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Genetic analysis of tomato accessions with pleiotropic genes affecting post-harvest attributes

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ABSTRACT. Plants of five tomato accessions (*Lycopersicon esculentum* Mill.) derived from diallel crossing, carrying modifier genes for normal fruit ripening and their F_1 hybrids, except reciprocals, were evaluated. The pleiotropic effect of 'stolid' mutant and the nature and magnitude of genetic effects involved in the determination of those traits are provided. Although 'stolid' mutant increased shelf life, fruit firmness and the levels of carotenoid pigments, soluble solids contents decreased. Excepting fruit pH, all traits followed the additive-dominant model with additive genetic effects. Dominance deviations were also important, with the exception of fruit firmness. Additive genetic effects were more important for shelf life and titratable acidity and there was no predominance of any other particular genetic effect for remaining characteristics. Recessive genes were very important to increase shelf life and fruit firmness, while dominant genes increased the level of soluble solids and titratable acidity. Regression analysis of W_i on V_i, showed that cv. Santa Clara and 'stolid' mutant were divergent in shelf life, firmness and soluble solids of fruits. Selection can only be efficient for shelf life. Cross P_1 ('stolid' mutant) with P_5 (BGH-6913 - *nor*) has been shown to favour an increase of post-harvest conservation.

Key words: *Lycopersicon esculentum*, ripening mutants, *rin, nor*, *alc*, diallel.

RESUMO. **Análise genética de acessos de tomateiro com genes pleiotrópicos que modificam caracteres pós-colheita de frutos.** Cinco acessos de tomateiro (*Lycopersicon esculentum* Mill.) contendo genes que alteram o processo normal de amadurecimento de frutos e seus híbridos F_1, obtidos do cruzamento dialélico, sem os recíprocos, foram avaliados com os objetivos de verificar a ocorrência de pleiotropia promovida pelo mutante 'firme' e estudar a natureza e magnitude dos efeitos gênicos envolvidos na determinação desses caracteres. Os resultados evidenciaram que o mutante 'firme' promove aumento da vida de prateleira, firmeza e os níveis de pigmentos carotenóides, e reduz o teor de sólidos solúveis. Com exceção do caráter pH de frutos vermelhos, todos os caracteres seguiram o modelo aditivo-dominante, sendo os efeitos gênicos aditivos envolvidos no controle de todos os caracteres. Os desvios de dominância também foram importantes, exceto para o caráter firmeza de frutos. Nos caracteres vida de prateleira e acidez titulável os efeitos gênicos aditivos foram mais importantes, enquanto nos demais não houve predominância de particular efeito gênico. Constatou-se ainda maior importância de genes recessivos no aumento das expressões da vida de prateleira e firmeza de frutos, e dos genes dominantes, no aumento do teor de sólidos solúveis e da acidez titulável. Pela análise de regressão W_i em V_i, o cv. Santa-clara e o mutante 'firme' apresentam comportamento discrepante para os caracteres vida de prateleira, firmeza de frutos e teor de sólidos solúveis de frutos vermelhos. No entanto, a seleção pode ser eficiente apenas quanto a vida de prateleira, sendo que a combinação de P_1 (mutante 'firme') com P_5 (BGH-6913 - *nor*) pode promover aumento do período de conservação pós-colheita.

Palavras-chave: *Lycopersicon esculentum,* mutantes do amadurecimento, *rin, nor, alc*, dialelo.

Tomato breeders endeavour to obtain the development of productive varieties which carry resistant genes against diseases and plagues, together with higher horticultural qualities, such as fruit flavour, colour and shelf life.

The tomato post-harvest conservation has been intensified by pleiotropic mutants that modify the normal process of fruit ripening (Kopeliovitch *et al*., 1979, Stevens and Rick, 1986, Tigchelaar *et al*., 1978) and by transgenics of antisense ripening genes (Oeller *et al*., 1991; Gray *et al*., 1994). In Brazil, the use by seed companies of pleiotropic mutations in hybrids has been remarkable.

Potentiality of pleiotropic mutants for breeding programs has been thoroughly examined. Kopeliovitch *et al*. (1979) studied the effects of pleiotropic mutations crossed with normal genotypes. He detected that colour rate decreased owing to ripening inhibitor *(rin)* and, to a less extent, by the non-ripening one (*nor*). Since these two factors are in the heterozygous condition, they indicate the involvement of mutations in lycopene content. It was found that in the combination of *rin* and *nor* mutations, in the heterozygous condition, fruits presented good post-harvest conservation and could be stored for about 50 days at 20°C. However, the fruits colour was light red. On the other hand, high pigment (*hp*) genes in the homozygous condition, associated to *rin* and *nor* genes, both heterozygous, caused a better effect on fruit colour.

