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Effect of Mutant Genotypes *hp* og^c and *dg* og^c on Tomato Fruit Quality

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Abstract. **The mutant genotypes *high-pigment/crimson (hp* og^c*)* and *dark-green/crimson (dg* og^c*)* were evaluated for their effect on tomato fruit quality. Mature green fruit from the mutant genotypes had 2 to 3 times the amount of chlorophyll in the pericarp and mesocarp tissue as their genetically related but normal counterparts or the control 'Flora-Dade'. Fruit of the mutant lines were firmer than 'Flora-Dade' or their normal counterparts at the mature green stage, as well as when fully ripe. The mutant genotypes conditioned an intense red color in the ripe fruit; the result of a 2-fold increase in carotenoid pigments, especially the red pigment lycopene. Ripe fruit of the mutant genotypes were higher in ascorbic acid (Vitamin C) and beta-carotene (Provitamin A) than normal, but they were slightly lower in total soluble solids. There were no significant differences in pH, alcohol insoluble solids, or total pectin among normal or mutant genotypes. Enzyme analyses showed higher polygalacturonase and β-galactosidase activity in the mutant genotypes than 'Flora-Dade' or the normal counterparts. Line T4077 *dg* og^c had significantly less activity for these 2 enzymes than line T3995 *hp* og^c. The *dg* og^c line also had less activity for pectinesterase and invertase than any of the other lines, including its normal counterpart. The 2 mutant genotypes had similar effects on fruit quality characteristics. A sensory panel (n = 48) indicated no significant taste preference differences among the 5 lines.**

Numerous genetic mutations have been identified and described in tomato (*Lycopersicon esculentum* Mill.) that affect its fruit ripening and fruit quality characteristics (1, 3, 8, 17, 18, 19, 20, 22, 23). Thompson et al. (16, 18) studied the effect of the mutant *high-pigment (hp)* on carotenoid pigments and showed that it caused a 2-fold increase in lycopene and carotene in ripe fruit. They reported that *hp* caused the flesh of the fruit to be firmer and to possess a higher level of protopectins than normal. Plants of the homozygous *hp* genotype had reduced plant vigor, and the immature fruit and foliage were a darker green than normal. Another mutant, very similar to *hp* in phenotype, was described by Konsler (8). Genetic studies showed that it was distinct from *hp* and, therefore, was given the designation *dark-green* (*dg*) (8). The effect of *dg* on the tomato plant and fruit is almost identical to that of *hp*, except that the immature fruit have a darker green color than those of *hp*. Neither *hp* nor *dg* have been used in cultivars, principally because of their negative effect on plant vigor, and they produce an orange color in the ripe fruit when conditions are not optimum for lycopene development.

A color variation in tomato was discovered by Butler (3) and given the name "crimson" for its bright red color. Genetic studies by Thompson et al. (17) showed that the crimson character was conditioned by a single recessive factor that was allelic with the gene (*og*) conditioning "old-gold" flower color (15). The gene symbol og^c was adopted to designate the *old-gold/crimson* characteristic, which appears to be a pleiotropic effect (17). Fruit from plants homozygous for og^c contained about 75% more lycopene than normal and half as much beta-carotene. The result was a higher lycopene: carotene ratio, especially in the locular region of the fruit, giving it a brilliant red color at full ripeness (19).

Based on several detailed evaluations that have been reported (8, 16, 23, 24), it seems that neither *hp* nor *dg* alone would contribute significantly toward improving tomato fruit quality.

Tomes and Thompson (23) reported that fruit produced on plants with the *hp* og^c combination had a distinct red color and high lycopene content. Wann et al. (24) reported that genes *hp* og^c when combined enhanced color development in detached immature tomatoes. These genes seemed to have a beneficial effect in tomatoes harvested at the mature-green stage for shipping. When fully ripe, high-pigment/crimson tomatoes have a deep red color, and the beta-carotene (Provitamin A) content is slightly above that of normal tomatoes. Preliminary observations by the authors indicate that the *dg* og^c genotype also would have a similar beneficial effect in fresh-market tomatoes.

The usefulness of the *hp* og^c and *dg* og^c genotypes in commercial cultivars is questionable at this time, due to slow seed germination and reduced plant vigor in the mutant lines. The economic value of the mutant genotypes and their acceptance in commercial cultivars remains to be determined. The purpose of these investigations was to evaluate the *hp* og^c and *dg* og^c genotypes in improved breeding lines and to determine their effect on pigmentation, enzyme systems, consumer preference, and other economically important fruit quality characteristics.

