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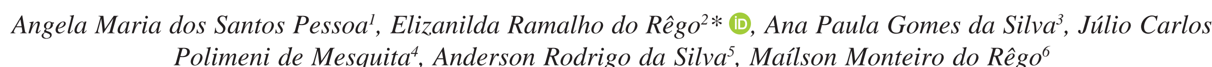
Genetic diversity in F3 population of ornamental peppers (*Capsicum annuum* L.)



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ABSTRACT

Peppers belong to the Solanaceae family and present a wide genetic variability that may be accessed through phenotypic and genotypic traits. This work aimed to study the genetic divergence in an ornamental pepper F_3 population by multivariate methods based on the individual and simultaneous analyses of qualitative, quantitative, and molecular data. The work was developed in the Center of Agrarian Sciences (CCA) of the Universidade Federal da Paraíba (UFPB), Paraíba state, Brazil. 44 progenies from an F_3 generation were used and the characterization of 30 qualitative traits, 16 quantitative traits, and 18 pairs of microsatellite primers was performed. Individual and simultaneous analyses of the variables were performed by using the clustering method of Ward's algorithm. Tocher's method was used based on distance matrixes. A non-metric multidimensional scaling was applied (nMDS) for the graphic representation of the distance matrixes in the bidimensional space. Data analysis was efficient to separate the genotypes into distinct groups. There is genetic variability among the individuals of the *C. annuum* F_3 population, verified by individual and joint trait measurements. The three clustering methods were efficient to represent the genetic distance between individuals of ornamental pepper plants. Individuals 1, 2, 7, 8, 10, 11, 21, 27, 29, 35, and 38 are indicated to advance generation and continue the breeding program.

Keywords: multivariate analysis; pepper; genetic variability; molecular markers; quantitative traits.

INTRODUCTION

Peppers belong to the Solanaceae family, genus *Capsicum*, and are found in regions of tropical and temperate climate in several regions of the world (Pickersgill *et al*., 1997; Stommel & Bosland, 2006). These plants have presented a growing and continuous reception by the consuming market (Rêgo *et al*., 2016; Rêgo & Rêgo, 2018).

Peppers also present a wide genetic diversity for the phenotypic traits of size, leaves, and fruits, with potential for utilization in the food industry and use as a medicinal (Stommel & Griesbach, 2008; Mongkolporn & Taylor, 2011) and ornamental plant (Rêgo & Rêgo, 2018).

The species *Capsicum annuum* comprises the bell peppers and the sweet and spicy peppers, as well as ornamental varieties (Stommel & Bosland, 2006). It is used as an ornamental plant for possessing variegated foliage, compact size, and fruits with varied coloring in the different maturation stages (Melo *et al*., 2014; Rêgo & Rêgo, 2018; Pessoa *et al*., 2018).

The knowledge of the degree of genetic variability, through divergence studies, is essential in the

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identification process for new sources of genes of interest (Falconer & Mackay, 1996). To indicate this variability, sufficient genetic diversity is needed to allow the selection of individuals that can be used in breeding programs. (Costa *et al*., 2011).

Among the ways to evaluate this diversity in pepper plants, quantitative and qualitative traits stand out in the identification of superior genotypes and more recently by molecular markers, which allow to characterize and determine the existing genetic divergence within and between vegetal species (Bonett *et al*., 2006; Villela *et al*., 2014).

In order to determine the genetic distance between individuals based on the collected data, biometric models are utilized, based on multivariate techniques that allow to combine several information data of a set of characteristics (Mesquita *et al*., 2016), thus allowing the choice of divergent individuals (Sudré *et al*., 2005).

The generation of several data of different categories (phenotypical or genotypical) may be a factor that complicates both the analysis and interpretation of the characterization results, often resulting in the incomplete distinction between accessions (Rocha *et al*., 2010). In this perspective, the joint analysis of the variables might provide a better indication of the variability potentiality existing in germplasm banks (Torres *et al*., 2015).

The technique that allows the joint analysis of qualitative and quantitative data was proposed by Gower (1971). Therefore, there are no reports on the use of this strategy for the quantification of the genetic dissimilarity using Gower's coefficient or in a genetic divergence study using Ward's algorithm, Tocher's method, and non-metric multidimensional scaling in *Capsicum* pepper plants for the joint analysis of quantitative, qualitative, and molecular data.