Combined mutations studied by Araújo (1997) on *alcobaça* (*alc*), *crimson* (og^{c+}) and *high pigment* (*hp*) genes with regard to lycopene and beta carotene levels of tomato under high temperature (taking into account the fruit to be homozygous recessive for *alc* gene) resulted in a yellow-rosy colour fruit with no commercial acceptance. The results revealed the existence of epistatic effects in large magnitude, while the most promising combinations of *alcobaça* gene were in the heterozygous condition with *crimson* and *high pigment* loci alternating in homozygous and heterozygous condition. Kopeliovitch *et al*. (1981) suggested modifier genes interacting with *alc* gene, enhancing colour development in a wide spectrum. In that case, the presence of determined genetic factors is essential for fruit colour development.

Since the identification of the 'stolid' mutant which modifies the process of fruit ripening (Schuelter *et al*., 1997ab; Schuelter, 1999), the pleiotropic nature of the mutation must be known. The mutation combined with *rin*, *nor* and *alc* may promote useful effects to be used in future breeding programs. In the present work, a system of diallel crossings was carried out, since the nearly-isogenetic lines cv. Santa Clara and 'stolid' mutant, and the other progenitors on another genetic base were available. To achieve these objectives, there were employed orthogonal contrasts to study pleiotropy and the Hayman (1954ab) diallel approach to verify character inheritance and identify useful progenitors for future breeding programs through hybridisation.

Material and methods

Materials and growing conditions. Plants of tomato accessions from the Germplasm Bank at the Federal University of Viçosa (BGH-UFV), selected for pleiotropic genes that modify shelf life, were crossed in diallel. All possible hybrid combinations except reciprocals were obtained. Parents were: P_1 - 'stolid' mutant; P_2 - cv. Santa Clara; P_3 - (BGH-6915; *alc*); P_4 - (BGH-6914; *rin*); P_5 - (BGH-6913; *nor*). The 'stolid' mutant, derived from cv. Santa Clara, contained a recessive gene that promoted early leaf senescence, yellowish stigmas and fruits with yellow-pale and red colour, respectively, in the immature and mature stage. This mutant also showed firm and increased shelf life fruits than the Santa Clara cv. One (Schuelter *et al.,* 1997; Schuelter, 1999). Parents BGH-6915, BGH-6914 and BGH-6913 were accessions that contained pleiotropic mutations, as alcobaça (*alc*), ripening (*rin*) and non-ripening (*nor*) inhibitor.

The genotypes of F_1 generations and parents were evaluated in field conditions at Viçosa MG Brazil from the winter to the summer of 1997, in a randomised block design, with three replications. Each experimental plot of parental generations and F1s consisted of 4 useful plants. During the experiment, the growing conditions were maintained according to recommendations for commercial tomato crops.

Evaluation of post-harvest characteristics. The following characteristics were evaluated: level of soluble solids (°Brix) of red fruits (SST); pH of red fruits (PH); titratable acidity (percentage of citric acid) of red fruits (TA); total carotenoid level (μg/g of fresh matter) (TC), beta carotene level (μg/g of fresh matter) (BC) and lycopene level (μg/g of fresh matter) (LC) of red fruits; shelf life (days of storage) (SL); firmness after six days of storage (FIRM), expressed in mega Pascal (Mpa). Analyses were carried out with eight fruits per plot.

Fruits were harvested in the red stage. After harvest, 10g of fresh matter of locular tissue and pericarp were excised from each fruit so that all determinations, except shelf life (SL) and firmness

(FIRM) could be undertaken. In the case of SL and FIRM evaluation, fruits were harvested in the green-pinkish stage and stored at 25°C and 90% relative humidity.

The following procedures were used: *carotenoid* - TC, LC, and BC were determined as described by Zscheile and Porter (1947), based on spectrophotometric analysis; *total level of soluble solids* - using a Abcc refractometer; *pH* - pH was determined by DM21 (Digimed) pHmeter; *titratable acidity* - by NaOH 0,1N and phenolphthalein as indicator; *fruit firmness* - as described by Calbo and Nery (1995); *shelf life* - number of storage days till consumption (Buescher *et al*., 1976).

Statistical analysis. A variance analysis was initially carried out for each evaluated characteristic, considering blocks, genotypes and residual as sources of variation. Genotypes consisted of parents and hybrids produced in the diallel crossings.

With regard to parental mean, heterosis were calculated on average values. Comparison tests were done by orthogonal contrasts. Contrasts of interest were thus planned and tested with respect to orthogonality. The orthogonality of two contrasts was defined by Steel and Torry (1980) in terms of its coefficients. $Q_1 = \sum c_{1i} Yi.$ and $Q_2 = \sum c_{2i} Yi.$ were considered linear functions, respectively. $\sum c_i$ was the total of coefficients. Each contrast was equal to zero. Yi. the total of the treatment indicated that two contrasts are orthogonal as $\sum c_{1i} c_{2i} = 0$.

For diallel analysis (Hayman, 1954ab), the following statistics were initially estimated: V_p (variance of parents); V_i (variance in the *i*th line or column); V (mean of line or column variances); V_m (variance of line means); W_{ci} (co-variance of column means and the *i*th line); W_i (co-variance of the parents and the *i*th line); and M^2 [the square of the difference between the general mean (ML_1) and the mean of the parents (ML_0)].

