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## Prospecting of tomato hybrids for table and industry via mixed modeling and multivariate analysis

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### ABSTRACT

Dual-purpose tomato breeding, for table and industry, has great importance for tomato production and strong impact on Brazilian socioeconomic development. In the present study, we used the methodology of mixed models to estimate the combinatorial ability of five tomato lines evaluated in complete diallel scheme without reciprocal, and cluster analysis to prospect new double and triple hybrids constitutions. Thus, we observed that positive values of general combining ability occur in 'B13LD', 'Viradoro' and 'Rio Grande' (for fruit firmness), 'B13LD', 'Castone' and 'Massag-72' (for soluble solids content); and 'Viradoro' and 'Rio Grande' (for productivity). The crosses 'B13LD x Rio Grande' and 'Massag-72 x Viradoro' showed positive estimates of specific combination ability for soluble solids content, fruit firmness and productivity. The hybrid 'B13LD x Rio Grande' showed itself promising to be among those with the highest estimates of the genetic value of the cross for the three aforementioned traits. For the analysis of genetic divergence and estimate of combining ability it was possible to indicate two double and three triple hybrids.

**Keywords:** *Solanum lycopersicum*, diallel analysis, productivity, plant breeding.

### RESUMO

#### Prospecção de híbridos de tomateiro para mesa e indústria via modelagem mista e análise multivariada

O melhoramento genético do tomateiro para dupla finalidade, mesa e indústria, é de relevante importância para a produção com forte impacto no desenvolvimento socioeconômico do Brasil. No presente estudo utilizou-se a metodologia de modelos mistos para estimar a capacidade combinatória de cinco linhagens de tomateiro avaliadas em esquema de dialélico completo sem recíproco, e análise de agrupamento para prospectar novas constituições híbridas (híbridos duplos e triplos). Sendo assim, foi possível observar que valores positivos de capacidade geral de combinação ocorrem em 'B13LD', 'Viradoro' e 'Rio Grande' (para firmeza do fruto), 'B13LD', 'Castone' e 'Massag-72' (para teor de sólidos solúveis); e, 'Viradoro' e 'Rio Grande' (para produtividade). Dos cruzamentos avaliados apenas 'B13LD x Rio Grande' e 'Massag-72 x Viradoro' apresentaram estimativas positivas de capacidade específica de combinação para teor de sólidos solúveis, firmeza do fruto e produtividade. O híbrido 'B13LD x Rio Grande' mostrou-se promissor por figurar entre os que apresentaram as maiores estimativas do valor genético do cruzamento para as três características já referidas. Pela análise de divergência genética e da estimativa da capacidade combinatória foi possível indicar dois híbridos duplos e três híbridos triplos.

**Palavras-chave:** *Solanum lycopersicum*, análise dialélica, produtividade, melhoramento de plantas.

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Tomato (*Solanum lycopersicum*) is one of the most popular vegetables worldwide. FAO (2014) shows that tomato world production, in 2012 harvest, was 161.79 million tons. Brazil is the eighth largest producer worldwide with production of 3.88 million tons in growing area covering 63.86 mil ha, with an estimated productivity of 60.66 t/ha (FAO, 2014). Also with regards to Brazilian tomato production, the progress achieved in this crop is remarkable. The Country increased the productivity from 37 t/ha, in 1990, to

61 t/ha, in 2012 and large part of this increase is due to the success of breeding programs carried out in Brazil by the public and private institutions, being most hybrids produced by multinational institutions (Marinho *et al.*, 2011).

Among the main methodologies used in tomato breeding programs, diallelic crosses stood out (Maciel *et al.*, 2010; Pádua *et al.*, 2010; Souza *et al.*, 2012). Diallelic cross system is widely used to evaluate the performance of the parents and to find the most promising crosses within a gene pool. Cruz *et*

*al.* (2012) mention Griffing's method (1956), used to estimate the effects and the sums of squares of the effects related to general and specific combining ability, as the most used methodology in diallelic analysis. Other diallelic analysis methodologies were also frequently used, such as the methods proposed by Gardner & Eberhart (1966) and Hayman (1954); however, these methodologies show some restrictions, and their use is limited.

