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Interaction of the mutant genes *B*, *og*^{c}, *hp* and *t* in the coloring of tomato fruit

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Abstract The aim of this study was to assess the effect of the mutant genes *B*, *og*^{c}, *hp*, and *t* on the fruit color of tomato genotypes. Two tomato lines (TOM-498 and TOM-499) with BB genotype composition (high β-carotene homozygous) were used in hybrid combinations with 7 tomato lines with different genotype compositions in loci *og*^{c}, *hp*, or *t*—Floradade, TOM-596, TOM-544, NC-8276, NC-2Y, Florida 7775, and Florida 7781. A randomized complete block design with 24 treatments and four replications was used. Both external and internal fruit color were assessed with a Minolta CR-400 colorimeter in the CIE *L**, *a** and *b** mode. Hue and chroma readings were taken at four different points (epidermis, pericarp, placenta, and columella) of the fruit. For high β-carotene heterozygous hybrids B^+/B, hue angle indicated an orange color (since these hybrids did not come to equal the genotypes of normal coloring, red), even when genes promoting lycopene synthesis (*og*^{c}, *hp*) were deployed as heterozygous genotypes. Fruit of high β-carotene heterozygous hybrids, without *t*, *og*^{c}, or *hp*, had chroma values similar to fruit of homozygous high β-carotene lines. The use of the *og*^{c} allele in heterozygosity led to higher chroma values in high β-carotene heterozygous genotypes in relation to the values found in high β-carotene heterozygous genotypes not bearing the *og*^{c} gene. The *og*^{c} homozygous lines had hue values that did not differ from those in normal genotypes in the epidermis and pericarp, but showed a significant shift towards red in the placenta and in the columella.

Keywords *Solanum lycopersicum* · Enriched food · Lycopene · Beta-carotene · Plant breeding

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Introduction

Tomato (*Solanum lycopersicum*), widely consumed both *in natura* and in processed form, is one of the main sources of antioxidants in the human diet. The colors of the mature fruit of the tomato plant may range from intense red to orange-red to orange,

depending on the lycopene/beta-carotene ratio (Botella-Paíva and Rodríguez-Concepción 2006). These two carotenoids have important functions in nutrition; lycopene is a powerful antioxidant in prevention of carcinogenesis and atherogenesis; beta-carotene is the most important plant metabolite as a source of vitamin A and in cancer prevention (Agarwal and Rao 2000; Thurnham 2007).

The mature red tomato in general contains a greater quantity of lycopene than beta-carotene, which results in the predominantly red color. The lycopene/beta-carotene ratio may be affected by the presence of mutant coloring and maturation genes (Shami and Moreira 2004). There are diverse mutant genes for carotenoid content and type in tomato plants. Among them, the mutation B = high β-carotene stands out (which may significantly increase the beta-carotene content, making the fruit an orange color when mature), and the mutations og^c (old-gold crimson) and *hp* (high pigment) (which increase the lycopene content and lend intensification to the usual red color of the fruit (Araújo et al. 2002; Giordano et al. 2003).

Fruit with mutant *hp* homozygous genes has more intense pigmentation compared to fruit of the normal genotype, at all stages of development. The og^c gene is mutant recessive and provides an intense red color to the fruit pulp (Thompson et al. 1967). Reports from Thompson et al. (1965) show that lycopene content is around 75 % greater in og^c mutant homozygous fruit (og^c/og^c) when compared to normal fruit (that does not have this mutant gene); however, it has reduced beta-carotene, especially in the locular region of the fruit.

Another mutant gene that interferes in tomato fruit color is tangerine (*t*). Fruit with this mutant allele accumulates zeta-carotene and prolycopene, instead of lycopene (Isaacson et al. 2002) and, therefore, it exhibits orange coloring in mature fruit. A mutant gene which is responsible for raising beta-carotene levels is the mutant *B* or high B-carotene. Tomes et al. (1954) observed that red tomato varieties that before had 10 % β-carotene, when crossed with high β-carotene material, exhibited hybrids with a mean value of 61 % β-carotene content, and in the F_2 generation, the lowest values were from 20 to 30 %, and the highest from 70 to 80 % of β-carotene.

Obtaining tomato cultivars with fruit that has greater nutraceutical value, with greater contents of β-carotene and lycopene in the fruit, is of great importance since cultivars with these traits are currently rare in the Brazilian market. However, since high β-carotene tomato fruit has orange color, it might not be well accepted by the consumer. In the attempt at improving the final color toward the standard red, combinations of high β-carotene with og^c and *hp* may be an alternative in improving color, since og^c and *hp* genes intensify red color in tomato fruit.

The aim of this study is to assess the effect of the *B* gene in homozygosis and in heterozygosis on the expression of internal and external fruit color, as well as the effects of the genes og^c, *hp*, and *t* on modification of this expression.

Materials and methods

The study was developed at the Experimental Station of HortiAgro Sementes S.A. on the Palmital Farm in the municipality of Ijaci, MG, Brazil at the coordinates 21°14′16″ latitude south and 45°08′00″ longitude west, at an altitude of 920 m, in the years 2009 and 2011. In the first year, the experimental hybrids were obtained and, after that, the crop experiment was set up and carried out with these hybrids. The assessments of internal color of the fruit were made in the Plant Product Laboratory of the Food Science Department of the Universidade Federal de Lavras.

Lines homozygous for the genes *B*, og^c, *hp*, and *t* were used from the germplasm bank of the HortiAgro Sementes S.A. company. These lines, described in Table 1, were used as parental lines in obtaining hybrids, together with the normal lines that do not exhibit any of the four allele combination (B^+/B^+, og^{c+}/og^{c+}, hp^+/hp^+, t^+/t^+).

