpene carotenoids. This model would suggest that the *B* gene functions at the control crossroads of diterpene−tetraterpene metabolism and would possibly implicate the GGPP dimerization step of phytoene synthase in the function of gene *B*.

A role for phytoene synthase has been shown in studies of transgenic tomatoes expressing the gene and subsequently exhibiting increased carotenoid levels (*36*). This study demonstrated competition of the metabolic flow through the different branches of the isoprenoid pathway. Plants overexpressing phytoene synthase contained higher carotenoid contents but displayed reduced chlorophyll levels (possibly due to lack of phytol) and dwarfing of vegetative growth, possibly due to a lack of another diterpene derivative, gibberellin (*36*). Dwarf phenotypes and reduced chlorophyll levels were also detected in tobacco plants overexpressing phytoene synthase genes and therefore accumulating high phytoene levels (*37*). The competition between branches of isoprenoid metabolism is also suggested by the *rp/rp* carrot mutant that is characterized by whitish yellow roots having reduced carotenoid synthesis but containing an enhanced level of tocopherol as compared with normal orange roots (*38*). Similarly, in transgenic *Brassica napus*, seed-specifically overproducing bacterial phytoene synthase, a sig-

nificant increase of carotenoid accumulation accompanied by a significant reduction of tocopherol content was recorded (*39*). Our observations on the increased carotenoid accumulation and limitation of diterpene synthesis by the dominant *B* allele in *C. pepo* (**Table 1**) support the hypothesis that this allele is somehow implicated in the phytoene synthase metabolic step, perhaps, but not only, via its expression.

Alternatively, *B* may be acting at the plastid organellar level rather than directly at the enzymatic level. Externally, the ovaries of *b/b* plants are green early in their development, with developed chloroplasts. The ovaries can remain green or become yellow or orange as they mature, through chloroplast-to-chromoplast transformation. *B/B* ovaries are yellow as soon as they are visible to the unaided eye and are devoid of chlorophyll or well-developed chloroplasts (*28*); they can remain yellow or become orange as they mature. Thus, *B* may be acting on chloroplast genesis, causing direct proplastid-to-chromoplast maturation (*34*). Accordingly, the characteristic chloroplast components, chlorophyll and tocopherol, are absent, whereas the chromoplast carotenoids are even increased. Starch content will also be decreased in genotypes in which starch accumulation is dependent on the presence of chloro-amyloplasts (*40*).

Carotenoid contents have previously been compared in some *C. pepo* cultivars and their near-isogenic *B/B* lines (*40*, *41*), but this is the first study that systematically studies the effect of *B* in defined and characterized genetic backgrounds differing only for fruit pigmentation loci. The results of this study clearly show that the intensity of internal color and the level of carotenoids can be most strikingly increased in a genetic background in which the dominant *L-2* allele is present (**Table 1**), leading to a highly significant complementary gene action.

The effects of the *B* allele on carotenoid contents were previously observed to range from nil to as much as a 6-fold increase, depending on the cultivar (*40*). These effects occurred regardless of whether the *B* allele was homozygous or heterozygous (*41*). In light of the present study we can implicate the complementary gene action of *L-2* in explaining the results of our previous study; only those genotypes harboring *L-2* showed a significant increase in carotenoids in response to *B*. In addition, the separation by HPLC in the present study has allowed for greater resolution of the carotenoid pigments of *C. pepo* fruit mesocarp than in earlier work (*40*, *41*). The results (**Table 1**) reaffirm that xanthophylls are present in greater concentration than carotenes. β-Carotene is the major component of the hydrocarbon fraction (data not shown), as has already been observed in a cultivar of *C. maxima* Duchesne and in a cultivar of *C. moschata* Duchesne (*42*). Similarly, Khachik has identified the carotenoid components of pumpkin, both in their esterified forms and after saponification (*32*). Our study indicates that there may be little genetic variation for carotenoid makeup within *C. pepo* and that the genetic variability present within the species may be limited to quantitative rather than qualitative variation.

The dominant *B* is a relatively recent mutation (*27*). *C. pepo* is native to North America and was unknown to Europeans prior to their contact with the New World (*43*). Among the first kinds of *C. pepo* encountered by Europeans apparently were pumpkins grown by native North Americans along the Atlantic seaboard of what is now the United States (*44*). The direct descendants of those pumpkins have the highest carotenoid content of all *b/b* cultivars of the species heretofore surveyed (*40*, *41*) and are of genotype *D/D l-1/l-1 L-2/L-2* (*26*). In the comparison of near-isogenic lines (**Table 1**), the *b/b D/D l-1/l-1 L-2/L-2* genotype was the one that had the highest possible carotenoid content together with measurable amounts of tocopherol production in the fruit mesocarp.

