Genetic divergence among eggplant genotypes under high temperatures

Ricardo de N Valadares1; Daniela N Nobrega1; Lilian B de Lima1; Adonis Q Mendes2; Fabian S Silva1; Roberto de A Melo3; Dimas Menezes3

1Universidade Federal Rural de Pernambuco (UFRPE), Recife-PE, Brasil; rnvaladares@yahoo.com.br (corresponding author); dany.an@hotmail.com.br; lilianbonfim53@gmail.com; fabianufpe@gmail.com; robertoagronomo@yahoo.com.br; dimasmenezes@superig.com.br.

2Instituto Federal de Pernambuco (IFPE), Vitória de Santo Antão-PE, Brasil; adonis@agronomo.eng.br.

3Universitário Federal de Pernambuco (IFPE), Vitória de Santo Antão-PE, Brasil; adonis@agronomo.eng.br.

ABSTRACT

The aim of this study was to estimate the genetic divergence among eggplant genotypes for agronomic traits in order to gather information for the selection of genotypes in eggplant breeding programs for tolerance to high temperatures. Ten traits recommended by the International Board for Plant Genetic Resources were analyzed in 24 genotypes, arranged in a randomized complete block design with four replicates. Data were submitted to analysis of variance (P<0.01) and later to the UPGMA and Tocher grouping methods, using the generalized Mahalanobis distance (D2) as dissimilarity measure. Three and six groups of similarity were obtained, respectively, for the multivariate techniques used, UPGMA and Tocher, with concordance in the grouping of 87.50% of the genotypes. The characters fruit length (34.71%), fruit width (35.96%) and fruit length/width ratio (14.08%) were the main contributors to genetic divergence, explaining 90.72% of total genetic dissimilarity. The genotypes presented considerable genetic variability for all agronomic traits analyzed and can be used in eggplant genetic breeding programs for high temperatures.

Keywords: Solanum melongena, genetic variability, protected cultivation.

RESUMO

Divergência genética entre genótipos de berinjela sob altas temperaturas

O objetivo deste trabalho foi estimar a divergência genética entre genótipos de berinjela para caracteres agronômicos, visando gerar informações para a escolha de genótipos em programas de melhoramento genético para tolerância a altas temperaturas. Foram analisados dez caracteres recomendados pelo International Board for Plant Genetic Resources em 24 genótipos, dispostos no delineamento de blocos ao acaso, com quatro repetições. Os dados foram submetidos à análise de variância (P<0,01) e posteriormente aos métodos de agrupamento de UPGMA e Tocher, utilizando-se a distância generalizada de Mahalanobis (D2) como medida de dissimilaridade. Obteve-se três e seis grupos de similaridade, respectivamente, para as técnicas multivariadas utilizadas, UPGMA e Tocher, havendo concordância no agrupamento de 87,50% dos genótipos. Comprimento do fruto (34.71%), largura do fruto (35.96%) e a relação comprimento/largura do fruto (14.08%) foram os caracteres que mais contribuíram para a divergência genética, explicando 90.72% da dissimilaridade genética total. Os genótipos apresentaram considerável variabilidade genética para todos os caracteres agronômicos analisados e podem ser utilizados nos programas de melhoramento genético de berinjela para altas temperaturas.

Palavras-chave: Solanum melongena, variabilidade genética, cultivo protegido, correlações genéticas.

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In Brazil, the area cultivated with eggplant (1550 ha/year) is concentrated mainly in the Center-South region (Boiteux et al., 2016). In the Northeast, where temperatures are relatively high, averaging around 28°C and peaking around 40°C (Ramilho, 2013) crop yields have been unpredictable. This is mainly due to flowering coinciding with warmer periods of the year, increasing the occurrence of malformation and/or fruit abortion. In greenhouse crops, where the internal temperatures are higher than the outside, there is a considerable reduction in crop yield in the region (Valadares et al., 2019ab).

The optimal temperature for crop growth and development is in the range of 22 to 30°C (Adamczewska-Sowińska & Krygier, 2013). When the temperature exceeds 32°C, productivity is drastically reduced (Baswana et al., 2006). Adoption of strategies for evaluation and selection of eggplant genotypes and knowledge of the genetic variability involved in traits of agronomic importance are extremely important for the choice of genotypes to compose eggplant breeding programs for high temperature tolerance.