The 14 experimental hybrids were obtained from seven lines (Floradade, TOM-596, TOM-544, NC-8276, NC-2Y, FLORIDA-7775, and FLORIDA-7781) which were used as female parents in combination with two lines (TOM-498, TOM-499) used as male parents. The female parent was emasculated before receiving pollen from the male parents and crosses were made manually in the greenhouse. Since TOM-498 and TOM-499 have a BB genotype composition (high β-carotene), all the hybrids obtained were heterozygous in the B locus and, presumably, rich in beta-carotene. Floradade was taken as the standard tomato with normal lycopene contents. The lycopene contents and fruit color in the hybrids may depend on the presence of the genes og^c, *hp*, or *t* in the female

Table 1 Lines used and their respective descriptions

Line	Description
TOM-498	Purdue-88-96-1 Line, provided by Edward C. Tigchelaar/Purdue University in August/1989; line with *B sp/B sp* genotype composition (high B, determinate growth habit); oblong fruit and rich in β-carotene
TOM-499	Purdue-88-100A-1 Line, provided by Edward C. Tigchelaar/Purdue University in August/1989; line with *B sp/B sp* genotype composition (high β-carotene, determinate growth habit); oblong fruit and rich in β-carotene
Floradade	Normal line (not bearing *B*, og^c, *hp*, or *t* alleles), originating from the University of Florida; multilocular fruit, with 4–5 locules, slightly flattened; determinate growth habit; without abscission layer at the peduncle (=jointless, *j2/j2* genotype); with green shoulders on the fruit (u^+/u^+ genotype); resistance to *Verticillium dahliae*, *Fusarium oxysporum* f.sp. *lycopersici* races 1 and 2, and *Stemphylium solani*
TOM-596	Line which is nearly isogenic to Floradade, differing from it through having an og^c *sp*/og^c *sp* genotype composition (old-gold crimson, determinate growth habit); fruit presumably rich in lycopene
TOM-544	Line which is nearly isogenic to Floradade, differing from it through having an og^c *sp*/og^c *sp* genotype composition (old-gold crimson, determinate growth habit), *hp*/*hp* (high pigment); fruit presumably rich in lycopene; with intense green shoulders when immature (pleiotropic effect of the *hp/hp* genotype)
NC-8276	Normal line (not bearing *B*, og^c, *hp*, or *t* alleles), originating from North Carolina State University; multilocular fruit, with 4–5 locules, flattened, firm, larger than those of Floradade; fruit with abscission layer at the peduncle ($j2^+/j2^+$ genotype), without green shoulders (*u/u* genotype); resistance to *Verticillium dahliae*, *Fusarium oxysporum* f.sp. *lycopersici* races 1 and 2
NC-2Y	NC-2Y Line, originating from North Carolina State University, with determinate growth habit (*sp/sp*), with *t/t* genotype composition (=tangerine), which brings about an orange color on the mature fruit; large fruits, round to flattened, with some susceptibility to splitting; fruit with abscission layer at the peduncle ($j2^+/j2^+$ genotype), without green shoulders (*u/u* genotype); resistance to *Verticillium dahliae*, *Fusarium oxysporum* f.sp. *lycopersici* races 1 and 2
Florida-7775	Line obtained at the University of Florida by J.W. Scott; with og^c *sp*/og^c *sp* genotype composition (old-gold crimson, determinate growth habit); multilocular fruit, flattened, rich in lycopene; without abscission layer at the peduncle (=jointless, *j2/j2* genotype); without accentuated green shoulders (*ug/ug* genotype, uniform green); resistance to *Verticillium dahliae*, *Fusarium oxysporum* f.sp. *lycopersici* races 1 and 2
Florida-7781	Line obtained at the University of Florida by J.W. Scott; with og^c *sp*/og^c *sp* genotype composition (old-gold crimson, determinate growth habit); multilocular fruit, flattened, rich in lycopene; with abscission layer at the peduncle (=jointed, $j2^+/j2^+$ genotype); without accentuated green shoulders (*ug/ug* genotype, uniform green); resistance to *Verticillium dahliae*, *Fusarium oxysporum* f.sp. *lycopersici* races 1 and 2

parent lines, which in homozygosis affect the carotenoid content in the fruit.

The 14 experimental hybrids obtained, the 9 parental lines, and a commercial hybrid (Giselle F1) constituted the 24 treatments, all with a determinate growth habit. The material with the name Giselle F1, from the Sakata Seed Sudamerica company, is a cultivar for salad tomatoes with long shelf life and high yield (Table 2).

The experiment was carried out in a randomized block design with 24 treatments and four replications. The plots consisted of a single row containing ten plants.

Seedling production of the 24 genotypes was in expanded polystyrene (styrofoam) trays with 128 cells, containing the commercial substrate Multi-plant®. The seedlings were transplanting in the greenhouse 30 days after seeding, with a spacing of 0.5 m between plants and 1.5 m between rows. Plants were trained to a stake and drip irrigation was used. Top dress fertilization was carried out through the aid of drip fertigation. In addition, manual weeding and spraying with products recommended for the tomato crop were performed whenever necessary. Fruit was harvested eight times from 24/01/2011 to 09/03/2011.