For the best analyzed diallelic date, it is recommended to use the mixed

models methodology. In this approach, the diallelic analysis becomes even more interesting, since using BLUP (the Best Linear Unbiased Estimation), proposed by Henderson (1974), pedigree information can be included and the simple hybrids, which were not tested, can be predicted. BLUP methodology is considered the best technique for animal and forestry genetic evaluation (Resende, 2007), and it is regularly used in annual and semiannual crop breeding, as shown in the review by Piepho *et al.* (2008). In Brazil, the authors found reports of this methodology used in beans (Carbonel *et al.*, 2007), sugar cane (Bastos *et al.*, 2007), sweet potato (Borges *et al.*, 2010), corn (Viana *et al.*, 2011) and papaya (Pinto *et al.*, 2013, Vivas *et al.*, 2014). However, models of diallelic analysis based on genotypic values (BLUP) or based on mixed models (REML/BLUP) are still rare, despite its broad utility (Vivas *et al.*, 2014).

According to Maluf *et al.* (1983), inclusion of data analysis of genetic distance in order to help indicate parents to be used in crosses, aiming to exploit heterosis, is possible. The authors mention reports which describe that, in tomato, the greater the genetic divergence between parents, the higher heterosis and, consequently, an increase in average of hybrid. No reports on the use of diallelic analysis through mixed models, in tomato, can be found, which makes this study a pioneering work. Given the above, the aim of this study was to use the mixed models methodology to select tomato hybrids, for both fresh market and industry, in diallelic cross scheme among five parents, as well as add information on genetic divergence in order to search new hybrid constitutions (double and triple hybrids).

## MATERIAL AND METHODS

In this study, the authors used complete diallelic scheme without reciprocal crosses, involving five determinate-growth parents ('B13LD', 'Castone', 'Massag-72', 'Viradoro' and 'Rio Grande'). 'B13LD' shows resistance to *Verticillium* spp., *Fusarium*

spp. and *Pseudomonas syringae* pv. *tomato*; its fruits are firm, elongated oblong shaped, absence of peduncle abscission layer, bright red color and high soluble solids content (°Brix). 'Castone' is resistant to *Verticillium* spp., *Fusarium* spp., *Pseudomonas syringae* pv. *tomato* and *Stemphylium* spp.; its fruits show early ripening, median firmness, high uniformity and bright red color; also, the plant is compact. 'Massag-72' shows resistant genes to *Verticillium* spp. and *Fusarium* spp.; elongated and pear-shaped fruits, showing good firmness. 'Viradoro' shows resistance to tospovirus; the fruits have good firmness. And, 'Rio Grande' is resistant to *Verticillium* sp. and *Fusarium* sp. races 1 and 2.

Hybridization was carried out in a greenhouse at the Research Support Unit at Universidade Estadual do Norte Fluminense Darcy Ribeiro, in the county of Campos dos Goytacazes, Rio de Janeiro State, Brazil. After obtaining the hybrids, the authors carried out an experiment at Estação Experimental of PESAGRO-Rio, in Campos dos Goytacazes. Randomized block design with four replications was used. Fifteen treatments (five parents and ten hybrids) were randomized in plots, spacing of 1.0x0.5 m between rows and plants, respectively. Six plants per plot were used; each plot of the experiment consisted of two rows, 3 m long. Fertilization was carried out according to the soil analysis; dripping irrigation system was used and the cultural practices were carried out according to the recommendations for the crop (Filgueira, 2008).

