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Multivariate techniques in the determination of genetic diversity in pest-resistant mini tomato genotypes

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ABSTRACT

The objective of this study was to compare methods of multivariate analysis on the evaluation of genetic diversity of mini tomato and to identify promising genotypes with resistance to pests. The experiment was conducted at the Vegetable Experiment Station of the Universidade Federal de Uberlândia, Monte Carmelo campus, from April 2013 to November 2016. The experimental design was a randomized complete block design with 16 treatments and four replications totaling 64 plots, and each plot represented by five plants. Sixteen genotypes were characterized, 12 from the F₂RC₁ generation, obtained through the interspecific crossing between the wild access LA-716 (*Solanum pennellii*) and pre-commercial lines of mini tomato (UFU-73 and UFU-2) (*Solanum lycopersicum*) and the UFU-2 lines. The content of acyl sugar, the amount of glandular trichomes (types I, IV, VI and VII), twospotted spider mite and whitefly resistance were evaluated. We concluded that there exist genetic variability between the genotypes. The number of groups formed by the canonical variated analysis was higher (four groups) than that obtained by the Tocher method (three groups) and UPGMA (three groups), demonstrating a greater discrimination power. The Tocher and UPGMA methods were consistent in the analysis of the genetic divergence in pest resistant germplasm of tomato, with the acyl sugar content being the most important variable. Genotype UFU-73-F₂RC₁ # 11 is resistant to pest attack, while the other studied lines have intermediate resistance.

Keywords: *Solanum pennellii*, *Solanum lycopersicum*, acyl sugar, genetic variability.

RESUMO

Técnicas multivariadas na determinação da diversidade genética em genótipos de minitomate resistentes a pragas

A pesquisa foi realizada com o objetivo de comparar diferentes métodos de análise multivariada na avaliação da diversidade genética em minitomate e identificar genótipos promissores com resistência a pragas. O experimento foi conduzido na Estação Experimental de Hortaliças da Universidade Federal de Uberlândia, campus Monte Carmelo, no período de abril de 2013 a novembro de 2016. O delineamento experimental foi em blocos casualizados com 16 tratamentos e quatro repetições totalizando 64 parcelas, sendo cada parcela representada por cinco plantas. Foram caracterizados 16 genótipos, sendo 12 provenientes da geração F₂RC₁, obtidos por meio do cruzamento interespecífico entre o acesso selvagem LA-716 (*Solanum pennellii*) versus linhagens pré-comerciais de minitomate (UFU-73 e UFU-2) (*Solanum lycopersicum*) e as linhagens UFU-2. Avaliou-se o teor de acilaçúcar, a quantidade de tricomas glandulares (tipos I, IV, VI e VII), a resistência ao ácaro e à mosca branca. Pode-se concluir que existe variabilidade genética entre os genótipos. O número de grupos formados pelo método de variáveis canônicas foi superior (quatro grupos) ao obtido pelo método de Tocher (três grupos) e UPGMA (três grupos), demonstrando maior poder de discriminação. Os métodos de Tocher e UPGMA foram coerentes quanto à análise da divergência genética em germoplasma de tomateiro resistente a pragas, sendo o teor de acilaçúcar a variável de maior importância. O genótipo UFU-73-F₂RC₁ # 11 é resistente ao ataque de pragas, enquanto as outras linhagens estudadas apresentam resistência intermediária.

Palavras-chave: *Solanum pennellii*, *Solanum lycopersicum*, acilaçúcar, variabilidade genética.

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The tomato crop (*Solanum lycopersicum*) stood out in recent years as an important agrarian activity in Brazil and in the world. In 2016, the area destined for tomato production in Brazil surpassed 57,000 hectares, with an estimated increase of 8% in 2017, surpassing 62,000 hectares (IBGE, 2017). In this context, the cultivation

of mini tomato has also increased due to its productivity and particularities related to the fruit such as reduced size, rounded and elongated shapes, various colors and high soluble solids content among others (Preczenhak *et al.*, 2014; Maciel *et al.*, 2015, 2016).