Measurements of internal color of the fruit were made in fully mature fruit of the third harvest, collecting 6 fruits per plot, and the mean value was calculated to obtain the value of the corresponding plot. Readings were made with the colorimeter Minolta CR-400 in the mode CIE L^*, a^*, and b^*, where: * L is "lightness", brightness coordinate (z axis), ranging from 0 (black) to +100 (white). Higher values indicate brighter colors; * a is hue coordinate (x axis), ranging from +60 (red) to −60 (green);

Table 2 Description of the treatments

Treatment	Name of treatment	Characteristics
T1	FLORADADE	Normal
T2	FLORIDA-7775	og^c/og^c
T3	FLORIDA-7781	og^c/og^c
T4	NC-2Y	*t/t*
T5	NC-8276	Normal
T6	TOM-544	og^c/og^c; *hp/hp*
T7	TOM-596	og^c/og^c
T8	TOM-498	*B/B*
T9	TOM-499	*B/B*
T10	F1(FLORADADE × TOM-499)	og^{c+} normal; hp^+ normal; B^+/B
T11	F1(FLORIDA-7775 × TOM-499)	og^{c+}/og^c; B^+/B
T12	F1(FLORIDA-7781 × TOM-499)	og^{c+}/og^c; B^+/B
T13	F1(NC-8276 × TOM-499)	og^{c+} normal; hp^+ normal; B^+/B
T14	F1(TOM-544 × TOM-499)	og^{c+}/og^c; t^+/t; B^+/B
T15	F1(TOM-596 × TOM-499)	og^{c+}/og^c; B^+/B
T16	F1(NC-2Y × TOM-499)	t^+/t; B^+/B
T17	F1(FLORADADE × TOM-498)	og^{c+} normal; hp^+ normal; B^+/B
T18	F1(FLORIDA-7775 × TOM-498)	og^{c+}/og^c; B^+/B
T19	F1(FLORIDA-7781 × TOM-498)	og^{c+}/og^c; B^+/B
T20	F1(NC-8276 × TOM-498)	og^{c+} normal; hp^+ normal; B^+/B
T21	F1(TOM-544 × TOM-498)	og^{c+}/og^c; t^+/t; B^+/B
T22	F1(TOM-596 × TOM-498)	og^{c+}/og^c; B^+/B
T23	F1(NC-2Y × TOM-498)	t^+/t; B^+/B
T24	Giselle F1	Normal

* b is hue coordinate (y axis), ranging from +60 (orange) to −60 (blue).

From the values of a* and b* were obtained:

"Hue angle" = defined as arc tg (b/a);

"Chroma" = saturation = square root of $(a^2 + b^2)$.

The hue angle is defined as of the **a** axis and is expressed in degrees; 0° is defined as +a (red), 90° as +b (orange), 180° as −a (green), and 270° as −b (blue). Mature tomato fruits in general range from 0° (red) to 90° (orange); the nearer the values to zero, the redder the fruit; the nearer to 90°, the orangeer the fruit (Color Glossary 2012; Konica Minolta 2012).

For saturation or chroma, the values range from 0 to 60. Values of zero correspond to the center of origin of the coordinates; values near zero indicate colors that are not very saturated, and values of 60 indicate maximum saturation (Color Glossary 2012; Konica Minolta 2012).

Readings were taken in four parts (external and internal) of the 6 tomato fruits from each plot. For external color, reading was made of the epidermis without the clear skin that covers the fruit of the tomato. For internal color, a cross-section was done in fruit and readings were made on the pericarp, placenta, and columella.

The data were subjected to analysis of variance and the mean values were compared by the Scott-Knott test at 5 % probability by the statistical application SAS®. Contrasts of interest were also calculated for comparisons between genotypes and/or group of genotypes with different genotype compositions at the loci *B*, og^c, *hp*, and *t* (Table 3).

Results and discussion

Differences were observed among the treatments ($\alpha = 0.05$) for all the characteristics evaluated

Table 3 Contrasts of interest used for comparisons between genotypes and/or group of genotypes with different genotype compositions at the loci *B*, og^c, *hp*, and *t*

Contrasts	Estimated contrasts	Description
C1	(T1 + T2 + T3 + T5 + T6 + T7 + T24)/7 − (T8 + T9 + T10 + T11 + T12 + T13 + T14 + T15 + T16 + T17 + T18 + T19 + T20 + T21 + T22 + T23)/16	Normal genotypes, og^c homozygous, versus High β genotypes (homozygous and heterozygous)
C2	(T1 + T2 + T3 + T5 + T6 + T7 + T24)/7 − T4	Normal genotypes, og^c homozygous, versus Tangerine genotype
C3	(T1 + T5 + T24)/3 − (T8 + T9 + T10 + T11 + T12 + T13 + T14 + T15 + T16 + T17 + T18 + T19 + T20 + T21 + T22 + T23)/16	Normal genotypes versus High β genotypes (homozygous and heterozygous)
C4	(T1 + T5 + T24)/3 − (T8 + T9)/2	Normal genotypes versus High β homozygous genotypes
C5	(T1 + T5 + T24)/3 − (T10 + T11 + T12 + T13 + T14 + T15 + T16 + T17 + T18 + T19 + T20 + T21 + T22 + T23)/14	Normal genotypes versus High β heterozygous genotypes
C6	(T1 + T5 + T24)/3 − T4	Normal genotypes versus Tangerine genotype
C7	(T1 + T5 + T24)/3 − (T2 + T3 + T7)/3	Normal genotypes versus og^c homozygote genotypes, hp^+hp^+
C8	(T1 + T5 + T24)/3 − T6	Normal genotypes versus og^c homozygote genotypes, *hp* homozygous
C9	(T2 + T3 + T7)/3 − T6	og^c homozygous genotypes, *hp* + *hp* + versus og^c homozygote genotype, *hp hp*
C10	(T8 + T9)/2 − (T10 + T13 + T17 + T20)/4	High β homozygous genotypes versus High β genotypes heterozygote, t^+normal og^{c+}normal, hp^+normal
C11	(T8 + T9)/2 − (T16 + T23)/2	High β homozygous genotypes versus High β heterozygous genotypes, tangerine heterozygous
C12	(T8 + T9)/2 − (T11 + T12 + T15 + T18 + T19 + T22)/6	High β homozygous genotypes versus High β heterozygous genotypes, og^c heterozygous, hp^+hp^+
C13	(T8 + T9)/2 − (T14 + T21)/2	High β homozygous genotypes versus High β heterozygous genotypes, og^c heterozygous, *hp* heterozygous
C14	(T10 + T13 + T17 + T20)/4 − (T16 + T23)/2	High β genotypes heterozygotes, t^+normal og^{c+}normal, hp^+normal versus High β heterozygous genotypes, tangerine heterozygous
C15	(T10 + T13 + T17 + T20)/4 − (T11 + T12 + T15 + T18 + T19 + T22)/6	High β genotypes heterozygotes, t^+normal og^{c+}normal, hp^+normal versus High β heterozygous genotypes, og^c heterozygous, hp^+hp^+
C16	(T10 + T13 + T17 + T20)/4 − (T14 + T21)/2	High β genotypes heterozygotes, t^+normal og^{c+}normal, hp^+normal *versus* High β heterozygous genotypes, og^c heterozygous, *hp* heterozygous