The evaluated traits were days to fruiting (DF): number of days after transplanting until the moment 50% of the plants of the plot presented at least a ripe fruit at the first and/or second fork; fruit length (CF) and fruit diameter (DIAMF), in mm: length and diameter average of 15 fruits per plot, using a digital caliper; thickness of pericarp (EP), in mm: measurement of pericarp using a digital caliper after a transversal cut made in the fruit in 15 fruits per plot; fruit firmness (FF), in Newton (N): determined using a digital penetrometer and a 8 mm diameter

tip, using two readings per fruit on the equatorial side, in 15 fruits per plot; total number of fruits per plant (NTFP): counting all the fruits produced at the experimental unit; average fruit weight (PMF), in g: relationship between total number and weight of fruits per plot; productivity (PROD): the result of the multiplication of NTFP and PMF, in kg/plant, extrapolated to t/ha; total soluble solids content (TSS), expressed in °Brix, obtained with the aid of a digital refractometer, in a sample of 15 fruits per plot.

With phenotypic values of PROD, FF and TSS, considered the most important traits for fresh and industrial tomato, the authors obtained, using software Selegen-Reml/Blup (Resende, 2006), the estimates of predicted additive genetic effect of the parents and specific combining ability of the crosses. Thus, the authors used the following model:

$$y = Xb + Zg + Ws + Tp + \varepsilon$$

where y, b, g, s, p and  $\varepsilon$  were vectors of data observed, vector of block effects (assumed to be fixed), vector of general combining ability of the parents involved in the crosses (assumed to be random), vector of plot effects (assumed to be random) and random errors, respectively. X, Z, W and T corresponded to incidence matrices for b, g, s and p, respectively.

Variance distributions and structures related to the model terms are:

$$y|b, V \sim N(Xb, V)$$

$$g | I\sigma_g^2 \sim N(0, I\sigma_g^2)$$

$$s | \sigma_s^2 \sim N(0, I\sigma_s^2)$$

$$p | \sigma_p^2 \sim N(0, I\sigma_p^2)$$

$$\varepsilon | \sigma_\varepsilon^2 \sim N(0, I\sigma_\varepsilon^2)$$

$$V = Z \sigma_g^2 Z' + W I \sigma_s^2 W' + T I \sigma_p^2 T' + I \sigma_\varepsilon^2$$

Estimates of general and specific combining ability effects were obtained through the following estimators:

$$\hat{\sigma}_g^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_y^2}$$

estimate of determination coefficient

of general combining ability of the parents involved in the crosses.

$$\hat{c}_s^2 = \frac{\hat{\sigma}_s^2}{\hat{\sigma}_y^2}$$

estimate of determination coefficient of specific combining ability of the crosses.

Phenotypic values of the traits evaluated were used to analyze the genetic diversity among genotypes, using computer program Genes. For this, variance analyses were carried out, and then, Mahalanobis distance, between all pairs of genotypes, was estimated ( $D_{ij}^2$ ), defined by:

$$D_{ij}^2 = \sum_{j=1}^n \sum_{j'=1}^n w_{jj'} d_j d_{j'}$$

where  $n$  = number of traits;  $W_{jj'}$  = element of the  $j$ -th line and  $j$ -th column of the inverse residual variance-covariance matrix between genotypes; and,  $d_j$  = difference between means of  $j$ '-th trait in the two populations or individuals considered (Cruz *et al.*, 2012). Based on the genetic distance matrix, the authors carried out the grouping analysis considering the Tocher method. The method consisted of forming the first group, identified by the pair of genotypes which showed the lowest distance value ( $D_{jj'}^2$ ). From this stage, the possibility of inclusion of other genotypes in the first group was evaluated. The inclusion or non-inclusion of the new genotype in the group was allowed in the case the increase in the value of the intra-group average distance did not exceed the maximum value of  $D_{jj'}^2$ , obtained in the set of the shorter distances involving each pair of individuals (Cruz *et al.*, 2012). Still based on the genetic distance matrix, a dendrogram was obtained using the unweighted pair group method with arithmetic mean (UPGMA). The adjustment between the distance matrix and the dendrogram was estimated by the cofenetic correlation coefficient (CCC), developed by Sokal & Rohlf (1962).