Despite this relevance, the tomato cultivation is considered a high risk

activity due to a great variety of pests that occur during its cultivation. Among the main arthropod pests, the whitefly (*Bemisia tabaci*) and the twospotted spider mite (*Tetranychus urticae*) causes damage to the crop productivity (Neiva *et al.*, 2013; Maciel *et al.*, 2017). An alternative to control these arthropods is using genotypes with a

broad spectrum of pest resistance (Maluf *et al.*, 2010). It is possible to increase the genetic variability in tomatoes aiming at resistance to pests in breeding programs and to increase the number of accesses available in germplasm bank (Neiva *et al.*, 2013; Maciel & Silva, 2014; Maciel *et al.*, 2017). Gruber (2017) reported that one of the priorities in tomato breeding programs should be to obtain pest-resistant plants. However, we have not found reports of the applicability of multivariate analysis in germplasm banks of tomato with different levels of resistance to pests.

The variability among genotypes can be estimated by measures of genetic dissimilarity, standing out the Mahalanobis distance (D^2_{ii}). This technique considers residual variances and covariance between quantitative characters (Cruz *et al.*, 2012). To characterize this divergence, the Tocher's optimization method and the hierarchical method Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA) are routinely used in tomato (Mattedi *et al.*, 2014; Araújo *et al.*, 2016). However, there is no consensus regarding the use of these techniques in mini-tomato germplasm whose genetic variability refers to the different levels of resistance to pests. The studies comparing methods of multivariate analysis in mini-tomato resistant to pests are scarce.

Considering the importance of tomato crops in national and international context, the evaluation of genetic diversity of mini tomato populations for pest resistance will provide the knowledge of the best combinations to obtain segregating or hybrid generations, allowing the obtention of superior genotypes. Then, the objective of this study was to compare methods of multivariate analysis on the evaluation of genetic diversity of mini tomato and to identify promising pest resistant genotypes.

MATERIAL AND METHODS

The experiment was conducted at the Experimental Station of Vegetables (18°42'43"S, 47°29'55"W, 873 m

altitude) and at LAGEN (Genetic Resources Laboratory) of Universidade Federal de Uberlândia (UFU), Campus Monte Carmelo, from April 2013 to November 2016.

Sixteen genotypes were characterized: twelve F_2RC_1 genotypes from the interspecific cross between wild access LA-716 (*S. pennellii*) versus pre-commercial lines of mini tomato (UFU-73 and UFU-2) (*S. lycopersicum*) followed by backcrossing and self-fertilization. These lines from free market were chosen because of their desirable agronomic characteristics as high soluble solids content.

Inbred lines UFU-2 have indeterminate growth habit (homozygous dominant, SP/SP), fruits with 11⁰ Brix soluble solids contents, red, average weight of 15 g and susceptible to pests. The mini-tomato lines UFU-73 have determinate growth habit (homozygous recessive, sp/sp), fruits with 10⁰ Brix soluble solids content, yellow, average weight of 18 g and susceptible to pests. The access LA-716 is rich in the allelochemical acyl sugar, capable of providing broad spectrum of resistance to pests in tomato and the commercial cultivar Santa Clara (susceptible to pests).

Genotypes were sown on May 2016, using 200-cell polystyrene trays filled with commercial coconut-based substrate. After 35 days of sowing, the seedlings were transplanted in 5 L pots filled with the same substrate used to produce them.

The experiment was conducted in randomized complete block design, with four repetitions totaling 64 plots, each plot represented by 5 plants, totaling 320 plants in the experiment. The plants were conducted in an arctype greenhouse, with dimensions 7x21 m and 4 meters ceiling height, covered with 150-micron transparent polyethylene film and white anti-aphid screen side curtains. The same plants were used to quantify the levels of acyl sugar, foliar trichrome, twospotted spider mite and whitefly repellency.

After 75 days of sowing, a sample composed of 6 leaf discs (equivalent to 4.2 cm²) was collected in each of

the five plants of the plot. The discs were collected from leaflets present in the upper third of the plants and packed in test tubes. The extraction and quantification of the allelochemical acyl sugar followed the methodology described by Resende *et al.* (2002) and adapted by Maciel & Silva (2014).