(epidermis, pericarp, placenta, and columella) for both hue angle and chroma.

Hue angle

The genotypes NC-2Y (*t* homozygote), and TOM-499 and TOM-498 (high β-carotene homozygotes) exhibit hue angles nearer the color orange, with values near or greater than 70° in the epidermis, the pericarp, the placenta, and the columella (Table 4). The hybrids F1(FLORADADE × TOM-499), F1(FLORIDA-7775 × TOM-499), F1(FLORIDA-7781 × TOM-499), F1(NC-8276 × TOM-499), F1(TOM-544 × TOM-499), F1(TOM-596 × TOM-499), F1(NC-2Y × TOM-499), F1(FLORADADE × TOM-498), F1(FLORIDA-7775 × TOM-498), F1(FLORIDA-7781 × TOM-498), F1(NC-8276 × TOM-498), F1(TOM-544 × TOM-498), F1(TOM-596 × TOM-498), and F1(NC-2Y × TOM-498) high β-carotene heterozygote's exhibited hue angles which in general were less than the high β-carotene homozygote genotypes, with values generally in the range of 60° to 70° in the epidermis as well as the pericarp, the placenta, and the columella. All the other genotypes exhibited hue values of less than 50°, and in general less than 40°, in the epidermis, as well as the pericarp, the placenta, and the columella (Table 4).

These values are clearly reflected in both the external and internal color of the mature fruit—orange (hue angle >60°) in the genotypes NC-2Y (tangerine homozygote), TOM-499 and TOM-498 (high β-carotene homozygotes), and in the high β-carotene heterozygous hybrids; and reddish (hue angle <50°) in the other genotypes. The estimates of the contrasts C1, C2, C3, C4, C5, and C6 indicated that all the treatments with reddish fruits exhibited hue angles significantly more distant from the orange color than the *t* homozygote, high β-carotene homozygote, and high β-carotene heterozygote genotypes (Table 5), whether

Table 4 Mean values of the Hue angle (°degrees) for the epidermis, pericarp, placenta, and columella in tomato fruit

Treatments		Epidermis	Pericarp	Placenta	Columella
T1	FLORADADE	42.87d	42.28e	48.17d	38.26e
T2	FLORIDA-7775	39.51d	35.86f	41.31e	43.22d
T3	FLORIDA-7781	45.24d	37.39e	37.40f	33.36e
T4	NC-2Y	75.84a	75.58a	75.82a	82.72a
T5	NC-8276	43.04d	35.02f	41.93e	44.87d
T6	TOM-544	41.09d	34.05f	32.25f	34.79e
T7	TOM-596	38.44d	31.07f	39.97f	32.34e
T8	TOM-498	71.61a	71.05a	66.08b	74.61b
T9	TOM-499	71.16a	75.83a	67.25b	72.28b
T10	F1(FLORADADE × TOM-499)	61.45b	66.28b	58.82c	61.38c
T11	F1(FLORIDA-7775 × TOM-499)	64.02b	64.47b	57.95c	66.71c
T12	F1(FLORIDA-7781 × TOM-499)	66.57a	69.97b	61.13c	70.49b
T13	F1(NC-8276 × TOM-499)	61.78b	60.51c	53.45c	64.43c
T14	F1(TOM-544 × TOM-499)	60.24c	60.02c	56.47c	64.28c
T15	F1(TOM-596 × TOM-499)	55.39c	50.62d	50.05d	60.65c
T16	F1(NC-2Y × TOM-499)	64.4b	64.97b	59.70c	65.26c
T17	F1(FLORADADE × TOM-498)	56.89c	66.64b	55.20c	62.97c
T18	F1(FLORIDA-7775 × TOM-498)	65.58b	57.54c	50.45d	59.69c
T19	F1(FLORIDA-7781 × TOM-498)	60.31c	65.97b	59.60c	62.10c
T20	F1(NC-8276 × TOM-498)	64.09b	62.90b	54.95c	66.06c
T21	F1(TOM-544 × TOM-498)	62.53b	57.34c	48.50d	63.03c
T22	F1(TOM-596 × TOM-498)	58.17c	54.52d	43.47e	66.89c
T23	F1(NC-2Y × TOM-498)	70.52a	66.66b	57.34c	67.56c
T24	Giselle F1	41.84d	30.77f	43.30e	38.10e

Mean values followed by the same letter in the column are not different by the Scott-Knott test at 5 % probability

in the epidermis or in the pericarp, placenta, and columella.