Genetic divergence studies provide these parameters and allow the correct choice of parents which, when crossed, result in high heterotic effect on progenies, maximizing the chances of obtaining superior genotypes in segregating generations (Rotili et al., 2012). These genotypes can be obtained by biometric techniques based on quantification of heterosis or by predictive processes (Nardino et al., 2017).

Among the biometric techniques are diallel analyzes, which generate information about the specific combining ability and heterosis manifested in hybrids and in the prediction of genetic divergence, also keeping in mind
that several multivariate methods can be applied, including agglomerative methods.

Agglomerative methods (Cruz et al., 2012) seek to genetically discriminate individuals and allow them to be separated into groups by analyzing a set of characters inherent to each individual, grouping them by some classification criteria, so that there is homogeneity within each group and heterogeneity between them. They also basically involve two stages, the first refers to the estimation of a similarity or dissimilarity measure and the second refers to the adoption of a grouping technique.

As dissimilarity measures, we can point out the Euclidean distance, the average Euclidean distance, the weighted distance and the generalized Mahalanobis distance ($D^2$) (Cruz et al., 2012, 2014).

Genotype grouping can be done by optimization and hierarchical clustering methods. Among the optimization methods are the modified Tocher and Tocher (Vasconcelos et al., 2007; Cruz et al., 2014). Hierarchical clustering methods include the methods of the nearest neighbor, the farthest neighbor, UPGMA (Unweighted Pair-Group Method using Arithmetic Averages), the centroid, the median (or WPGMC), and the Ward’s minimum variance (Cruz et al., 2012).

Finally, we can adopt the cophenetic correlation analysis to increase the reliability of the conclusions regarding interpretation based on dendrograms. This establishes a correlation between the similarity or dissimilarity matrix with the generated dendrogram, i.e., compares the actual distances obtained between the accessions with the distances graphically represented (Kopp et al., 2007). The higher the correlation value, the smaller the distortion caused by grouping.

Given the above, the present work aimed to estimate genetic divergence between eggplant genotypes for agronomic traits, aiming to generate information for the choice of genotypes in eggplant breeding programs for high temperature tolerance.

### MATERIAL AND METHODS

The experiment was conducted between May and September 2016 at Universidade Federal Rural de Pernambuco (UFRPE), Recife-PE.

Seeds were sown in 128-cell expanded polystyrene trays filled with inert substrate (sifted coconut powder). Trays were kept in greenhouse in the hydroponic system by sub-irrigation until reaching the point for transplantation, plantlets with three definite leaves. Seedlings were individually transplanted to 5 L pots, containing inert substrate (coconut powder), spaced 1.75 m between rows and 0.60 m between plants.

Plants were cultivated in open hydroponics with substrate, under a 30 m long, 14 m wide, 3 m ceiling height arch, with 50% shading side screens and roof covered with a low-density polyethylene film, 150 micrometers thick.

Mineral nutrition and water requirement of plants were supplied by balanced nutrient solution at each plant development stage. A drip irrigation system was used with 2 L h⁻¹ emitter, automatically controlled by a digital timer, with irrigation amounts and duration adjusted according to environmental conditions of the region and the amount of nutrient solution absorbed by the plants.

Throughout the experiment period, relative air temperature (average, maximum and minimum) and relative air humidity were recorded using a HOBO mini datalogger. The environmental conditions in which the experiment was performed are characterized by high temperatures, since in all phenological phases temperatures exceeded the optimum range of the culture.

Eighteen eggplant accessions from the Embrapa Hortaliças’ germplasm bank and six commercial cultivars (Ciça F1, Choryoku F1, Kokushi Onaga F1, Ajimurasaki F1, Ajishirakawa F1 and Florida Market) were evaluated, coming to a total of 24 treatments arranged in randomized block design with four replications and four plants per experimental plot.