Glandular trichomes (types I, IV, VI and VII) were quantified according to the methodology of Glas *et al.* (2012), in the periods of 30, 45, 60, 75 and 90 days after sowing. Five young and expanded leaflets were collected from the upper third of each plant and the number of epidermal glandular trichomes per cm² was evaluated on the abaxial and adaxial surfaces. Trichomes were quantified with a stereomicroscope (40x), with a micrometric scale of 1 cm² area.

The twospotted spider mite resistance was quantified according to Weston & Snyder's (1990) repellency test, measuring the distances covered by arthropods on the leaflet surfaces of the genotypes at 5, 10, 15 and 20 minutes. The test was done with the placement of five twospotted spider mites on a thumbtack attached to the center of each leaflet.

The presence of whitefly was verified according to the methodology described by Maluf *et al.* (2010). After 90 days of sowing, the number of eggs and nymphs per cm² of leaf area were quantified with the aid of a stereomicroscope (40x). Five leaflets of the upper third of the plant were evaluated by each genotype. For counting the number of adults, a mirror was used to visualize the insects before the escape to the abaxial part of each leaflet.

The data were submitted to multivariate analysis with the objective of determining the genetic dissimilarity between the genotypes. The dissimilarity matrix was obtained by Mahalanobis distance (D^2_{ii}). From Mahalanobis distance matrix, the genetic divergence was represented by a dendrogram obtained by the hierarchical method Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA), validated by the cophenetic correlation coefficient (CCC) with Mantel's test (1967). Tocher's optimization method was also used to cluster the genotypes.

The relative contribution of the quantitative traits was calculated according to Singh (1981). For the analysis by canonical variables, the genetic divergence was demonstrated by the dispersion of the scores in graphs, with the axes represented by the first canonical variables. All analyzes were performed using the software Genes v. 2015.5.0 (Cruz, 2013).

RESULTS AND DISCUSSION

The genetic dissimilarity measures (Table 1), estimated from the generalized distance of Mahalanobis (D^2), ranged from 44.02 to 53257.93. This implies the presence of genetic divergence among the studied genotypes (Azevedo *et al.*, 2015). We observed that 87.5% of the studied genotypes presented greater divergence when compared to *S. pennellii* genotype. However, the Santa Clara genotype presented a shorter distance in relation to the wild access.

For UPGMA dendrogram, the value of the cophenetic correlation

coefficient (CCC) was 0.77 with 29.64% of distortion, which shows an adequate relation between the matrix distance and the dendrogram produced.

When a cut considering 35% of dissimilarity was made, we observed the formation of three clusters (Figure 1). The first group consisted of 81.25% of the genotypes under study (UFU-22-F2RC1#1; UFU-22-F2RC1#2; UFU-22-F2RC1#3; UFU-22-F2RC1#4; UFU-22-F2RC1#5; UFU-22-F2RC1#6; UFU-22-F2RC1#7; UFU-22-F2RC1#8; UFU-22-F2RC1#9; UFU-22-F2RC1#10; UFU-22-F2RC1#12; UFU-73-2-3-10-1 and UFU-F4-2-2-2). The Santa Clara genotype, characterized as being susceptible to pest attack, formed an isolated group. The third cluster consisted of the UFU-73-F2RC1#11 genotype and the wild-access *S. pennellii* genotype characterized by being tolerant to insect attack. Using this same method, Lucatti *et al.* (2013), evaluating the genetic diversity of 35 accessions of tomato on resistance to *B. tabaci*, detected divergence between them, observing the formation of two

groups.

The clustering of the 16 mini tomato genotypes by Tocher's optimization method allowed the formation of three groups: I: Inbred lines UFU-22-F2RC1#4, UFU-22-F2RC1#5, UFU-22-F2RC1#3, UFU-73-F2RC1#12, UFU-22-F2RC1#7, UFU-22-F2RC1#9, UFU-22-F2RC1#10, UFU-22-F2RC1#1, UFU-73-2-3-10-1, UFU-22-F2RC1#8, UFU-22-F2RC1#6, UFU-F4-2-2-2 and UFU-22-F2RC1#2; II: Santa Clara genotype, III: UFU-73-F2RC1#11 and *S. pennellii* (Table 2).