In contrast, the homozygous old-gold crimson genotypes (=og^c homozygous) did not differ from the normal genotypes in regard to hue angles in the epidermis and pericarp, but showed a significant deviation in the red direction in the placenta and columella (C7 and C8 contrasts, Table 5). This deviation in the red direction occurred in the og^c/og^c genotypes, both in the presence of the high pigment allele in homozygosis (*hp/hp*) (Contrast C8, Table 5) and in its absence (hp^+/hp^+) (Contrast C7, Table 5). In og^c/og^c genotypes, the presence of the *hp* allele in homozygosis (*hp/hp*) led to a slight additional deviation in the red direction, observable only in the placenta (Contrast C9, Table 5).

In the absence of other alleles (*t*, og^c, or *hp*) that may affect fruit color, the high β-carotene heterozygotes exhibited significant deviations in the hue angle in the red direction, as compared to the high β-carotene homozygote genotypes (Contrast C10, Table 5) in the epidermis, pericarp, placenta, and columella, indicating that, although dominant, the high β-carotene allele does not have complete dominance. The greater tendency to orange of the high β-carotene homozygous genotypes in relation to the high β-carotene heterozygous ones may be observed whether the latter are also heterozygous for

Table 5 Estimates of contrasts of interest for hue angle of the epidermis, pericarp, placenta, and columella de tomato fruit

	Estimates[a]				Description of the contrast
	EP	PE	PL	CO	
C1	−21.70**	−28.19**	−16.08**	−27.67**	Normal genotypes, og^c homozygous, versus High β genotypes (homozygous and heterozygous)
C2	−34.12**	−40.37**	−35.63**	−44.86**	Normal genotypes, og^c homozygous, versus Tangerine Genotype
C3	−20.84**	−27.38**	−11.81**	−25.11**	Normal genotypes versus High β genotypes (homozygous and heterozygous)
C4	−28.80**	−37.42**	−22.20**	−33.03**	Normal genotypes versus High β homozygous genotypes
C5	−19.70**	−25.95**	−10.32**	−23.98**	Normal genotypes versus High β heterozygous genotypes
C6	−33.25**	−39.55**	−31.35**	−42.30**	Normal genotypes versus Tangerine genotype
C7	1.51ns	1.24ns	5.90**	4.10*	Normal genotypes versus og^c genotypes homozygotes, hp^+hp^+
C8	1.49ns	1.97ns	12.21**	5.61*	Normal genotypes versus og^c genotypes homozygotes, *hp* homozygous
C9	−0.02ns	0.72ns	6.31**	1.51ns	og^c homozygous genotypes, *hp* + *hp* + versus og^c genotype homozygote, *hp hp*
C10	10.33**	9.36**	11.06**	9.73**	High β homozygous genotypes versus High β genotypes heterozygotes, t^+normal og^{c+}normal, hp^+normal
C11	3.90*	7.62**	8.14**	7.03**	High β homozygous genotypes versus High β heterozygous genotypes, tangerine heterozygous
C12	9.71**	13.07**	12.89**	9.03**	High β homozygous genotypes versus High β heterozygous genotypes, og^c heterozygous, hp^+hp^+
C13	10.01**	14.76**	14.18**	9.79**	High β homozygous genotypes versus High β heterozygous genotypes, og^c heterozygous, *hp* heterozygous
C14	−6.43**	−1.73ns	−2.91ns	−2.71ns	High β genotypes heterozygotes, t^+normal og^{c+}normal, hp^+normal versus High β heterozygous genotypes, tangerine heterozygous
C15	−0.62ns	3.71**	1.82ns	−0.71ns	High β genotypes heterozygotes, t^+normal og^{c+}normal, hp^+normal versus High β heterozygous genotypes, og^c heterozygous, hp^+hp^+
C16	−0.33ns	5.41**	3.11ns	0.06 ns	High β genotypes heterozygotes, t^+normal og^{c+}normal, hp^+normal versus High β heterozygous genotypes, og^c heterozygous, *hp* heterozygous

[a] *EP* epidermis; *PE* pericarp; *PL* placenta, and *CO* columella; *ns* non-significance

**, * Significance at 1 and 5 % by the *t* test, respectively

t (Contrast C11, Table 5), heterozygous for og^c (Contrast C12, Table 5), or heterozygous both for og^c and for *hp* (Contrast C13, Table 5). The presence of alleles (*t*, og^c, *hp*) that may affect fruit color in high β-carotene heterozygous hybrids only marginally affected the color of these hybrids—in the hybrids also bearing the *t* allele in heterozygosis, it resulted in a deviation in the orange direction at the epidermal level (Contrast C14, Table 5); in the hybrids bearing the og^c allele in heterozygosis, whether in the presence of *hp* in heterozygosis or not, it resulted in deviations in the red direction in the pericarp (Contrasts C15 and C16, Table 5).

The results show that the use of the high β-carotene gene in homozygosis condition leads to an orange color in the fruit, both internally and externally, which is partially attenuated with the use of the high β-carotene gene in heterozygosis condition. Even in this latter case, however, the basic color continues to be orange since the high β-carotene heterozygote hybrids are not equal to the genotypes with normal color (red), not even when genes that could promote lycopene synthesis (like og^c or og^c + *hp*) are used in heterozygosis condition (Table 4).