Six quantitative traits were evaluated: fruit length (cm), fruit width (cm), fruit length/width ratio, number of fruits per plant, yield per plant (g) and fruit mass (g); and four qualitative traits: fruit color at commercial maturity (1 = green; 2 = white; 3 = yellow; 4 = light red; 5 = dark red; 6 = grayish purple; 7 = purple; 8 = dark purple; 9 = black), fruit color distribution at commercial maturity (1 = uniform; 3 = mottled; 5 = lacy; 7 = streaked), fruit curvature (1 = none (straight fruit); 3 = slightly curved; 5 = curved; 7 = snake-shaped; 8 = sickle-shaped; 9 = U-shaped) and the presence of thorns in the fruit’s cup (0 = none; 1 = very few (<3); 3 = few (~5); 5 = intermediate (~10); 7 = many (~20); 9 = very many (~30)) (IBGPR, 1990).

Quantitative data were initially submitted to univariate analysis of variance (p<0.01) and from the means and residual variance and covariance matrix was obtained the genetic dissimilarity matrix based on the generalized Mahalanobis distance ($D^2$). The genotype clustering was obtained by the method of ascending hierarchical classification algorithm UPGMA (Unweighted Pair-Grouped Method Average) and by the Tocher’s optimization method.

The relative importance of traits in the prediction of genetic diversity was also studied through the participation of $D^2$ components, related to each trait in the total dissimilarity observed, and the diversity between genotypes was estimated by Mahalanobis distance. (Singh, 1981).

To test the efficiency of the hierarchical clustering method, we estimated the cophenetic correlation coefficient, obtained with 1,000 simulations, analyzed by the “t” test. The cutoff point (Cp) of the dendrogram formed by the UPGMA method was defined as proposed by Mojema (1977), following the formula $Cp = m + ksd$, where $m$ = the mean distance values of the fusion levels corresponding to the stadiaums; $k = 1.25$ (Milligan & Cooper, 1985); $sd$ = standard deviation.

All statistical analyzes were performed using the GENES software, version 1990.2018.75 (Cruz, 2013).
RESULTS AND DISCUSSION

The micrometeorological data obtained during the experiment period showed that the maximum air temperature in the greenhouse ranged between 29.8 and 41.4°C and the minimum temperature between 18.6 and 23.7°C. The average temperature ranged between 23.7 and 28.5°C. Thus, the environment was classified as high temperature for eggplant cultivation. Relative humidity ranged from 83.7 to 95.4%.

Significant differences were verified by F test ($p<0.01$) between genotypes for all analyzed traits (Table 1). This result refers to the existence of phenotypic variability between genotypes, and it is necessary to identify the superior genotypes to be crossed in eggplant breeding programs.

Dissimilarities ($D^2$) between genotypes ranged from 1.07 to 728.53, with an average of 133.47. The largest distances were recorded between CNPH 135 and Ajishirakawa F1 genotypes. On the other hand, genotypes CNPH 47 and Florida Market were the least genetically distant (Figure 1). Thus, crossings between the most divergent groups are indicated for formation of segregating populations and with greater genetic variability for the analyzed traits.

The dendogram obtained by UPGMA hierarchical method showed the formation of three groups, considering a significant cut of 44.32% (Mojena, 1977). Group 1 was composed of most genotypes, approximately 84% (Figure 1). Among quantitative traits, those that most contributed to the genetic divergence stood out (Table 3). In this sense, the fruits of this group had an average length of 14.11 cm, with averages ranging from 6.89 (CNPH 668) to 18.09 cm (CNPH 51). For fruit width, the average was 5.84 cm, with values between 3.64 (CNPH 84) and 8.55 cm (CNPH 135), reflecting in the length/width ratio of the fruit, which was between 1.48 (CNPH 668) and 4.91 (CNPH 84) with a mean of 2.59 (Table 1). Results similar to those were reported by Valadares et al. (2019b).

In the morphological description of the genotypes of group 1, for qualitative traits (Table 1), considerable levels of phenotypic variability were observed only for fruit color at commercial maturity, with a predominance of dark purple, followed by grayish purple.