The Tocher's method was similar to the UPGMA method. The group I in both methods had the highest number of genotypes (87.5%). The genotype UFU-73-F2RC1#11 was allocated in the same group as the wild-type *S. pennellii*, representing the genotype with the highest tolerance to pest attack. The second group was formed by the Santa Clara hybrid, susceptible to pest attack. In contrast, the group III was represented by genotypes with intermediate level of resistance.

Using Tocher and UPGMA clustering methods, Luz *et al.* (2016) in a genetic diversity study of 53 tomato hybrids for industrial processing, and Faria *et al.* (2012) evaluating genetic divergence in peppers, reported predominance of genotypes in one group and formation of groups with only one genotype. The results observed in the present study corroborate with those obtained by these authors.

The importance of the use of analysis of characters stands out for studying the total available variation of the genotypes based on the evaluated characteristics. Based on the criteria proposed by Singh (1981), in terms of the relative contribution of each character to the genetic divergence (Table 3), we verified that the most important characteristics for discrimination of the genotypes were: acyl sugar content (37.05%), number of nymphs per plant (12.48%) and number of eggs per plant (12.32%), and the characters of lower contribution were the distance covered by the twospotted spider mite during five minutes (0.05%) and the counting of trichomes with thirty days (0.07%).

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Table 1. Estimates of the nearest and further distance of 16 mini tomato genotypes, based on the Mahalanobis distance (D^2). Monte Carmelo, UFU, 2013-2016.

Genotypes ¹	Smaller D^2	Closer	Higher D^2	Less close
1	72.80	8	24022.13	16
2	13.94	6	31588.91	16
3	115.06	5	21355.78	16
4	44.02	5	20684.83	16
5	44.02	4	19998.79	16
6	68.84	8	28198.82	16
7	142.27	9	16639.61	16
8	68.84	6	26207.23	16
9	142.27	7	15045.47	16
10	194.62	7	16505.18	16
11	3318.14	9	26882.11	13
12	123.80	5	17826.78	16
13	2862.08	15	53257.93	16
14	141.82	8	26482.89	16
15	238.45	6	31774.25	16
16	53257.93	11	5611.72	13

¹1= UFU-22-F2RC1#1; 2= UFU-22-F2RC1#2; 3= UFU-22-F2RC1#3; 4= UFU-22-F2RC1#4; 5= UFU-22-F2RC1#5; 6= UFU-22-F2RC1#6; 7= UFU-22-F2RC1#7; 8= UFU-22-F2RC1#8; 9= UFU-22-F2RC1#9; 10= UFU-22-F2RC1#10; 11= UFU-73-F2RC1#11; 12= UFU-73-F2RC1#12; 13= Santa Clara; 14= UFU-73-2-3-10-1; 15= UFU-F4-2-2-2; 16= *Solanum pennellii*.

of characters obtained by means of Canonic Variables, the acyl sugar content was the one that contributed the most to the genetic divergence among the accessions. This result is relevant, because it discriminates the existence of wide variability of the degree of resistance between the accesses,

allowing the selection of accesses with greater potential for resistance to pests.

The relative importance of canonical variables was measured by the percentage of their eigenvalues in relation to the total eigenvalues. The values of the canonical variables analysis, obtained by the correlation matrix of the evaluated characters, revealed that the first two canonical variables were sufficient to explain 98.74% of the variation observed. The first variable absorbed 96.06% of the variation obtained by the characteristics of greater contribution: the acyl sugar content. The second canonical variable represented 2.68% of the variation obtained based on the variables of greatest contribution: twospotted spider mite covering during fifteen minutes.

Silva *et al.* (2016), evaluating the F2 generation, from interspecific crossing between *S. lycopersicum* (TOM-684) and *S. galapagense* (LA-1401), observed that genotypes selected for high density of type IV glandular trichomes presented resistance to the caterpillar *Helicoverpa armigera*. These trichomes are associated with the production of acyl sugar. Silva *et al.* (2017) and Maciel *et al.* (2018a) also observed that tomato genotypes with higher acyl sugar content presented resistance to *Liriomyza trifolii* and *Spider mite*, respectively.