Materials and Methods

The cultivar 'Flora-Dade', a widely grown commercial cultivar for fresh market production, was used as a control in these studies. Normal and mutant (*hg* og^c and *dg* og^c) counterparts of experimental breeding lines T3995 and T4077 were used for genotype comparisons. Experimental lines T3995 hp^+og^{c+} and T3995 *hp* og^c were derived from a cross between 'Patriot' (og^c)

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and a related breeding line carrying the mutant *hp* gene from Univ. of Illinois breeding line No. 1252 (16). Lines T4077 dg^+ og^{c+} and T4077 *dg* og^c were derived from a cross between 'Patriot' (og^c) and North Carolina dark-green (*dg*) (8), selected for the double-mutant genotype, and outcrossed to 'Flora-Dade'. From the F_2 progenies, sib lines were produced with either normal or the double mutant genotypes. The experimental lines were in the F_4 generation when these studies were made. The tomatoes were grown in field plots (16 plants/plot, replicated 4 times) at Charleston, S. C., using standard cultural practices.

Fruit samples were harvested at the mature green stage. Fruit maturity was judged initially by the position of the fruit on the plant relative to others showing most recent color development on the same plant. Further, a fruit was judged to be mature green when whitish lines were visibly radiating from the blossom end of the fruit and a corky ring was present at the stem scar. The samples were placed on open trays in a ripening room held at 20° ± 2°C. Subsamples of 4 fruit each were taken immediately without ripening from each tray and used for total chlorophyll determinations.

Total chlorophyll was determined in the mature green fruit using the procedure of Holden (6) with some modifications. Representative samples of pericarp and mesocarp tissue were removed from the fruit in sections extending from the stem scar to the blossom end. The chlorophyll was extracted with 80% aqueous acetone using a Brinkman Polytron tissue homogenizer. The homogenate was filtered through No. 3 Whatman paper to produce a clear chlorophyll extract. Absorbence of the solution was measured at 645 and 663 nm. The chlorophyll content in milligrams per 100 g of fresh tissue was calculated according to the formula developed by Holden (6).

After ripening for 7–10 days, a uniform sample of 6 ripe fruit was taken from each plot. Each fruit was cut laterally in half for flesh firmness and soluble solids determinations. Flesh firmness was measured by forcing a 1 cm plunger connected to an Ametek Hunter force gauge into the carpel wall tissue. Resistance was measured in kilograms per square centimeter. Soluble solids were measured by expressing a few drops of clear juice onto the prism of a Bausch and Lomb Abbe desk refractometer. The fruit then were quartered, and opposite quarters from each fruit were frozen at 0°C for later carotenoid analyses. The remaining quarters were blended for 3 min in a Waring Blendor for pH and ascorbic acid determinations. The pH of the blended puree was determined with a standard laboratory pH meter. Reduced ascorbic acid was determined using the procedure developed by Heinze et al. (5), in which 1% oxalic acid was used for extraction. This method does not measure dehydroascorbic acid which was considered insignificant in the tomato fruit.

Carotenoid pigment determinations (lycopene and beta-carotene) were made from the frozen samples by techniques developed by McCollum (10), except that the lycopene fraction was eluted from the chromatographic column and measured separately (25).

A separate set of samples was harvested mature green from the plots at Charleston, S.C., transported to the Richard Russell Research Center at Athens, Ga., and ripened as described previously. Representative subsamples were used for enzymatic analyses and determination of alcohol-insoluble solids, total pectin, and consumer preference.

Alcohol insoluble solids (AIS) were determined by extracting the tomato tissue with 80% ethanol and drying the insoluble fraction under vacuum (14). The total pectin content was determined by hydrolysis of the uronic acid in the AIS with fungal pectinase (14). The solubilized uronic acid was measured by the hydroxydiphenyl method (2).

Extracts for the enzymatic analyses were prepared by blending 100 g of tissue with 100 ml of cold water. The homogenate was adjusted to pH 4, stirred 1 hr and centrifuged at 20,000 g for 20 min. The pellet was suspended in 150 ml of cold 1.0 M NaCl, adjusted to pH 6, and stirred for 1 hr. The suspension then was centrifuged at 20,000 g for 20 min and 5 ml of the supernatant solution was dialyzed overnight against 1.0 M NaCl. All of the above steps were conducted at about 3°C.

Polygalacturonase (PG) was assayed by measuring the formation of reducing groups from polygalacturonic acid (13). Pectinesterase (PE) was assayed by titration of the carboxyl groups released from pectin at pH 7 (13). Invertase (INV) was assayed by measuring the formation of reducing sugars from sucrose (11). β-Galactosidase (βG) was assayed by measuring the p-nitrophenol released from p-nitrophenyl-galactoside (12).