As Hayman's approach (1954ab) imposes the assumptions of diploid segregation, parental homozygosis and absence of maternal effect, multiple allelism and epistasis, as well as independent distribution of genes among the parents, values of W_i and V_i were tested for data adequacy to the additive-dominance model. The following tests were done: a) the significance of the slope coefficient of the regression line was tested by the regression of W_i on V_i (H_0:b=1 vs H_a:b≠1); b) weighting of Wi and V_i through 45° rotation of the axes represented by those statistics, taking new W_i and V_i values; slope coefficient of regression line was tested after rotation (H_0:b=0 vs H_a:b≠0).

When data adequacy to the additive-dominance model was tested for all the evaluated characters, statistics obtained from the diallel table were used to estimate genetic components of variation $\tilde{H}_1$, $\tilde{H}_2$, $\tilde{D}$, $\tilde{h}^2$ and $\tilde{F}$, where $\tilde{H}_1$ and $\tilde{H}_2$ gave the variation caused by the dominance effects; $\tilde{D}$ was the variation due to additive genetic effects; $\tilde{h}^2$ was the variation for dominance effects, as well as the square of the difference between the mean of the n^2 elements in the diallel table and the means of parents; $\tilde{F}$ is the mean co-variance among additive and dominance effects.

The significance of the components was tested by *t* test, dividing the estimates by the respective standard deviations. Values that exceeded 1,96 were considered significant at 5% probability level, (Singh and Chaudhary, 1979; Ferreira, 1985).

The association between the genetic components was also used for the estimation of parameters and interpretation of results. Thus, $(\tilde{H}_1/\tilde{D})^{1/2}$ was the mean degree of dominance at all loci; $\tilde{H}_2/4\tilde{H}_1$ measures average value of the frequency of positive alleles multiplied by frequency of negative alleles in dominant loci with a maximum value of 0.25; $K_D/K_R = \left(\sqrt{4\tilde{D}\tilde{H}_1} + \tilde{F}\right) \Big/ \left(\sqrt{4\tilde{D}\tilde{H}_1} - \tilde{F}\right)$ measured the most frequent type of allele which, in a ratio close to unity, indicated equality between the number of dominant and recessive alleles in the parents; $\tilde{h}^2/\tilde{H}_2$ measured the number of genes or groups of genes which controlled possible dominant trait; $\tilde{h}^2_R = \left(\tilde{D} - \tilde{F} + \tilde{H}_1 - \tilde{H}_2\right) \Big/ \left(\tilde{D} - \tilde{F} + \tilde{H}_1 - \frac{1}{2}\tilde{H}_2 + 2\tilde{\varepsilon}\right)$ was heritability in the narrow sense; $\tilde{h}^2_A = \left(\tilde{D} - \tilde{F} + \tilde{H}_1 - \frac{1}{2}\tilde{H}_2\right) \Big/ \left(\tilde{D} - \tilde{F} + \tilde{H}_1 - \frac{1}{2}\tilde{H}_2 + 2\tilde{\varepsilon}\right)$ is the heritability in broad sense.

The statistic (W_i + V_i), which ranks all parents according to their number of dominant genes, based on the principle that parents containing most dominant genes have the lowest values of this statistic and the reverse for recessive genes, was calculated. The correlation (r) between (W_i + V_i) and Y_{ii} (mean values of the parents), which is an indicator of the relationship between the favourable alleles and dominance, was also calculated. The theoretical limits of selection, referring to the mean of the completely dominant or recessive parents for the segregating genes of the diallel, was obtained by regression of Y_{ii} on (W_i + V_i).

Statistical analyses were done by GENES - Software for Experimental Statistics in Genetics (Cruz, 1997), Department of Biology of the Federal University of Viçosa, Brazil.

Results and discussion

Mean values and orthogonal contrasts. Significant differences in all traits between parents and F_1 hybrids were detected by test *F* ($\alpha < 0,01$). Mean values of shelf life (SL) were quite different (Table 1). Fruit shelf life of 'stolid' mutant (P_1) was similar to accessions of pleiotropic mutations P_4 (BGH-6914, *rin*) and P_5 (BGH-6913, *nor*). There was a high reduced shelf life of F_1 hybrids when compared to that to that of parents P_1, P_4 and P_5. Orthogonal contrasts (Table 2) pointed out shorter shelf life fruits of cv. Santa Clara (P_2), 'stolid' mutant (P_1) and $F_{1(1x2)}$ hybrid when compared to that of parents with *rin* and *nor* pleiotropic genes. It was also verified that the 'stolid' mutant (P_1) increased its shelf life when compared to those of cv. Santa Clara (P_2) and $F_{1(1x2)}$ hybrid, which presented similar behaviour. Although the mean values of hybrids containing 'stolid' mutant were, in absolute value, superior to its absence, it was verified that the combination of P_1 with P_3 , P_4 e P_5 had no effect on extending fruit shelf life. Probably the control of gene expression, affected by mutations, is the same, necessarily requiring only one factor to initiate the full ripening and senescence process. Consequently, increase of storage life is not allowed. Research results on *rin* and *nor* mutants support the hypothesis of lesions in genes related to regulation of ethylene biosynthesis closely linked to shelf life increase (Tigchelaar *et al*., 1978; Giovanonni *et al*., 1989; Gray *et al*., 1992).