## RESULTS AND DISCUSSION

After carrying out the procedures,

the authors observed that 'B13LD', 'Viradoro' and 'Rio Grande' showed the highest values of general combining ability (predicted additive genetic effect) for fruit firmness (Figure 1), which indicates that these parents have the potential to increase fruit firmness. Fruit firmness is a trait which indicates resistance to physical damage during shipping, which is usually done in bulk. Thus, fruits which present little firmness are more subject to deformation and breakage of the skin causing loss to the producer.

Soluble solid content, concerning industrial processes, is one of the main traits of tomato; thus, the higher the soluble solids content, the higher is the industrial yield and lower energy expenditure in the process of pulp concentration. For soluble solid content, the authors observed that 'B13LD', 'Castone' and 'Massag-72' showed positive estimates of general combining ability. On the other hand, 'Viradoro' and 'Rio Grande', presented positive estimates for fruit firmness, showed negative estimates of general combining ability for soluble solid content. When the authors analyzed the productivity, they observed for the evaluated parents, discrepancy between estimates of general combining ability between soluble solids and productivity, which means, parents which present positive estimates for soluble solid content show negative estimates for productivity, and vice versa (Figure 1). This discrepancy in effect of general combining ability (predicted additive genetic effect) reflect into lower, or higher genetic values of crosses in which these parents participate, since genetic value of cross is estimated by predicted additive genetic effect of the parents, as well the effect of specific combining ability for a specific cross.

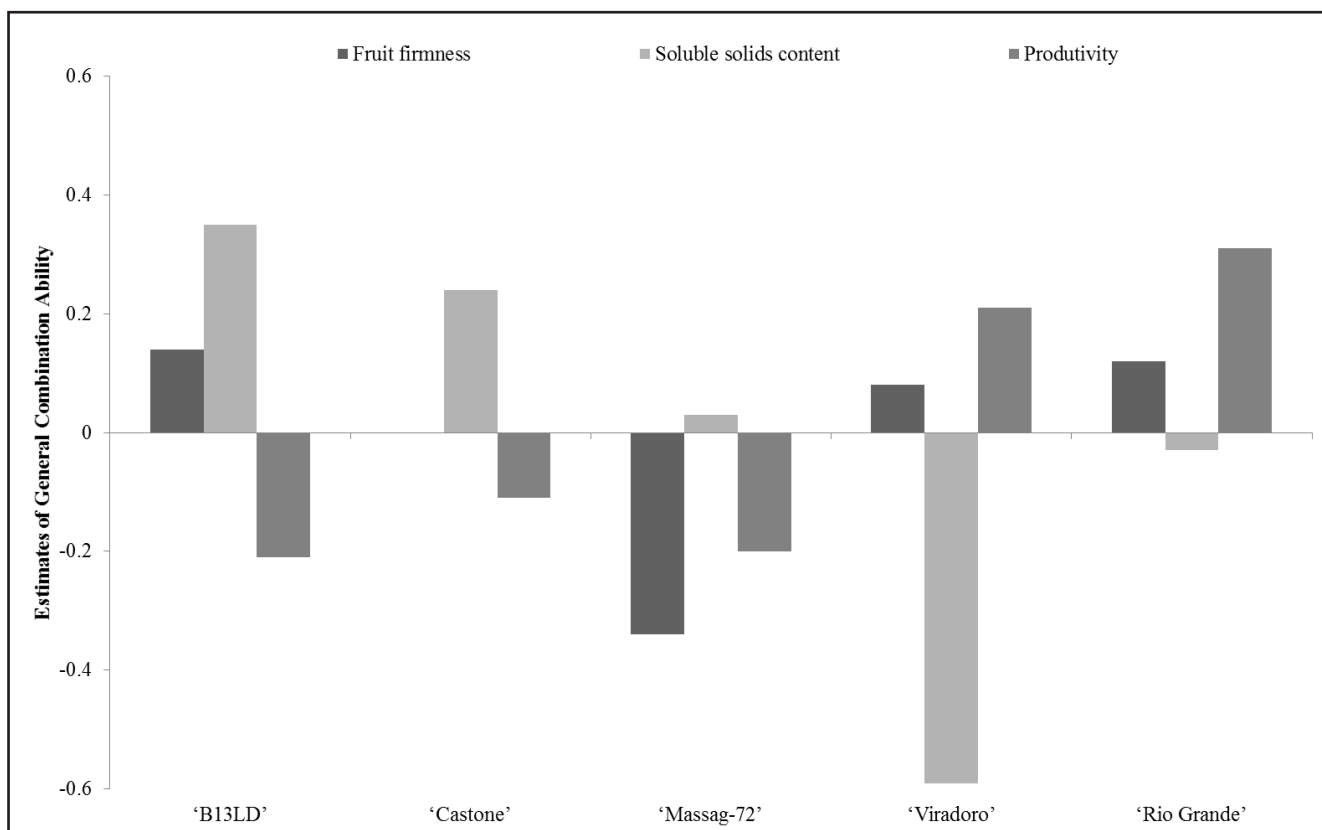
The estimates of additive values in figure 1 allow inferring that each parent is able to contribute for a specific cross; however, it does not infer the genetic value of cross. For this, knowledge of specific combining ability of crosses which compose the diallel is required. The effect of specific combining ability is interpreted as a deviation of performance of the hybrid in relation to