### Table 1. Description of eggplant genotypes under high temperatures. Recife, UFRPE, 2016.

| Genotypes       | LF (cm) | FW (cm) | FLWR | NFP  | YP (g) |
|-----------------|---------|---------|------|------|--------|
| CNPH 135        | 12.96   | 8.56    | 1.52 | 1.89 | 470.03 |
| CNPH 60         | 13.51   | 6.77    | 2.00 | 2.14 | 301.03 |
| CNHP 51         | 18.09   | 5.00    | 3.67 | 1.57 | 203.20 |
| CNHP 410        | 15.29   | 5.48    | 2.80 | 1.39 | 149.72 |
| CNPH 84         | 17.70   | 4.91    | 3.56 | 283.50 |
| CNPH 71         | 16.01   | 4.09    | 3.94 | 1.85 | 161.08 |
| CNPH 668        | 6.89    | 4.67    | 1.48 | 4.08 | 209.79 |
| CNPH 146        | 12.63   | 5.84    | 2.17 | 2.35 | 249.95 |
| CNPH 140        | 13.42   | 5.27    | 2.83 | 2.09 | 191.20 |
| CNPH 93         | 13.82   | 4.61    | 2.99 | 1.97 | 173.23 |
| CNPH 47         | 14.15   | 7.68    | 1.85 | 1.26 | 242.36 |
| CNPH 141        | 11.56   | 5.86    | 1.97 | 3.83 | 383.69 |
| CNPH 67         | 13.06   | 6.38    | 2.05 | 3.00 | 341.20 |
| CNPH 107        | 15.56   | 5.58    | 2.80 | 2.24 | 317.69 |
| CNPH 53         | 11.90   | 7.77    | 1.55 | 1.00 | 151.28 |
| CNPH 109        | 14.47   | 6.18    | 2.34 | 1.72 | 225.65 |
| CNPH 79         | 13.16   | 5.33    | 2.60 | 2.00 | 101.41 |
| Ciça F1         | 16.59   | 6.21    | 2.68 | 1.42 | 242.56 |
| CNPH 100        | 17.35   | 4.56    | 3.91 | 1.16 | 110.99 |
| Florida Market  | 14.26   | 7.50    | 1.91 | 1.39 | 257.40 |
| Ajishirakawa F1 | 23.65   | 3.21    | 7.41 | 1.30 | 130.94 |
| Choryoku F1     | 30.02   | 3.99    | 7.51 | 1.13 | 154.54 |
| Kokushi Onaga F1| 27.95   | 4.42    | 6.39 | 1.67 | 223.60 |
| Ajimurasaki F1  | 28.36   | 2.84    | 9.97 | 4.13 | 316.13 |
| CV (%)          | 7.58    | 6.50    | 10.86 | 28.78 | 32.29 |
| QM (treatments) | 128.83**| 8.83**  | 19.82** | 3.65** | 32586.12** |
| Mean            | 16.35   | 5.48    | 3.47 | 2.08 | 233.01 |
purple and green. However, the fruits showed color distribution at commercial maturation predominantly uniform with no curvature and no thorns in the fruit’s cup (Table 1). This distribution indicates that, in relation to the evaluated traits (quantitative and qualitative), most genotypes presented high levels of similarity, including the commercial cultivars Ciça F1 and Florida Market, contemplated in this group 1.

According to Guedes et al. (2013), individuals are grouped in pairs, using arithmetic means of dissimilarity, and the dendrogram prioritizes genotypes with greater similarity. This explains why the Kokushi Onaga F1, Ajishirakawa F1 and Choryoku F1 genotypes formed group 2 and the Ajimurasaki F1 genotype alone group 3, consisting of fruits longer than 23.64 cm, fruit width less than 4.41 cm and length/width ratio of the fruit greater than 6.38 (Table 1). Averages for fruit length in group 2 were between 23.65 (Ajishirakawa F1) and 30.01 cm (Choryoku F1) and for fruit width between 3.20 (Ajishirakawa F1) to 4.41 cm (Kokushi Onaga F1). For length/width ratio of the fruit, the variation ranged from 1.48 (CNPH 668) to 4.91 (CNPH 84) (Table 1). For fruit color, Ajishirakawa F1 genotype presented white, Choryoku F1 green and Kokushi Onaga F1, black fruits. However, predominantly of uniform distribution and without any thorn in the fruit’s cup. About fruit curvature, Ajishirakawa F1 and Choryoku F1 genotypes presented curved fruits and Kokushi Onaga F1 snake-shaped fruits (Table 1). No non-commercial genotype showed considerable similarity with these commercial cultivars.