The fruit firmness after six days of storage (FIRM), which was highly correlated with SL, was partially similar (Tables 1 and 2). Orthogonal contrasts indicated that cv. Santa Clara, 'stolid' mutant and $F_{1(1x2)}$ hybrid were not significantly different with respect to firmness from the other parents. On the other hand, mean of 'stolid' mutant was significantly higher than that of cv. Santa Clara and $F_{1(1x2)}$. Combination of 'stolid' mutant with other mutants did not increase the firmness of fruits, except for $F_{1(1x5)}$ hybrid.

According to Amaral Jr. (1996), cv. Santa Clara is directly descended from Santa Cruz population characterised by high yield potential, broad adaptation and quite hard pericarp. Therefore, mean firmness in cv. Santa Clara (P_2), 'stolid' mutant (P_1) and $F_{1(1x2)}$ and other parents are probably due to fruit texture from the Santa Cruz background. However, the difference of firmness of 'stolid' mutant fruits compared with the mean of $F_{1(1x2)}$ and cv. Santa Clara can be attributed to mutation effects.

Orthogonal contrasts for PH (Tables 1 and 2) was largely in accordance with data of TA. There was no change in pH and titratable acid levels of 'stolid' mutant (P_1) in comparison with that of cv. Santa Clara (P_2). Fruit pH of 'stolid' mutant, cv. Santa Clara and $F_{1(1X2)}$ hybrid was higher but contents of titratable acids were lower than those of parents. Among parents, P_3 (BGH-6915, *alc*) had lowest pH mean value, followed by P_5 (BGH-6913, *nor*). However, P_5 was a parent of higher titratable acid contents (TA). Among F_1 hybrids, $F_{1(1X3)}$ and $F_{1(2X3)}$ hybrids, pH values increased. The same occurred to titratable acid as far as $F_{1(1X4)}$, $F_{1(2X4)}$, $F_{1(1X5)}$ and $F_{1(2X5)}$, according to *F* test, at 5% probability level.

Table 1. Mean values and percentage heterosis, in relation to parental means (H_{MP}) for shelf life (SL), firmness after six days of storage (FIRM), level of soluble solids (SST), pH (PH), titratable acidity (TA), total carotenoid (CT), lycopene (LC) and beta carotene (BC), evaluated in a diallel, among five tomato accesses

Populations[1]	SL		FIRM		SST		PH		TA		CT		LC		BC	
	Means	H_{MP} (%)	Means	H_{MP} (%)	Means	H_{MP} (%)	Means	H_{MP} (%)	Means	H_{MP} (%)	Means	H_{MP} (%)	Means	H_{MP} (%)	Means	H_{MP} (%)
P_1 ('stolid' mutant)	30,75	-	0,083	-	3,979	-	4,391	-	0,474	-	234,741	-	147,342	-	87,396	-
P_2 (cv. Sta Clara)	15,333	-	0,0357	-	4,625	-	4,435	-	0,523	-	149,834	-	87,561	-	62,272	-
P_3 (BGH6915; *alc*)	16,667	-	0,034	-	4,099	-	3,996	-	0,894	-	43,944	-	19,142	-	24,802	-
P_4 (BGH6915; *rin*)	32,417	-	0,061	-	4,837	-	4,228	-	0,566	-	5,184	-	1,240	-	3,944	-
P_5 (BGH6915;*nor*)	36,25	-	0,088	-	4,821	-	4,031	-	0,975	-	53,015	-	27,049	-	25,966	-
$F_{1(1x2)}$	21,63	-6,1	0,036	-56,4	4,804	11,7	4,406	-0,2	0,55	9,1	214,094	2,4	127,982	0,1	86,076	6,1
$F_{1(1x3)}$	19,00	-19,9	0,05	-14,4	4,637	14,8	4,046	-3,45	0,913	-23,9	50,074	-64,2	22,609	-72,8	27,464	-51,0
$F_{1(1x4)}$	23,167	-26,6	0,038	-47,2	4,646	5,6	4,296	-0,2	0,634	-16,1	170,878	42,5	105,913	42,6	64,964	42,2
$F_{1(1x5)}$	19,50	-41,8	0,068	-20,4	5,233	18,9	4,219	4,2	0,858	-14,2	266,401	198,3	153,067	75,5	113,335	99,9
$F_{1(2x3)}$	18,167	13,5	0,029	-15,8	4,762	14,2	4,026	-4,5	0,771	8,9	45,22	-60,1	19,25	-69,8	25,971	-47,9
$F_{1(2x4)}$	20,667	-13,4	0,049	2,7	4,892	7,8	4,316	-0,4	0,672	23,3	171,436	82,1	113,181	106,6	58,256	47,9
$F_{1(2x5)}$	17,00	-34,1	0,042	-31,0	4,804	6,0	4,278	1,0	0,708	-5,4	287,864	143,8	174,943	158,5	112,921	124,0
$F_{1(3x4)}$	25,867	5,4	0,05	5,5	4,417	-1,2	4,599	11,8	0,699	-4,2	33,099	34,9	17,133	68,1	15,967	11,1
$F_{1(3x5)}$	24,667	-6,8	0,055	-9,4	4,50	0,9	4,50	12,1	0,867	-7,2	35,5	-26,8	24,183	4,7	11,317	-55,4
$F_{1(4x5)}$	28,833	-16,0	0,058	-21,6	4,75	-1,6	4,75	15,0	0,800	3,8	33,833	11,4	10,567	-25,3	23,267	55,6