what was expected based on the general combining ability of its parents (Cruz *et al.*, 2012).

Considering the estimates of specific combining ability for fruit firmness, the authors observed positive estimates in 'B13LD x Castone' (1.08), 'B13LD x Rio Grande' (1.48), 'Massag-72 x Viradoro' (0.66) and 'Massag-72 x Rio Grande' (0.01) (Table 1). With respect to soluble solid content (TSS), the highest estimates of specific combining ability were observed in the combinations 'B13LD x Castone' (0.16), 'B13LD x Rio Grande' (0.09), 'Castone x Rio Grande' (0.03), 'Massag-72 x Viradoro' (0.34) (Table 1). For productivity, the crosses with positive values of specific combining ability were observed in 'B13LD x Viradoro' (17.26), 'B13LD x Rio Grande' (14.51), 'Castone x Viradoro' (8.95), 'Massag-72 x Viradoro' (4.12), 'Massag-72 x Rio Grande' (3.81), 'Viradoro x Rio Grande' (3.08) and 'Castone x Rio Grande' (2.69) (Table 1).

Concerning the hybrid combinations mentioned above only 'B13LD x Rio Grande' and 'Massag-72 x Viradoro' showed positive estimates of specific combining ability for the three traits studied (Table 1). Given the above, the authors could infer about the possibility of breeding practices aiming to achieve gains of these traits. Resende *et al.* (2012) report two main phases in which breeding is based, such as, the identification of superior individuals and creation of new superior genetic combinations through crosses between these individuals. The same authors also state that in both stages, selecting individuals plays a relevant role and it is carried out based on the genetic evaluation of individuals, allowing inferring about their genetic values of the individuals and then order them.

The authors observed that the cross 'B13LD x Rio Grande' was allocated in the first positions in the three traits evaluated (Table 1), the highest estimates of the genetic values of crosses. According Resende (2006), the genetic values of the cross is obtained by the predicted additive effect of the parents and the value of estimate of specific ability of a cross; thus, the authors could



**Figure 1.** Estimates of general combining ability (CGC) (predicted additive genetic effect) predictable for fruit firmness (FF), soluble solids content (TSS) and productivity (PROD). Campos dos Goytacazes, UENF, 2012.