Group 3 included only the Ajimurasaki F1 genotype with the second longest fruit length among the evaluated genotypes (28.35 cm), smallest fruit width (2.83 cm) and highest fruit length/width ratio (9.96). Averages for fruit length in group 2 were between 23.65 (Ajishirakawa F1) and 30.01 cm (Choryoku F1) and for fruit width between 3.20 (Ajishirakawa F1) to 4.41 cm (Kokushi Onaga F1). For length/width ratio of the fruit, the variation ranged from 1.48 (CNPH 668) to 4.91 (CNPH 84) (Table 1). For fruit color, Ajishirakawa F1 genotype presented white, Choryoku F1 green and Kokushi Onaga F1, black fruits. However, predominantly of uniform distribution and without any thorn in the fruit’s cup. About fruit curvature, Ajishirakawa F1 and Choryoku F1 genotypes presented curved fruits and Kokushi Onaga F1 snake-shaped fruits (Table 1). No non-commercial genotype showed considerable similarity with these commercial cultivars.

**Table 1.** Genetic divergence among eggplant genotypes under high temperatures

| Genotypes       | FM (g)    | FCCM          | DFCCM                        | FC                        | TFC                        |
|-----------------|-----------|---------------|------------------------------|---------------------------|----------------------------|
| CNPH 135        | 248.16    | dark purple   | uniform                      | none (straight fruit)     | intermediate               |
| CNPH 60         | 139.12    | dark purple   | uniform                      | none (straight fruit)     | none                       |
| CNHP 51         | 127.49    | dark purple   | uniform                      | slightly curved           | none                       |
| CNPH 410        | 105.68    | purple        | uniform                      | none (straight fruit)     | none                       |
| CNPH 84         | 77.69     | purple        | uniform                      | curved                    | none                       |
| CNPH 71         | 89.06     | purple        | streaked                     | curved                    | none                       |
| CNPH 668        | 51.47     | green         | streaked                     | none (straight fruit)     | intermediate               |
| CNPH 146        | 108.66    | grayish purple| uniform                      | none (straight fruit)     | intermediate               |
| CNPH 140        | 91.53     | grayish purple| uniform                      | none (straight fruit)     | few                        |
| CNPH 93         | 77.35     | grayish purple| uniform                      | none (straight fruit)     | none                       |
| CNPH 47         | 174.82    | dark purple   | uniform                      | none (straight fruit)     | none                       |
| CNPH 141        | 98.41     | dark purple   | uniform                      | none (straight fruit)     | few                        |
| CNPH 67         | 111.23    | grayish purple| uniform                      | none (straight fruit)     | none                       |
| CNPH 107        | 140.49    | dark purple   | uniform                      | none (straight fruit)     | none                       |
| CNPH 53         | 167.53    | dark purple   | uniform                      | none (straight fruit)     | none                       |
| CNPH 109        | 134.09    | purple        | uniform                      | none (straight fruit)     | none                       |
| CNPH 79         | 47.84     | grayish purple| uniform                      | none (straight fruit)     | none                       |
| Ciça F1         | 168.41    | dark purple   | uniform                      | none (straight fruit)     | none                       |
| Florida Market  | 187.18    | dark purple   | uniform                      | none (straight fruit)     | none                       |
| Ajishirakawa F1 | 101.27    | white         | uniform                      | curved                    | none                       |
| Choryoku F1     | 138.04    | green         | uniform                      | curved                    | none                       |
| Kokushi Onaga F1| 142.96    | black         | uniform                      | snake-shaped              | none                       |
| Ajimurasaki F1  | 77.68     | purple        | uniform                      | snake-shaped              | none                       |

CV (%) 24.60 - - - -
QM (treatments) 8472.63** - - - -
Mean 121.65 - - - -
that fruit length, fruit width and fruit 
length/width ratio presented the highest 
percentage of contribution to divergence 
among the 24 evaluated genotypes, 
explaining 90.72% of the total genetic 
dissimilarity (Table 3).

High contribution of fruit length to 
eggplant divergence has been reported 
by Babu & Patil (2004) and Mehta 
et al. (2004), while average fruit weight 
and number of fruits per plant traits 
have lower contributions as reported 
by Prabakaran et al. (2015). Bashar 
et al. (2016) also cited contributions 
of length and width of fruit traits in 
the genetic divergence of eggplant. 