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LITERATURE CITED

(1) Paris, H. S. Summer squash: history, diversity, and distribution. *HortTechnology* **1996**, *6*, 6−13.
(2) Duchesne, A. N. *Essai sur l'histoire naturelle des courges*; Panckoucke: Paris, France, 1786; 46 pp.
(3) Naudin, C. Nouvelles recherches sur les caractères spécifiques et les variétés des plantes du genre *Cucurbita*. *Ann. Sci. Nat. Bot. IV* **1856**, *6*, 5−73.
(4) Paris, H. S.; Yonash, N.; Portnoy, V.; Mozes-Daube, N.; Tzuri, G.; Katzir, N. Assessment of genetic relationships in *Cucurbita pepo* (Cucurbitaceae) using DNA markers. *Theor. Appl. Genet.* **2003**, *106*, 971−978.
(5) Emerson, R. A. The inheritance of sizes and shapes in plants. *Am. Nat.* **1910**, *44*, 739−746.
(6) Sinnott, G. W. A developmental analysis of inherited shape differences in cucurbit fruits. *Am. Nat.* **1936**, *70*, 245−254.
(7) Paris, H. S. *Quiescent intense* (*qi*), a gene that affects young but not mature fruit color intensity in *Cucurbita pepo*. *J. Hered.* **2000**, *91*, 333−339.
(8) Murphy, E. F.; Hepler, P. R.; True, R. H. An evaluation of the sensory qualities of inbred lines of squash (*Cucurbita maxima*). *Proc. Am. Soc. Hortic. Sci.* **1966**, *89*, 483−490.
(9) Francis, F. J. Relationship between flesh color and pigment content in squash. *Proc. Am. Soc. Hortic. Sci.* **1962**, *81*, 408−414.
(10) Kubicki, B.; Walczak, B. Variation and heritability of beta-carotene content in some cultivars of the *Cucurbita* species. *Genet. Polon.* **1976**, *17*, 531−544.
(11) Demmig-Adams, B.; Gilmore, A. M.; Adams, W. W. Carotenoids 3: in vivo function of carotenoids in higher plants. *FASEB J.* **1996**, *10*, 403−412.
(12) Cunningham, F. X., Jr.; Gantt, E. Genes and enzymes of carotenoid biosynthesis in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1998**, *49*, 557−583.
(13) Croteau, R.; Kutchan, T. M.; Lewis, N. G. Natural products (secondary metabolites). In *Biochemistry and Molecular Biology of Plants*; Buchanan, B., Gruissem, W., Jones, R., Eds.; American Society of Plant Physiologists: Rockville, MD, 2000; pp 1250−1318.
(14) Hirschberg, J. Carotenoid biosynthesis in flowering plants. *Curr. Opin. Plant Biol.* **2001**, *4*, 210−218.
(15) Albrecht, M.; Klein, A.; Hugueney, P.; Sandmann, G.; Kuntz, M. Molecular cloning and functional expression in *E. coli* of a novel plant enzyme mediating ζ-carotene desaturation. *FEBS Lett.* **1995**, *372*, 199−202.
(16) Hirschberg, J. Production of high-value compounds: carotenoids and vitamin E. *Curr. Opin. Biotechnol.* **1999**, *10*, 186−191.
(17) Isaacson, T.; Ronen, G.; Zamir; D.; Hirschberg, J. Cloning of *tangerine* from tomato reveals a carotenoid isomerase essential for the production of carotene and xanthophylls in plants. *Plant Cell* **2002**, *14*, 333−342.
(18) Bauernfeind, J. C.; Adams, C. R.; Marusich, W. L. Carotenoids and other vitamin A precursors in animal feed. In *Carotenoids as Colorant and Vitamin A Precursors*; Bauernfeind, J. C., Ed.; Academic Press: New York, 1981; pp 563−743.
(19) West, C. E. Meeting requirements for vitamin A. *Nutr. Rev.* **2000**, *58*, 341−345.
(20) Combs, G. F. *The Vitamins*; Academic Press: San Diego, CA, 1992.
(21) Traber, M. G.; Sies, H. Vitamin E in humans: demand and delivery. *Annu. Rev. Nutr.* **1996**, *16*, 321−347.