Table 2. Values and significance of mean squares of orthogonal contrasts (MSC), difference between comparison of contrast means (D) and residual mean squares (MSR), involving five parents and F_1 hybrid combinations for shelf life (SL), firmness after six days of storage (FIRM), level of soluble solids (SST), pH (PH), titratable acidity (TA), total carotenoid (TC), lycopene (LC) e beta carotene (BC)

Orthogonal contrasts[1]	SL		FIRM		SST		PH	
	MSC	D	MSC	D	MSC	D	MSC	D
P_1 P_2 $F_{1(1x2)}$ vs P_3 P_4 P_5	155,2498**	-52,86	0,0004ns	-0,08	6,09**	-10,47	2,4537**	2,93
P_1 vs P_2 $F_{1(1x2)}$	301,0322**	73,61	0,0044**	0,28	1,0819**	-4,41	0,0017ns	-0,18
P_2 vs $F_{1(1x2)}$	59,4783ns	-18,89	1,35 x 10^{-7ns}	0,0009	0,0481ns	-0,54	0,0013ns	0,08
P_3 vs P_4 P_5	624,1044**	-105,99	0,0033**	-0,24	1,0658**	-4,38	0,0356**	-0,80
P_5 vs P_4	22,0378ns	11,50	0,0011ns	0,08	0,0004ns	-0,05	0,0582**	-0,59
$F_{1(1x3)}$ $F_{1(2x3)}$ vs $F_{1(1x4)}$ $F_{1(2x4)}$ $F_{1(1x5)}$ $F_{1(2x5)}$	9,00ns	-18,00	0,0004ns	-0,12	0,1509ns	-2,33	0,2328**	-2,89
$F_{1(1x4)}$ $F_{1(2x4)}$ vs $F_{1(1x5)}$ $F_{1(2x5)}$	4,482ns	7,33	0,0004ns	-0,07	0,1867ns	-1,49	0,0099ns	0,34
$F_{1(1x3)}$ vs $F_{1(2x3)}$	1,041ns	2,50	0,0007ns	0,06	0,0234ns	-0,37	0,0006ns	0,06
$F_{1(1x4)}$ vs $F_{1(2x4)}$	9,375ns	7,50	0,0002ns	-0,03	0,0908ns	-0,74	0,0006ns	-0,06
$F_{1(1x5)}$ vs $F_{1(2x5)}$	9,375ns	7,50	0,001*	0,08	0,2761*	1,29	0,0052ns	-0,18
MSR	18,0013		0,00022		0,0436		0,0036	

Orthogonal contrasts[1]	TA		TC		LC		BC	
	MSC	D	MSC	D	MSC	D	MSC	D
P_1 P_2 $F_{1(1x2)}$ vs P_3 P_4 P_5	0,3943**	-2,66	122930,1403**	1487,53	49755,6131**	946,36	16386,2925**	543,09
P_1 vs P_2 $F_{1(1x2)}$	0,0078ns	-0,37	5427,5697*	312,56	56369,6809**	237,42	6293,5662ns	79,33
P_2 vs $F_{1(1x2)}$	0,0011ns	-0,08	6187,0833**	-192,67	2450,7859**	-121,26	849,9456*	-71,41
P_3 vs P_4 P_5	0,0305ns	0,74	440,7184ns	89,07	49,95ns	29,98	193,9268ns	59,08
P_5 vs P_4	0,2509**	1,23	3431,7068*	143,49	999,1567ns	77,43	727,3206*	66,06
$F_{1(1x3)}$ $F_{1(2x3)}$ vs $F_{1(1x4)}$ $F_{1(2x4)}$ $F_{1(1x5)}$ $F_{1(2x5)}$	0,0615*	1,49	124606,882**	-2117,98	53681,6462**	-1390,17	14714,42**	-727,82
$F_{1(1x4)}$ $F_{1(2x4)}$ vs $F_{1(1x5)}$ $F_{1(2x5)}$	0,0507*	-0,78	33692,7377**	-635,86	1667,6225*	-141,46	7962,313**	-309,11
$F_{1(1x3)}$ vs $F_{1(2x3)}$	0,0302ns	0,43	35,3274ns	14,56	16,9243ns	10,08	3,3436ns	4,48
$F_{1(1x4)}$ vs $F_{1(2x4)}$	0,0022ns	-0,11	0,4704ns	-1,68	7147,71**	-207,09	67,4959ns	20,12
$F_{1(1x5)}$ vs $F_{1(2x5)}$	0,0337ns	0,45	690,9262ns	-64,39	717,8391ns	-65,63	0,2571ns	1,24
MSR	0,0086		719,2212		291,5351		163,2517	