**Table 1.** Estimates of specific combining ability (CEC), phenotypic value of the cross (VGC) and ranking of hybrid combinations (R), obtained for fruit firmness (FF), soluble solids content (TSS) and productivity (PROD). Campos dos Goytacazes, UENF, 2012.

Hybrids	Fruit firmness (N)			Soluble solids content (°Brix)			Productivity (t/ha)		
	CEC	VGC	R	CEC	VGC	R	CEC	VGC	R
B13LD x Castone	1.08	15.94	2	0.16	4.69	1	-11.49	38.94	8
B13LD x Massag-72	-1.91	12.78	9	-0.12	4.30	4	-12.55	37.84	9
B13LD x Viradoro	-1.90	13.00	8	-0.22	3.89	9	17.26	67.86	1
B13LD x Rio Grande	1.48	16.41	1	0.09	4.49	2	14.51	65.15	2
Castone x Massag-72	-2.79	11.83	10	-0.19	4.18	7	-13.53	36.91	10
Castone x Viradoro	-0.23	14.60	6	-0.02	4.03	8	8.95	59.60	3
Castone x Rio Grande	-1.38	13.47	7	0.03	4.36	3	2.69	53.39	7
Massag-72 x Viradoro	0.66	15.33	3	0.34	4.29	5	4.12	54.72	4
Massag-72 x Rio Grande	0.01	14.70	5	-0.04	4.19	6	3.81	54.46	5
Viradoro x Rio Grande	-0.18	14.71	4	-0.05	3.87	10	3.08	53.94	6

infer that for the productivity, 'Viradoro' and 'Rio Grande' tend to contribute to a greater magnitude, considering that these parents were the only ones to show positive predicted genotypic values (Figure 1). With respect to productivity, the crosses which were ranked the best, always had at least one of the aforementioned parents: 'B13LD

x Viradoro', 'B13LD x Rio Grande', 'Castone x Viradoro', 'Massag-72 x Viradoro' and 'Massag-72 x Rio Grande' (Table 1).

The grouping by the Tocher method separated 15 genotypes (five parents and ten hybrids) into eight different groups (Table 2). Considering that the parents were allocated into

different groups, this method led to the establishment of groups, so that the authors can notice homogeneity in the group and heterogeneity between groups. Moreover, it is an optimization technique which groups individuals keeping the criteria that intra-group distances are always shorter than inter-group distances (Cruz *et al.*,

2012). Thus, the formation of these groups represents valuable information regarding the choice of parents to be used in crosses aiming to exploit heterosis to create new hybrids.

Dendrogram obtained through the UPGMA method showed a cogenetic correlation coefficient of 0.62, showing good adjustment between the graphical representation of the distances and its original matrix (Sokal & Rohlf, 1962). The UPGMA method showed to be similar to the Tocher method as the separation of parents into different groups; as well as the presentation of the approach of 'Viradouro', 'Rio Grande' and the hybrid 'Viradouro x Rio Grande' (Table 2 and Figure 2).