(22) Burton, G. W.; Ingold, K. U. Auto-oxidation of biological molecules. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. *J. Am. Chem. Soc.* **1981**, *103*, 6472−6476.
(23) Erin, A. N.; Skrypin, V. V.; Kagan, V. E. Formation of alpha-tocopherol complexes with fatty acids: nature of complexes. *Biochim. Biophys. Acta* **1985**, *14*, 209−214.
(24) Sies, H.; Stahl, W. Vitamins E and C, β-carotene, and other carotenoids as antioxidants. *Am. J. Clin. Nutr.* **1995**, *62*, 1315S−1321S.
(25) Stampfer, M. J.; Hennekens, C. H.; Munson, J. E.; Colditz, G. A.; Rosner, B.; Willett, W. C. Vitamin E consumption and the risk of coronary disease in women. *N. E. J. Med.* **1993**, *328*, 1444−1449.
(26) Paris, H. S.; Nerson, H. Genes for intense pigmentation of squash. *J. Hered.* **1986**, *77*, 403−409.
(27) Shifriss, O. The unpredictable gourds. *Am. Hortic. Mag.* **1965**, *44*, 184−201.
(28) Boyer, C. Genetic control of chromoplast formation during fruit development of *Cucurbita pepo* L. In *Current Topics in Plant Physiology, Vol. 2, Physiology, Biochemistry and Genetics of Non-Green Plastids*; Boyer, C. D., Shannon, J. C., Richardson, R. C., Eds.; American Society of Plant Physiologists: Rockville, MD, 1989; pp 241−252,
(29) Paris, H. S. Complementary genes for orange fruit flesh color in *Cucurbita pepo*. *HortScience* **1988**, *23*, 601−603.
(30) Shifriss, O. Genetics and origin of the bicolor gourds. *J. Hered.* **1955**, *46*, 213−222.
(31) Tadmor, Y.; Larkov, O.; Meir, A.; Minkoff, M.; Lastochkin, E.; Edelstein, M.; Levin, S.; Wong, J.; Rocheford, T.; Lewinsohn, E. Reversed-phase high performance liquid chromatographic determination of vitamin E components in maize kernels. *Phytochem. Anal.* **2000**, *11*, 370−374.
(32) Khachik, F.; Beecher, G. R.; Lusby, W. R Separation and identification of carotenoids and carotenol fatty acid esters in some squash products by liquid chromatography. 2. Isolation and characterization of carotenoids and related esters. *J. Agric. Food Chem.* **1988**, *36*, 938−946.
(33) Schaffer, A. A.; Boyer, C. D.; Gianfagna, T. Genetic control of plastid carotenoids and transformation in the skin of *Cucurbita pepo* L. fruit. *Theor. Appl. Genet.* **1984**, *68*, 493−501.
(34) Schaffer, A. A.; Boyer, C. D. Effects of gene B on fruit development in *Cucurbita pepo* L. *J. Am. Soc. Hortic. Sci.* **1984**, **109**, 432−437.
(35) Goodwin, T. W.; Mercer, E. I. *Introduction to Plant Biochemistry*, 2nd ed.; Pergamon Press: Oxford, U.K., 1983; pp 400−479.
(36) Fray, R. G.; Wallace, A.; Fraser, P. D.; Valero, D.; Hedden, P.; Bramley, P. M.; Grierson, D. Constitutive expression of a fruit phytoene synthase gene in transgenic tomatoes causes dwarfism by redirecting metabolites from the gibberellin pathway. *Plant J.* **1995**, *8*, 693−701.
(37) Busch, M.; Seuter, A.; Hain, R. Functional analysis of the early steps of carotenoid biosynthesis in tobacco. *Plant Physiol.* **2002**, *128*, 439−453.
(38) Goldman, I. L. The *reduced pigment (rp)* gene and biosynthesis of alpha-tocopherol in carrot roots. *HortScience* **2000**, *35*, 474.
(39) Shewmaker, C. K.; Sheely, J. A.; Daley, M.; Colburn, S.; Yang Ke, D. Seed-specific overexpression of phytoene synthase: increase in carotenoids and other metabolic effects. *Plant J.* **1999**, *20*, 401−412.
(40) Schaffer, A. A.; Paris, H. S.; Ascarelli, I. M. Carotenoid and starch content of near-isogenic B^+B^+ and *BB* genotypes of *Cucurbita*. *J. Am. Soc. Hortic. Sci.* **1986**, *111*, 780−783.
(41) Paris, H. S.; Schaffer, A. A.; Ascarelli, I. M.; Burger, Y. Heterozygosity of gene *B* and the carotenoid content of *Cucurbita pepo*. *Crop Res.* **1989**, *29*, 11−18.
(42) Hidaka, T.; Anno, T.; Nakatsu, S. The composition and vitamin A value of the carotenoids of pumpkins of different colors. *J. Food Biochem.* **1987**, *11*, 59−68.
(43) Whitaker, T. W. American origin of the cultivated cucurbits. *Ann. Mo. Bot. Gard.* **1947**, *34*, 101−111.
(44) Paris, H. S. History of the cultivar-groups of *Cucurbita pepo*. *Hortic. Rev.* **2001** *25*, 71−170.

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