For the satisfactory interpretation of the variability found among the genotypes it is necessary that the first two canonical variables presented a minimum estimate of 80% of the total variation contained in the mentioned character (Cruz *et al.*, 2012). In this study the first two canonical variables explained more than 80% of the total variance of characters analyzed (98.74%). In this way the variability manifested among the genotypes can be explained by means of the dispersion graph (Figure 2). By the scatter plot method, four groups were formed. The Santa Clara and *S. pennelli* genotypes formed isolated and distant groups. The genotype UFU-73-F2RC1#11 formed an isolated group, being between *S. pennelli* and the group formed by the other genotypes. In this way the scatter plot method differed from the other

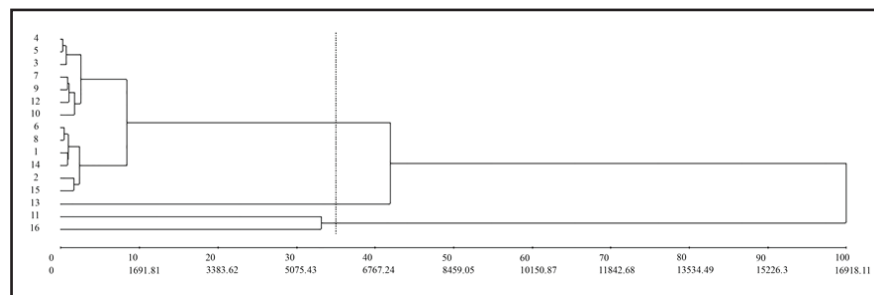


Figure 1. Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA) dendrogram of 16 mini tomato genotypes obtained with the Mahalanobis distance generated with thirteen characters. 1= UFU-22-F2RC1#1; 2= UFU-22-F2RC1#2; 3= UFU-22-F2RC1#3; 4=UFU-22-F2RC1#4; 5= UFU-22-F2RC1#5; 6= UFU-22-F2RC1#6; 7= UFU-22-F2RC1#7; 8= UFU-22-F2RC1#8; 9= UFU-22-F2RC1#9; 10= UFU-22-F2RC1#10; 11= UFU-73-F2RC1#11; 12= UFU-73-F2RC1#12; 13= Santa Clara; 14= UFU-73-2-3-10-1; 15= UFU-F4-2-2-2; 16= *Solanum pennellii*. Monte Carmelo, UFU, 2013-2016.

Table 2. Mini tomato genotypes clustered by Tocher's optimization method estimated with Mahalanobis distance. Monte Carmelo, UFU, 2013-2016.

Group	Genotypes
I	UFU-22-F2RC1#4, UFU-22-F2RC1#5, UFU-22-F2RC1#3, UFU-73-F2RC1#12, UFU-22-F2RC1#7, UFU-22-F2RC1#9, UFU-22-F2RC1#10, UFU-22-F2RC1#1, UFU-73-2-3-10-1, UFU-22-F2RC1#8, UFU-22-F2RC1#6, UFU-F4-2-2-2 e UFU-22-F2RC1#2
II	Santa Clara
III	UFU-73-F2RC1#11 and <i>Solanum pennellii</i>

Table 3. Relative contribution (%) of characteristics for genetic divergence in mini tomato genotypes, estimated by the method proposed by Singh (1981). Monte Carmelo, UFU, 2013-2016.

Variable	S.j (%)
Acyl sugar (nmol/cm ² of leaf area)	37.05
Number of nymphs	12.48
Number of eggs	12.32
Number of trichomes (90 DAS) ¹	8.90
Number of adult insects	8.79
Number of trichomes (60 DAS) ¹	7.89
DP twospotted spider mite (15 minutes) ²	6.71
DP twospotted spider mite (10 minutes) ²	3.92
Number of trichomes (75 DAS) ¹	1.08
Number of trichomes (45 DAS) ¹	0.49
DP twospotted spider mite (10 minutes) ²	0.27
Number of trichomes (30 DAS) ¹	0.07
DP twospotted spider mite (15 minutes) ²	0.03

¹DAS= days after sowing; ²DP= distance covered by the twospotted spider mite.