The allele og^c in homozygosis condition led to a red color in the placenta in genotypes not bearing the high β-carotene gene, something that was strengthened with simultaneous use of the *hp* allele also in homozygosis condition.

The development of cultivars richer in functional compounds like carotenoids has been the focus of genetic breeding studies of garden crops, particularly tomatoes, a species for which mutants have been described that may substantially increase the carotenoid content in the fruit. This has drawn the interest of various researchers and breeders (Araújo et al. 2002; Cá et al. 2006). Some studies have shown the possibility of the use of these mutant alleles in tomato breeding, for the purpose of increasing the carotenoid content in the fruit without affecting production characteristics (de Andrade Júnior et al. 2005; Araújo et al. 2002; Dias et al. 2003; Faria et al. 2003).

These effects on color have already been reported by Araújo et al. (2002) and Faria et al. (2003) and showed the og^c and *hp* mutants, in Floradade background, alone or in combination, both in homozygosis and heterozygosis, led to significant increases in internal and external color, as well as in β-carotene and lycopene content of fruit. Faria et al. (2003) also observed increases in external color and in lycopene content in nor^+/nor^A mature fruit (mutant maturity genes of non-ripening (*nor*) and *alcobaça* (nor^A)), brought about by the combination og^{c+}/og^c hp^+/hp.

Saturation (Chroma)

The genotypes NC-2Y (*t* homozygote), and TOM-499 and TOM-498 (high β-carotene homozygotes), especially the first two, tend to show the lowest chroma values among the treatments, mainly in the pericarp and in the columella (Table 6)—values less than 20. The effect of the *t* homozygote in reducing saturation was greater than that of the high β-carotene homozygotes, and was quite accentuated in both the epidermis and in the pericarp, placenta, and columella, with values always less than 13. For the saturations of the epidermis, pericarp, placenta, and columella, the high β-carotene heterozygote hybrids in general showed intermediate values among those of the high β-carotene homozygotes and those of the genotypes of red fruit color (normal red genotypes or red genotypes bearing og^c and *hp*) (Table 6).

Fruit from high β-carotene genotypes (homo or heterozygous), as well as the tangerine homozygote genotype, tend to produce fruit with significantly lower chroma values in the epidermis, pericarp, and columella than the genotypes with red color fruit, whether the genotypes with red color fruit bear the og^c or *hp* alleles or not (Contrasts C1, C2, C3, C4, C5, C6, Table 7). In the placenta, saturation in the tangerine homozygote genotype was also significantly less than in the genotypes with red color fruit (contrasts C2 and C6, Table 7), which did not occur in saturation of the high β-carotene genotypes (contrasts C3, C4, C5, Table 7), except when the genotypes are normal and/or homozygous for og^c (Contrast C1, Table 7).

Genotypes with the og^c allele in homozygosis led to a greater chroma value in the pericarp and placenta compared to the genotypes of red fruit not bearing the og^c allele (Contrast C7, Table 7). This greater chroma value brought about by og^c was strengthened by simultaneous use of the *hp* allele in homozygosis since the og^c/og^c *hp*/*hp* genotype showed more intense saturation than the normal genotypes with red fruit, not only in the pericarp and placenta, but also in the epidermis and in the columella (Contrast C8, Table 7). This strengthening of the effect of og^c by the action of

Table 6 Mean values of Chroma for epidermis, pericarp, placenta, and columella in tomato fruit

Treatments		EP	PE	PL	CO
T1	FLORADADE	24.47a	19.52b	20.66c	28.08b
T2	FLORIDA-7775	25.02a	20.66b	21.55c	21.31c
T3	FLORIDA-7781	25.32a	23.90a	30.60a	26.80b
T4	NC-2Y	12.72d	9.85d	12.66d	7.98e
T5	NC-8276	24.63a	18.89b	23.38b	20.55c
T6	TOM-544	26.73a	25.96a	31.82a	32.01a
T7	TOM-596	24.82a	24.54a	24.46b	28.89b
T8	*Purdue* 88-96-1 (=TOM-498)	17.09c	14.25c	19.34c	15.04d
T9	*Purdue* 88-100A-1 (=TOM-499)	21.63b	19.69b	22.41b	17.26d
T10	F1(FLORADADE × TOM-499)	21.11c	15.35c	20.77c	18.20d
T11	F1(FLORIDA-7775 × TOM-499)	19.74c	18.37c	19.93c	20.01c
T12	F1(FLORIDA-7781 × TOM-499)	19.34c	15.43c	21.62c	17.48d
T13	F1(NC-8276 × TOM-499)	20.29c	17.98c	23.75b	18.42d
T14	F1(TOM-544 × TOM-499)	21.42b	16.73c	21.55c	15.35d
T15	F1(TOM-596 × TOM-499)	25.40a	19.89b	23.45b	17.25d
T16	F1(NC-2Y × TOM-499)	20.31c	15.36c	21.06c	16.87d
T17	F1(FLORADADE × TOM-498)	21.78b	17.86c	19.23c	15.69d
T18	F1(FLORIDA-7775 × TOM-498)	22.45b	16.85c	23.58b	18.15d
T19	F1(FLORIDA-7781 × TOM-498)	20.55c	19.31b	22.93c	16.64d
T20	F1(NC-8276 × TOM-498)	19.59c	18.81b	20.33b	17.56d
T21	F1(TOM-544 × TOM-498)	21.81b	17.96c	23.07c	18.61d
T22	F1(TOM-596 × TOM-498)	22.60b	20.36b	24.39b	21.88c
T23	F1(NC-2Y × TOM-498)	17.39c	15.57c	21.15c	18.33d
T24	Giselle F1	22.59b	20.63b	24.46b	26.99b

EP epidermis; *PE* pericarp; *PL* placenta, and *CO* columella

Mean values followed by the same letter in the column are not different by the Scott-Knott test at 5 % probability

hp may also be noted in the significance of the Contrast C9 (Table 7) in the pericarp, placenta, and columella.