The consumer preference test was conducted using a rank order preference scale with 1 = most preferred to 5 = least preferred. Forty-eight judgments were obtained from employee volunteers, all were consumers of fresh tomatoes. Tomatoes from each line were selected for uniform color and firmness and cut into wedges. Wedges from each line were served to the panelists on individual plates coded with randomly determined 3-digit numbers. All 5 samples were presented simultaneously in a panel testing room equipped with individual stations, positive air pressure, and green lighting (40-W) to mask obvious color differences. The order in which samples were placed in the stations was randomized. Panelists were instructed to taste each sample in the order presented, to rinse their mouth with room temperature water between samples, to write comments on texture and taste, and finally, to indicate preference by placing the sample code number in the appropriate blank for 1 = most preferred to 5 = least preferred. Preference data were analyzed by rank sum analysis (7) and analysis of variance of converted rank scores (9).

Results and Discussion

The effects of the genes *hp* and *dg* were visible at all stages of growth from the dark green color in the stem, leaves, and immature fruit. Flowers on the *hp* og^c and *dg* og^c plants were distinctly "old-gold" with a brownish color in the petals. Yield and fruit size were not measured in this experiment. Our observations of the field plots at maturity, however, indicated that productivity and fruit size of the lines with mutant genotypes were below that of 'Flora-Dade' or their normal counterparts. Plants of the mutant genotypes also were smaller and more compact than normal determinate plants.

Fruit harvested from the *hp* og^c and *dg* og^c genotypes were different in many respects from their normal counterparts. At the mature green stage, they had 2 to 3 times more total chlorophyll in the pericarp and mesocarp tissue than normal (Table 1). The high concentration of chlorophyll produced an intensely dark green color, especially on the shoulders of the fruit. The *hg* og^c and *dg* og^c fruit took 1–3 days longer to reach full red color in the ripening room, and blotches of green occasionally persisted on the shoulders for several days after full ripeness. Color in their ripe fruit was deep red due to the high total pigment, enhanced by high lycopene (red) content. Beta-carotene (Provitamin A) and lycopene were significantly higher in *dg* og^c fruit than *hp* og^c (Table 1).

The fruit of *hg* og^c and *dg* og^c genotypes were judged to be extremely firm by feel, both in the mature green and fully rip-

Table 1. Tomato fruit quality characteristics of normal and high-pigmented gentoypes.[z]

Tomato lines	Total chlorophyll[y] (mg/100 g)	Fruit firmness (kg/cm^2)	Ascorbic acid (mg/100 g)	Beta-carotene (μg/g)	Lycopene (μg/g)	Soluble solids (%)	pH	Alcohol insoluble solids (mg/g FW)	Total pectin (% of AIS)
Flora-Dade	2.9	0.95 ± 0.06[x]	8.5	3.8	40.8	5.3	4.4	18.9	25.9
T3995 (*hp*$^+$ *og*$^{c+}$)	4.8	1.00 + 0.05	9.2	3.5	55.6	7.0	4.5	17.3	26.4
T3995 (*hp og*^{c})	8.8	1.32 + 0.08	17.9	6.2	96.0	5.7	4.6	18.3	24.8
T4077 (*dg*$^+$ *og*$^{c+}$)	2.9	1.00 + 0.08	7.9	2.7	51.4	5.7	4.4	19.3	24.8
T4077 (*dg og*^{c})	11.0	1.24 ± 0.06	14.7	8.9	117.5	5.1	4.5	19.9	26.4
LSD at 5%	0.8	---	3.7	1.6	20.4	0.5	0.1	NS	NS

[z]All measurements except total clorophyll were made on fruit samples harvested at the mature-green stage and ripened at 20°C for 7–10 days.
[y]Total chlorophyll was measured in the fruit wall at the mature-green stage.
[x]SE of the mean of 2 readings/fruit, 6 fruit/sample and 4 replications.

ened stages. The fruit firmness data in Table 1 confirm that the flesh of ripe fruit in the mutant genotypes was firmer than 'Flora-Dade', which is itself considered a firm fruited tomato. Ascorbic acid, beta-carotene, and lycopene also were elevated (about doubled) in the ripe fruit of the mutant lines (Table 1). The mutant genotypes apparently had a negative effect on soluble solids in the ripe fruit (Table 1). We noted, however, that T3995 *hg*$^+$*og*$^{c+}$ was significantly higher in total soluble solids than any of the other lines tested, which was unexplained and probably not related to fruit genotypes. Only small differences occurred in pH of the blended puree of the various genotypes, and they were not considered genetically significant. There were no significant differences among the lines for alcohol insoluble solids or total pectin.

Lines with the mutant genotypes *hp og*^{c} and *dg og*^{c} had higher levels of PG than their normal counterparts or 'Flora-Dade' (Table 2). Generally, firm lines of tomatoes contain less PG, but the relationship between PG activity and fruit firmness is complex and indirect (21). The lower PE in the mutant genotypes, compared with the normal counterparts (Table 2), may have had some effect on fruit firmness, although Hall and Dennison (4) did not find a significant correlation between PE and tomato firmness. The mutant genotypes also contained more βG activity than 'Flora-Dade'. Evidence was presented recently (13) that a particular form of βG may be involved in tomato softening. Although we did not measure the relative activities of the different forms of βG in these tests, it is possible that a nonsoftening form was present in the mutant genotypes.