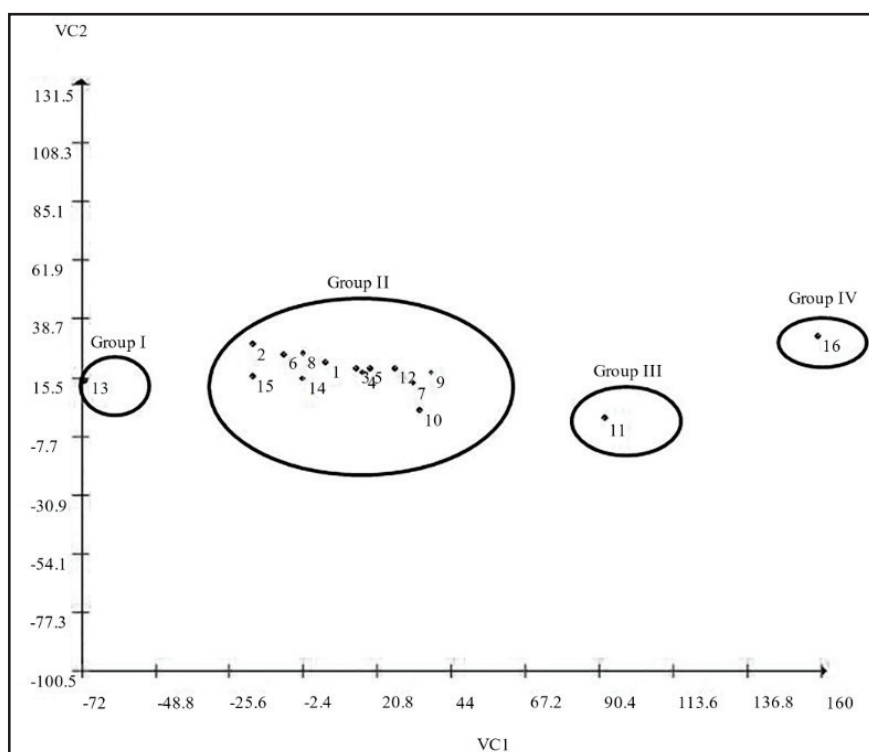


Figure 2. Dispersion graph of 16 mini tomato genotypes in relation to the scores of the first two canonical variables, VC1 (96.06%) and VC2 (2.68%). 1= UFU-22-F2RC1#1; 2= UFU-22-F2RC1#2; 3= UFU-22-F2RC1#3; 4= UFU-22-F2RC1#4; 5= UFU-22-F2RC1#5; 6= UFU-22-F2RC1#6; 7= UFU-22-F2RC1#7; 8= UFU-22-F2RC1#8; 9= UFU-22-F2RC1#9; 10= UFU-22-F2RC1#10; 11= UFU-73-F2RC1#11; 12= UFU-73-F2RC1#12; 13= Santa Clara; 14= UFU-73-2-3-10-1; 15= UFU-F4-2-2-2; 16= *Solanum pennellii*. Monte Carmelo, UFU, 2013-2016.

analyzed methods, due to the formation of a larger number of groups.

The formation of coincident groups, through the use of complementary methods of multivariate analysis, is also described in pepper (Negreiros & Miqueloni, 2013), passion fruit (Paiva et al., 2014), tomato (Luz et al., 2016; Amaral Júnior et al., 2017; Maciel et al., 2018b), which shows consistency of similarity results in the groups composition. The results obtained in the present study corroborate those found by these authors.

The application of the UPGMA hierarchical method, Tocher's optimization and canonical variables allowed to conclude the existence of genetic variability among the genotypes. The number of groups formed by the canonical variables method was higher (four groups) than that obtained by the Tocher method (three groups) and UPGMA (three groups), demonstrating a greater discriminating power. This

allows the identification of more groups containing similar accesses. In addition, there was consistency between Tocher and UPGMA methods for the analysis of genetic divergence in pest-resistant tomato germplasm, with the most important variable being the acyl sugar content. Genotype UFU-73-F2RC1 # 11 is resistant to pest attack, while the other lines studied have intermediate resistance.

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