[1] (P_1) 'stolid' mutant; (P_2) cv. Santa Clara; (P_3) BGH-6915 (*alc*); (P_4)BGH-6914(*rin*) e (P_5)BGH-6913(*nor*)

P_1, P_2 and $F_{1(1X2)}$ fruits had lower levels of soluble solid level (SST) than those of P_3, P_4 and P_5 (Tables 1 and 2). In addition, SST of P_1 and P_3 was significantly lower than that of P_2 and $F_{1(1X2)}$, and of P_4 and P_5, respectively. As for the presence or absence of mutation in a heterozygous condition, $F_{1(1X5)}$ presented higher mean value than $F_{1(2X5)}$.

According to Azanza *et al*. (1994), the soluble solid fraction is basically made up of glucose and fructose, organic acids citrate and malate. The pH is closely related to acid levels. Thereby, SST and TA contents assure that 'stolid' mutant decreased soluble solid without affecting titratable acid levels. This was confirmed by the unchanged pH. Hence, the hypothesis of mutations affecting sugar levels is put forward until further studies are done.

Synthesis of carotenoid pigments (Tables 1 and 2) is influenced by pleiotropic mutations under homozygous conditions (Gray *et al*, 1994; Kopeliovitch *et al*. 1981). Total carotenoid (TC), lycopene (LC) and beta carotene (BC) levels of red fruits of cv. Santa Clara, 'stolid' mutant and $F_{1(1X2)}$ hybrid were significantly higher than those of parents P_3, P_4 and P_5. Likewise, total carotenoid (TC) and lycopene (LC) levels of 'stolid' mutant were higher than fruits of cv. Santa Clara and $F_{1(1X2)}$. This fact denotes the effect of mutation on that pigment group. The contrast cv. Santa Clara vs $F_{1(1X2)}$ means that levels of TC, LC and BC were significantly higher in the hybrid, indicating the effect of mutation in the heterozygous condition. Carotenoid pigments were accumulated in the pericarp of F_1 hybrids, but $F_{1(2X5)}$ and $F_{1(1X5)}$ outstood the mean values. On the other hand, $F_{1(1X3)}$ and $F_{1(2X3)}$ hybrids of lowest carotenoid pigments levels yielded yellow-orange fruits. However, the fruit of $F_{1(2X4)}$ hybrid were higher in lycopene than the $F_{1(1X4)}$ ones.

Diallel analysis. Table 3 indicates the adequacy of the additive-dominance genetic model for all characteristics, except pH. Therefore, the genetic analysis of seven characteristics related to post-harvest fruits through diallel analysis is correct.

Table 3. Adequacy tests of standard additive-dominance model[1] for shelf life (SL), firmness after six days of storage (FIRM), level of soluble solids (SST), titratable acidity (TA), total carotenoid (TC), lycopene (LC) and beta carotene (BC)

Traits	Regression[2/]	Rotation of the $\hat{W}_i$ e $\hat{V}_i$ [3]
	t (H_0 : b = 1)	F = t^2 (H_0 : b - 1 = 0)
SL	-0,9162[ns]	0,7862[ns]
FIRM	0,0933[ns]	-0,6114[ns]
SST	-1,0753[ns]	0,4335[ns]
PH	-3,8258*	0,0197[ns]
TA	0,0257[ns]	-0,2923[ns]
CT	-1,7232[ns]	1,0433[ns]
LC	-1,3815[ns]	0,7702[ns]
BC	-2,4922[ns]	1,6017[ns]

[1] Using Hayman (1954) approach for eight characteristics; [2]*t* test, weighting the mean values of W_i and V_i at 1% and 5% probability; [3] *F* test, weighting W_i and V_i axes by 45° rotation, at 1% and 5% probability; [ns] Non-significant at 5% probability

Estimates of genetic and non-genetic components of variation of seven characteristics are presented in Table 4. The sign of estimates for contrasts between mean of parents (ML_o) and mean of elements (ML_1), in the diallel table, indicates dominance deviations toward highest mean. Besides dominance deviations, additive variation also contributed to the variation between parents and F_1, since dominance ($\tilde{H}_1$) and additive ($\tilde{D}$) components were significantly different from zero, except for FIRM, due to significance of additive variation.

The importance of genetic effects (Table 4) came from $\tilde{D} - \tilde{H}_1$. Thus, the variation associated with additive genetic effects was more important for SL and TA. The predominance of effects was not detected for the other characteristics. Estimation of average degree of dominance, $(\tilde{H}_1/\tilde{D})^{1/2}$ pointed towards partial dominance of alleles under control of SL, FIRM and TA, also pointed over-dominance of remaining characteristics (Table 5). In fact, the straight regression line intercepts the ordinate above the origin, which means agreement of results (Figure 1).