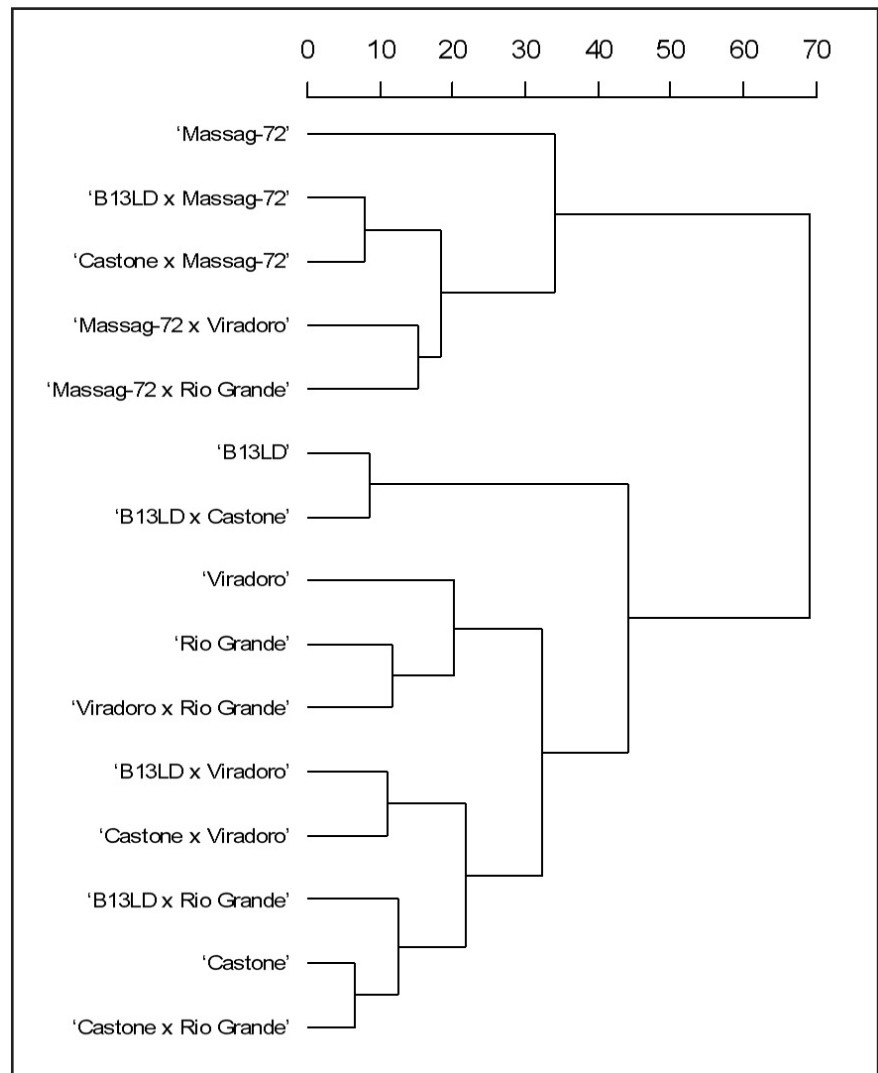
Based on groupings formed (Table 2 and Figure 2), as well as in estimates of general combining ability of the parents (Figure 1) and in specific combining ability of hybrids (Table 1), the authors can infer about the potential of these parents for creating new hybrids. Thus, the authors can recommend the combinations of 'B13LD x Castone' with 'Viradouro x Rio Grande' and 'B13LD x Rio Grande' with 'Massag-72 x Viradouro' to create double hybrids. They also recommend the combinations of parents 'B13LD' with hybrid 'Viradouro x Rio Grande' and hybrid 'B13LD x Castone' with parents 'Viradouro x Rio Grande' to create triple hybrids. The authors believe that these combinations have higher heterotic effect, due to genetic distance among genotypes, as they add genes which contributed to increased fruit firmness, soluble solids content and highest productivity.

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**Table 2.** Grouping of five parents and 10 tomato hybrids by the Tocher's grouping method using the Mahalanobis distance as a measure of genetic distance. Campos dos Goytacazes, UENF, 2012.

Group	Genotypes
1	Castone, Castone x Rio Grande, B13LD x Rio Grande, Castone x Viradouro
2	B13LD x Massag-72, Castone x Massag-72, Massag-72 x Rio Grande
3	B13LD, B13LD x Castone
4	Rio Grande, Viradouro x Rio Grande
5	Viradouro
6	B13LD x Viradouro
7	Massag-72 x Viradouro
8	Massag-72



**Figure 2.** Dendrogram resulting from five parents analysis and 10 tomato hybrids obtained by grouping by UPGMA and using the Mahalanobis distance as a measure of genetic distance. Campos dos Goytacazes, UENF, 2012.

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