We observed that genotype clustering 
was predominantly influenced by fruit 
length, fruit width and fruit length/width 
ratio, showing greater variability for 

Table 2. Grouping by Tocher method resulting from the analysis of 24 eggplant genotypes evaluated under high temperatures. Recife, UFRPE, 2016.

| Groups | Genotypes                                                                 |
|--------|---------------------------------------------------------------------------|
| 1      | CNPH 47, Flórida Market, CNPH 53, CNPH 60, CNPH 67, CNPH 109, CNPH 146,  |
|        | CNPH 141, Ciça F1, CNPH 146, CNPH 109, CNPH 84, CNPH 668, CNPH 107,     |
|        | CNPH 79, CNPH 410, CNPH 107, CNPH 140, CNPH 93, CNPH 51, CNPH 100, CNPH  |
|        | 71                                                                       |
| 2      | AjishirakawaF1, ChoryokuF1, Kokushi Onaga F1                              |
| 3      | CNPH 84                                                                   |
| 4      | Ajimurasaki F1                                                           |
| 5      | CNPH 668                                                                   |
| 6      | CNPH 135                                                                  |

among the most divergent genotypes 
(Table 2). Similarity between the 
different clustering techniques can 
be seen from the fact that genotypes 
belonging to Tocher’s group 1 were 
mostly the same ones from the UPGMA 
grouping, around 71% of the genotypes, 
including Ciça F1 and Florida Market.

There was also agreement in the 
formation of group 2 which included 
genotypes Kokushi Onaga F1, Ajishirakawa F1 and Choryoku F1 and the 
formation of group 4 composed 
only by genotype Ajimurasaki F1. 
Agreement between multivariate 
techniques is important in the study 
of genetic divergence, as it allows the 
recommendation of crossing between 
the most divergent parents possible, 
in order to broaden the genetic base and 
consequently increase genetic variability 
(Abreu et al., 2004). Disagreements 
occurred in the formation of groups 
3 (CNPH 84), 5 (CNPH 668) and 6 
(CNPH 135) by Tocher’s method.

The association of clustering 
techniques provides a more efficient 
support for determination of divergence, 
since Tocher discriminates each group 
and UPGMA discriminates each 
genotype and can more safely infer the 
use of parents in breeding programs 
(Bertan et al., 2006).

The relative importance of the 
analyzed traits in the genetic 
dissimilarity between genotypes was 
detected by Singh’s method (1981). 
This method considers that the most 
important characteristics express greater 
variability. In this respect, we found 
that fruit length, fruit width and fruit 
length/width ratio presented the highest 
percentage of contribution to divergence 
among the 24 evaluated genotypes, 
explaining 90.72% of the total genetic 
dissimilarity (Table 3).

High contribution of fruit length to 
eggplant divergence has been reported 
by Babu & Patil (2004) and Mehta et al. (2004), while average fruit weight 
and number of fruits per plant traits 
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we observed that genotype clustering 
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length, fruit width and fruit length/width 
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Table 3. Relative contribution of six quantitative traits to genetic divergence among 24 eggplant genotypes, using the Singh method, evaluated in 24 eggplant genotypes under high temperatures. Recife, UFRPE, 2016.

| Traits                        | Relative contribution (%) |
|-------------------------------|---------------------------|
| Fruit length (cm)             | 40.71                     |
| Fruit width (cm)              | 35.96                     |
| Fruit length/width ratio      | 14.08                     |
| Number of fruits per plant    | 5.56                      |
| Yield per plant (g)           | 2.88                      |
| Fruit mass (g)                | 0.81                      |
| Total                         | 100                       |

these traits (Table 3).

According to Rohlf (2000), the adjustment of cophenetic correlation coefficient is considered good when values are equal to or higher than (r) 0.70. In this case, the greater the (r) the smaller the distortion of the cluster, presenting a good fit between the matrix and the formed dendrogram (Cruz et al., 2012).

Eggplant genotypes, under high temperatures, showed significant genetic divergence for all evaluated traits. Töcher’s optimization methods and the hierarchical UPGMA agreed in 87.50% of genotypes clustering. The traits that the most contributed to divergence were fruit length, fruit width and fruit length/width ratio. The cophenetic correlation coefficient (r) was 0.79. Most genotypes showed genetic similarity with Ciça F1 and Florida Market cultivars.

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