Ratio K_D/K_R (Table 5) implies that dominant alleles are more frequent in parents, except for carotenoid contents (CT, LC and BC), because of high frequency of recessives. Except for SL, estimates of $\tilde{H}_2/4\tilde{H}_1$ (Table 5) call for asymmetry, as results show.

Statistics $\tilde{h}^2/\tilde{H}_2$ indicates one or two genes exhibiting dominance (Table 5). These results support the hypothesis that the number of genes involved in the determination of those characteristics with dominance is small. However, Cruz and Regazzi (1994) have pointed out that the number of genes for low or no dominance is not taken into consideration by the methodology of Hayman (1954ab). Estimates should be therefore interpreted with caution.

As a rule, estimates of heritability in the broad sense were high for all the characteristics, whereas heritability, in a strict sense, except for SST, had medium values (Table 5). High estimates of $\tilde{h}^2_A$ for SL, SST, TA, CT, LC and BC indicate the low sensitivity of those characteristicss to environmental effects. On the other hand, the greater the difference of heritability estimates, in the broad and strict sense, the more evident becomes the importance of the additive and non-additive genetic effects for character expression.

Table 4. Estimates of the genetic and non-genetic variation components, with their respective standard-deviations for shelf life (SL), firmness after six days of storage (FIRM), level of soluble solids (SST), titratable acidity (TA), total carotenoid (CT), lycopene (LC) and beta carotene (BC)

Traits	Genetic and non-genetic components							ML_1-ML_0
	$\tilde{D} \pm SD$	$\tilde{H}_1 \pm SD$	$\tilde{H}_2 \pm SD$	$\hat{h}^2 \pm SD$	$\tilde{F} \pm SD$	$\tilde{D} - \tilde{H}_1 \pm SD$	$\tilde{\varepsilon} \pm SD$	
SL	79,354±2,78★	38,035±8,56★	39,635±7,56★	7,157±4,55^{ns}	13,697±6,84★	41,319±8,1★	4,964±1,25★	1,656
FIRM	0,0006±0,0001★	0,0002±0,0003^{ns}	0,0002±0,0003^{ns}	0,0003±0,0002^{ns}	0,0003±0,0003^{ns}	0,0003±0,0003^{ns}	0,0001±0,0005^{ns}	0,009
SST	0,152±0,04★	0,259±0,11★	0,193±0,10ns	0,180±0,06★	0,157±0,09^{ns}	-0,107±0,09^{ns}	0,013±0,02^{ns}	0,218
TA	0,051±0,003★	0,019±0,009★	0,016±0,008★	0,008±0,005ns	0,021±0,007★	0,032±0,01★	0,002±0,001★	0,049
CT	8543,146±2645,32★	19795,850±8141,24★	15126,231±7191,30★	2724,025±4324,74^{ns}	-1743,917±6499,90^{ns}	-11252,703±8280,3^{ns}	192,441±1188,67^{ns}	26,795
LC	3554,752±969,79★	8045,092±2984,62★	6252,264±2636,37★	1006,617±1585,47^{ns}	-404,526±2382,89^{ns}	-4490,340±2955,95^{ns}	78,666±435,77^{ns}	16,333
BC	1073,284±438,73★	2733,615±1350,24★	2044,061±1192,69^{ns}	404,502±717,26^{ns}	-402,036±1078,02^{ns}	-1660,331±1275,13^{ns}	43,364±197,14^{ns}	10,462

★Estimate of the component divided by its standard-deviation (SD). When exceeding 1.96, it is significant at 5% probability (Singh and Chaudary, 1979); ^{ns} Non-significant

Table 5. Estimates of genetic parameters for shelf life (SL), firmness after six days of storage (FIRM), level of soluble solids (SST), titratable acidity (TA), total carotenoid (CT), lycopene (LC) and beta carotene (BC)

Traits	Genetic parameters									Means ordered from low to high[1]	Dominance ordered from high to low
	$(\tilde{H}_1/\tilde{D})^{1/2}$	$\tilde{H}_2/4\tilde{H}_1$	K_D/K_R	$\tilde{h}^2/\tilde{H}_2$	$\tilde{h}^2_R$	$\tilde{h}^2_A$	r	$\hat{Y}_D$	$\hat{Y}_R$		
SL	0,69	0,26	1,28	0,18	0,68	0,89	0,81	17,10	38,83	P_5(36,25), P_1, P_4,P_3,P_2 (15,33)	P_5, P_1, P_3, P_4, P_2
FIRM	0,64	0,21	2,40	1,69	0,58	0,77	0,69	0,04	0,10	P_5(0,09), P_1, P_4, P_2, P_3(0,03)	P_1, P_5, P_3, P_2, P_4
SST	1,30	0,19	2,31	0,93	0,33	0,86	-0,71	4,75	3,28	P_4 (4,84), P_5, P_2, P_3, P_1 (3,98)	P_1, P_4, P_3, P_5, P_2
TA	0,61	0,21	2,05	0,50	0,72	0,89	-0,59	0,83	0,41	P_5 (0,97), P_3, P_4, P_2, P_1 (0,50)	P_1, P_2, P_5, P_4, P_3
CT	1,52	0,19	0,87	0,18	0,65	0,98	-0,17	(116,72)[2/]	(57,75)	P_1 (234,76), P_2, P_5, P_3, P_4 (5,12)	P_5, P_4, P_1, P_2, P_3
LC	1,50	0,19	0,93	0,16	0,64	0,98	-0,14	(68,20)	(34,54)	P_1 (147,34), P_2, P_5, P_3, P_4 (1,24)	P_5, P_4, P_1, P_2, P_3
BC	1,59	0,19	0,79	0,20	0,66	0,97	-0,17	(47,19)	(24,89)	P_1 (87,40), P_2, P_5, P_3, P_4 (3,94)	P_5, P_4, P_1, P_2, P_3