Measurement of INV, which may be involved in sugar formation, was variable, yet both of the T4077 genotypes had significantly less INV activity than T3995 *hp og*^{c}. Line T4077 *dg og*^{c} had less INV activity than any of the lines tested, including its normal counterpart.

A sensory evaluation panel of 48 untrained individuals indicated no significant preference ($P = 0.05$) for any of the 5 lines of fresh tomatoes tested (Table 3). Ranked preference data were subjected to both rank sum analysis (7) and analysis of variance of converted rank scores (9). Neither test showed a significant probability of variance in data being attributed to true preferences, although in each analysis the preference scores for T3995 *hp*$^+$ *og*$^{c+}$ approached significance. Further steps to group panelists by associated correlations indicated 4 major ranking patterns among the panelists. The largest group, accounting for 44% of the panelists, gave a mean ranking of 1.7 for T3995 *hp*$^+$ *og*$^{c+}$ preference over the other tomato lines. Preference for T3995 *hp*$^+$ *og*$^{c+}$ may have been influenced by the exceptionally high soluble solids (Table 1) found in that particular line.

Tomato fruit of the *hp og*^{c} and *dg og*^{c} genotypes seemed to have several advantages over their normal counterparts. They were extraordinarily firm at all stages of maturity, had brilliant red color when ripe, and they were higher than normal in Vitamins A and C. They have the disadvantage of requiring 1–3 days longer to reach full ripeness, and the *dg og*^{c} genotype had lower soluble solids than normal. Plants of the mutant genotypes were less vigorous in the early stages of growth and obviously had lower yield than normal plants. Recent breeding work (unpublished) suggests, however, that improvements can be made in the mutant genotypes for plant vigor, yield, and fruit size.

Table 2. Enzyme activity in ripe tomato fruit from different genotypes affecting fruit quality characteristics.

Tomato lines	Units[z]/gram fresh wt: Poly-galacturonase (PG)	β-galactosidase (βG)	Pectin-esterase (PE)	Invertase (INV)
Flora-Dade	21	0.94	87	100
T3995 *hp*$^+$ *og*$^{c+}$	24	1.22	81	117
T3995 *hp og*^{c}	37	1.36	74	130
T4077 *dg*$^+$ *og*$^{c+}$	18	0.92	89	98
T4077 *dg og*^{c}	29	1.16	55	75
LSD 5%	4	0.20	17	22

[z]A unit of activity is defined as follows: For PG, the amount that releases 1 μmole of reducing groups from polygalacturonic acid in 15 min; for βG, the amount that hydrolyzes 1 μmol of p-nitrophenyl-β-galactoside in 15 min; for PE the amount that releases 1 μmole of carboxyl group from pectin in 5 min; for INV, the amount that releases 1 μmole of reducing groups from sucrose in 5 min.

Table 3. Results of sensory preference test for the 5 tomato lines.[z]

Tomato lines	Rank sums	Mean preference ranking
Flora-Dade	159	3.31
T3995 *hp*$^+$ *og*$^{c+}$	118	2.46
T3995 *hp og*^{c}	138	2.88
T4077 *dg*$^+$ *og*$^{c+}$	153	3.19
T4077 *dg og*^{c}	152	3.17

[z]Scale for ranking preference was 1 = most preferred to 5 = least preferred. N = 48 panelists.

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Fruit Temperature Effects on Mechanical Damage of Sweet Cherries

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Abstract. **The resistance of sweet cherries to compression damage as measured by the fruit firmness variables, [force to bioyield (FBY), slope of a compression curve, and maximum and residual forces of a compression-relaxation curve] decreased linearly with increasing fruit temperture. The incidence of impact-induced surface pitting decreased linearly as fruit temperature increased. The rate of decrease in impact damage per degree increase in fruit temperature was a function of the cultivar, contact surface, and drop height.**

The firmness and response to mechanical stress of a fleshy fruit is affected by temperature (1, 11). Further, whether the physical forces are applied instantaneously (impact) or gradually (compression) determines how temperature will affect the mechanical properties of the fruit.

Bourne (1) recently has established firmness-temperature coefficients (FT) for several commodities using quasi-static firmness measurements. Firmness-temperature coefficient is defined as the "percentage change in firmness per degree temperature increase." Most commodities showed a slight linear softening (negative FT) with increasing temperature. The FT coefficient, however, was highly variable within cultivar, among cultivars and species, and with stage of maturity.

This same negative relationship between temperature and

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