Fruit of high β-carotene heterozygous genotypes that do not bear *t*, og^c, or *hp*, had chroma values similar to fruit of high β-carotene homozygous genotypes (Contrast C10, Table 7), whether in the epidermis, pericarp, placenta, or columella. Saturation differences were also not observed between the high B-carotene homozygous genotypes and the high β-carotene heterozygous genotype bearing the *t* allele in heterozygosis (Contrast 11, Table 7). In contrast, high B-carotene heterozygous hybrids bearing og^c in heterozygosis exhibited significantly greater saturation in the epidermis and columella than the high β-carotene homozygote genotypes (Contrast C12, Table 7)—something also observed in the epidermis for high β-carotene heterozygous hybrids bearing og^c and *hp* in heterozygosis (Contrast 13, Table 7). The greater saturations of the high B-carotene heterozygous hybrids shown by the contrasts C12 and C13 (Table 7) appear to be only the effect of the action of the og^c allele in heterozygosis, and not of the *hp* allele in heterozygosis—which does not appear to have contributed at all to greater saturation since its presence did not even contribute to improvement in chroma values of the columella (Contrast C13, Table 7) and even seems to be against the action of og^c in heterozygosis.

This action of the heterozygous *hp* in countering the favorable effects of the heterozygous og^c on saturation of the heterozygous high β-carotene may also be observed by the lack of significance of contrast C16 on the placenta when compared to the significance of contrast C15 (Table 7).

In contrast, fruit of the high β-carotene heterozygous genotypes, not bearing *t*, og^c, or *hp*, had chroma values in the epidermis greater than that of the high β-carotene heterozygous hybrid bearing the *t* allele in heterozygosis (Contrast 14, Table 7), showing that *t*, even in heterozygosis, may contribute to lower chroma

Table 7 Estimates of contrasts of interest for Chroma of the epidermis, pericarp, placenta, and columella of tomato fruit

	Estimates				Description of the contrast
	EP	PE	PL	CO	
C1	4.02**	4.53**	3.48**	8.70**	Normal genotypes, og^c homozygous, versus High β genotypes (homozygous and heterozygous)
C2	12.08**	12.16**	12.61**	18.39**	Normal genotypes, og^c homozygous, versus Tangerine Genotype
C3	3.11**	2.19**	−1.04ns	7.53**	Normal genotypes versus High β genotypes (homozygous and heterozygous)
C4	4.53**	2.71*	1.95ns	9.05**	Normal genotypes versus High β homozygous genotypes
C5	2.91**	2.11**	0.91ns	7.32**	Normal genotypes versus High β heterozygous genotypes
C6	11.18**	9.83**	10.16**	17.22**	Normal genotypes versus Tangerine genotype
C7	−1.17ns	−3.35**	−2.70**	−0.46ns	Normal genotypes versus og^c genotypes homozygotes, hp^+hp^+
C8	−2.82*	−6.28**	−8.98**	−6.78**	Normal genotypes versus og^c genotypes homozygotes, *hp* homozygous
C9	−1.67ns	−2.92*	−6.28**	−6.33**	og^c homozygous genotypes, $hp + hp +$ versus og^c genotype homozygote, *hp hp*
C10	−1.33ns	−0.52ns	−0.14ns	−1.31ns	High β homozygous genotypes versus High β genotypes heterozygotes, t^+normal og^{c+}normal, hp^+normal
C11	0.51ns	1.50ns	−0.23ns	−1.45ns	High β homozygous genotypes versus High β heterozygous genotypes, tangerine heterozygous
C12	−2.31**	−1.39ns	−1.77ns	−2.41**	High β homozygous genotypes versus High β heterozygous genotypes, og^c heterozygous, hp^+hp^+
C13	−2.25*	−0.37ns	−1.43ns	−0.83ns	High β homozygous genotypes versus High β heterozygous genotypes, og^c heterozygous, *hp* heterozygous
C14	1.84*	2.03ns	−0.09ns	−0.13ns	High β genotypes heterozygotes, t^+normal og^{c+}normal, hp^+normal versus High β heterozygous genotypes, tangerine heterozygous
C15	−0.99ns	−0.87ns	−1.63*	−1.11ns	High β genotypes heterozygotes, t^+normal og^{c+}normal, hp^+normal versus High β heterozygous genotypes, og^c heterozygous, hp^+hp^+
C16	−0.91ns	0.15ns	−1.29ns	0.48ns	High β genotypes heterozygotes, t^+normal og^{c+}normal, hp^+normal versus High β heterozygous genotypes, og^c heterozygous, *hp* heterozygous

[a] *EP* epidermis; *PE* pericarp; *PL* placenta, and *CO* columella; *ns* non-significance

**, * Significance at 1 and 5 % by the *t* test, respectively

values. In the opposite direction, fruit of high β-carotene heterozygous genotypes, not bearing *t*, og^c, or *hp*, had chroma values in the placenta less than those of high β-carotene heterozygous hybrids bearing the og^c allele in heterozygosis (Contrast 15, Table 7)—confirming the tendency of the heterozygote og^{c+}/og^c genotype to promote increases in chroma values. This tendency of og^{c+}/og^c in promoting increases in saturation appears to have been reversed in heterozygote genotypes both for og^c and for *hp* simultaneously (og^{c+}/og^c hp^+/hp genotypes) (Contrast 16, Table 7), confirming the previous suggesting that the actions of the og^{c+}/og^c and hp^+/hp genotypes on chroma values of high β-carotene heterozygous hybrids may be in opposition.