According to methodology of Hayman (1954); [1] (P_1) 'stolid mutant'; (P_2) cv. Santa Clara; (P_3) BGH-6915 (*alc*); (P_4) BGH-6914 (*rin*); e (P_5) BGH-6913 (*nor*). The value between parentheses refers to the mean of the characteristic; [2] Predicted values by regression equation with low r (below 50%).

Table 5 shows that the estimates of coefficients of correlation (r) between ($W_i + V_i$) and the mean value of parents ($\overline{Y}_{ii}$) present different sign and magnitude values from the evaluated characteristics. The positive correlations, 0.81 and 0.69, for SL and FIRM, respectively, reveal that the recessive alleles are mainly responsible for the increase of the expression. This may be confirmed by comparing the mean and dominance order of the parents detected by $W_i + V_i$. Table 5 and Figures 1, 2 show that P_1 ('stolid' mutant) and P_5 (BGH-6913, *nor*), of greater shelf life and firmer fruits, present higher concentration of recessive genes. On the other hand, the P_2 (cv. Santa Clara) presents the highest concentration of dominant alleles for characteristic SL and the second highest for FIRM, indicating the importance of the mutant from cv. Santa Clara and of other pleiotropic mutants.

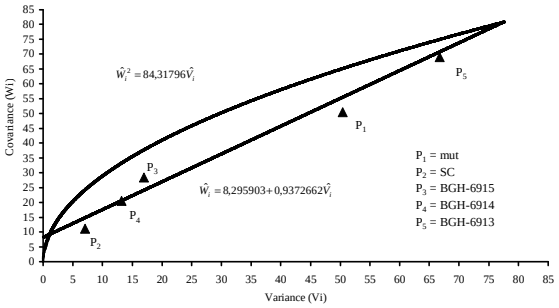

Figure 1. Regression of W_i, on V_i, for fruit shelf life (SL)

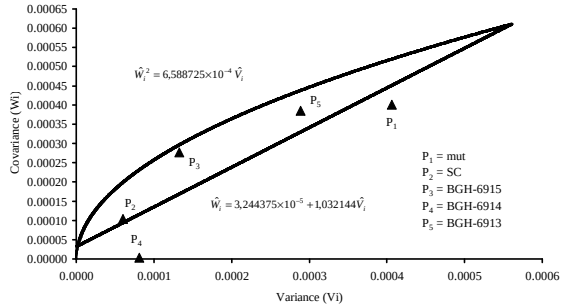

Figure 2. Regression of W_i on V_i for fruit firmness after six days of storage (FIRM)

In opposition, the negative and medium magnitude estimates of r, found for SST and TA, indicate that higher levels of soluble solids and titratable acidity are predominantly conditioned by dominant genes, albeit not exclusively (Table 5). Among parents, P_1 ('stolid' mutant) of higher concentration of recessive alleles was the smallest mean values of SST and TA. Really it is the effect of 'stolid' mutant on soluble solid levels. It is vulnerable due to sugar concentration, since titratable acid content was not on the increase.

Table 5 shows that the characteristics CT, LC and BC have negative correlation, albeit of low magnitude (below 0.2 in absolute value). Therefore, there is no evidence that alleles responsible for such mean increase are predominantly positive. However, nearly the majority of heterosis estimates for CT, LC and BC are positive (Table 1), indicating the importance of dominant alleles to raise characteristics expression. P_1, P_2 and P_3 presented higher concentration of dominant alleles, while P_4 and P_5 a larger number of recessive genes.

Except for SL, the estimates of r, varying from low to medium in the other characteristics, favouring the conclusion that the limits of possible selection may be reached in segregating populations derived from diallel, given by Y_D and Y_R (Table 5), are not very reliable. In that case, the crossing between P_1 ('stolid' mutant) and P_5 (BGH-6913, *nor*) may increase shelf life of fruit. However, those mutations under homozygous condition are of deleterious effects, such as fruit and plant colour. These are not well accepted by farmers and consumers. Nevertheless, they may be useful under heterozygous condition yielding hybrids.

Additive and dominance genetic variation of SL and FIRM indicate that breeding programs, looking for hybrids of post-harvest conservation, may be successful. In such cases, the combination 'stolid' mutant and *non-ripening*, under heterozygous conditions, would give rise to green fruits at earlier stages and red ones when ripen.

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