The carotenoids present in the fruit are the main agents responsible for beneficial properties in reducing the risk of chronic degenerative diseases, and within the cultivars on the market, lycopene makes the greatest contribution (Raupp et al. 2009). Obtaining tomato cultivars with fruit of greater nutraceutic value, with greater contents of beta-carotene and lycopene, is of great importance since cultivars with these characteristics are currently rare on the Brazilian market, and there is no tomato registered as being rich in lycopene and beta-carotene in the same cultivar.

However, as high β-carotene tomato fruit has an orange color, it might not be well accepted by the consumer. The use of combinations of high β-carotene with og^c and *hp* may be an alternative in improving color since these last two genes intensify the red color in the tomato fruit. In the present study, it may be observed that none of the hybrids obtained a red base color, but the hybrids with a hue angle between 50 and

60° are slightly red and may be placed on the market and would be accepted for sale with the advantage of a greater beta-carotene content from the presence of the high β-carotene gene which promotes the synthesis of this carotenoid.

Conclusions

The hybrids F1(TOM-596 × TOM-499), F1(FLORADADE × TOM-498), and F1(TOM-596 × TOM-499) are those that most drew near the red external color (hue angle <60°). Even with the use in heterozygosis of genes that could promote lycopene synthesis (og^c or $og^c + hp$), the basic color of the hybrids continues to be orange, whether in the epidermis, pericarp, placenta, or columella. In high β-carotene hybrids, the use of the og^c gene in heterozygosis associated with *hp* in heterozygosis counters the effect of the og^c alone in heterozygosis of improving the chroma values of the hybrids. In use of the *t* gene in hybrid combinations with the *B* gene, color is still orange, but less accentuated than in the homozygous lines and leads to a reduction in the chroma values in all the parts studied. The best combination of *B* in heterozygosis is with the mutant gene og^c for both general color (lower hue angle) and for chroma (greater chroma values).

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References

Agarwal S, Rao AV (2000) Tomato lycopene and its role in human health and chronic diseases. Can Med Assoc J 163(6):739–744

Araújo ML, Maluf WR, Gomes LAA, Oliveira ACB (2002) Intra and interlocus interactions between alcobaça (alc), *old-gold crimson* (og^c) and high pigment (*hp*) loci in tomato *Lycopersicon esculentum* Mill. Euphytica 125(2):215–225

Botella-Paíva P, Rodríguez-Concepción M (2006) Carotenoid biotechnology in plants for nutritionally improved foods. Physiol Plant 126(3):369–431

Cá JA, Maluf WR, Gomes LAA, Nascimento IR, Faria MV, Licursi V, Moretto P (2006) Híbridos de tomateiro longa-vida com frutos de maior intensidade de coloração. Pesqui Agropecu Brasil 41(9):1377–1384

Color Glossary (2012) Color glossary. http://www.sapdesignguild.org/resources/glossary_color. Accessed 23 October 2012

de Andrade Júnior VC, Maluf WR, Faria MV, Benites FRG, dos Santos Júnior AM (2005) Produção e qualidade de frutos de híbridos de tomateiro portadores de alelos mutantes de amadurecimento e coloração. Pesqui Agropecu Bras 40:555–561

Dias TJM, Maluf WR, Faria MV, Freitas JA, Gomes LAA, Resende JTV, de Azevedo SM (2003) *Alcobaça* allele and genotypic backgrounds affect yield and fruit shelf life of tomato hybrids. Sci Agric 60:269–275

Faria MV, Maluf WR, Azevedo SM, Andrade-Júnior VC, Gomes LAA, Moretto P, Licursi V (2003) Yield and post-harvest quality of tomato hybrids heterozygous at the loci *alcobaça, old-gold crimson* or *high pigment*. Genet Mol Res 2(3):317–327

Giordano LB, Aragão FAS, Boiteux LS (2003) Melhoramento genético do tomateiro. Inform Agropecu 24:43–57

Isaacson T, Ronen G, Zamir D, Hirschberg J (2002) Cloning of *tangerine* from tomato reveals a carotenoid isomerase essential for the production of β-carotene an xanthophylls in plants. Plant Cell 14:333–342

Konica Minolta (2012) Precise color communication. http://www.konicaminoltaeurope.com/pcc/en. Accessed 12 November 2012

Raupp DS, Gardingo JR, Schebeski LS, Amadeu CA, Borsato AV (2009) Processamento de tomate seco de diferentes cultivares. Acta Amazônica 39(2):415–422

Shami NJ, Moreira EA (2004) Licopeno como agente antioxidante. Revista de Nutrição 17(2):227–236

Thompson AE, Tomes ML, Wann EV, Mccollum JP, Stoner AK (1965) Characterization of *old-gold crimson* tomato fruit color. Proc Am Soc Hortic Sci 86(1):610–616

Thompson AE, Tomes ML, Erickson HT, Wann EV, Armstrong RJ (1967) Inheritance of crimson fruit color in tomatoes. Proc Am Soc Hortic Sci 91:495–504

Thurnham DI (2007) Bioequivalence of β-carotene and retinol. J Sci Food Agric 87(1):13–39

Tomes ML, Quackenbush FW, Mcquistan M (1954) Modification and dominance of the gene governing formation of high concentrations of beta-carotene in the tomato. Genetics 39:810–817