Under the hypothesis of distinction and characterization of promising individuals in the genetic breeding of ornamental peppers, this work aimed to assess the genetic diversity in an F_3 population of ornamental peppers through multivariate methods based on the individual and joint analysis of qualitative, quantitative, and molecular data.

MATERIAL AND METHODS

The work was developed in a plant nursery and in the Laboratory of Vegetal Biotechnology at the Center of Agrarian Sciences (CCA) of the Universidade Federal da Paraíba (UFPB), state of Paraíba, Brazil.

The vegetal material consisted of 44 progenies from an F_3 generation of ornamental peppers (*C. annuum* L.), belonging to the Germplasm Bank of the genus *Capsicum* of the UFPB, originated from the controlled self-fertilization of the F_2, obtained from the self-fertilization of the F_1 resultant of the crossing between the parental individuals: UFPB134 x UFPB77.2 (Table 1), with phenotypical diversity for the qualitative traits.

The seeds of the 44 progenies were sowed in polystyrene trays of 180 cells filled with commercial substrate, and when presenting six definitive leaves the seedlings were transplanted to plastic pots containing 900 mL of the same substrate.

The morpho-agronomic characterization was performed based on the *Capsicum* descriptors (IPGRI, 1995). 30 qualitative traits were evaluated: stem color, nodal anthocyanin, stem shape, stem pubescence, growth habit, branching density, tillering, leaf density, leaf color, leaf shape, leaf blade edges, leaf pubescence, flower position, corolla color, corolla stain color, corolla shape, filament color, anther color, stigma insertion, calix pigment, calix margin, calix ring constriction, immature fruit color, intermediary fruit color, mature fruit color, anthocyanin stain, fruit shape, fruit apex shape, fruit pedicel firmness and stem pedicel firmness, and 16 quantitative traits: plant height, canopy diameter, first bifurcation height, stem diameter, leaf length, leaf width, fruit length, largest fruit diameter, smallest fruit diameter, peduncle length, pericarp thickness, placenta length, fruit length/diameter relation, fruit weight, dry matter content and number of seeds per fruit.

For the molecular analyses, 200 mg of young foliar tissue from each individual of the F_3 population were subjected to DNA extraction, according to the protocol described by Doyle & Doyle (1990). The DNA amount was analyzed in agarose gel at 0.8%. Aliquots from each DNA sample were applied to the gel wells, and the concentration of the samples was estimated by visually comparing the fluorescence intensity of the DNA bands with bands of known pattern. The running was performed in TAE 1X buffer (Tris-acetate 0.04 M and EDTA 1 mM) at 80 V and the ethidium bromide gel was photographed under UV light in a Gel Logic 112® Imaging System.

For DNA purification, the samples were incubated in water bath at 37 ºC, with DNA in a proportion of 1:1/2 RNAse (40 ng / mL; v:v), for 12 min. Afterwards, NaCl 1:10 5 M was added, followed by 2/3 of the volume of cold isopropanol, and the samples were kept at -20 ºC for 2 h. After the incubation period, the samples were centrifuged for 10 min at 14.000 rpm. The supernatant was removed and the microtubes were washed twice with 70% ethanol and once with 95% ethanol, and then centrifuged at 14.000 rpm for 2 min per washing. Afterwards, the supernatant was carefully discarded and the microtubes were maintained at ambient temperature for total ethanol evaporation; the precipitate was resuspended in 40 µL of TE buffer.

The microsatellite markers were selected based on the information available in the literature for *C. annuum* (Portis *et al*., 2007). The microsatellite primers used in the experiment consisted of 18 pairs (Table 2).

The amplification reactions were conducted in a final volume of 25 µL, containing the following reagents: 2 µL of genomic DNA, 0.2U of Taq DNA polymerase enzyme, 2.5 µmol L^{-1} of buffer of the 10X enzyme (500 mM KCl, 100 mM Tris-HCl), 1.5 µmol L^{-1} of MgCl2 (50 mM), 0.5 µmol L^{-1} of dNTP (deoxyribonucleotides), 0.4 µM of the *forward* primer, 0.5 µM of the *reverse* primer, and 17.4 µL of ultrapure water. 2 µL of DNA was applied and, later, 23 µL of the previously described mix was added.

All PCR amplifications were performed in a thermal cycler (model TC-Plus Techne Bibby Scientific Ltd®), and the amplification reactions were conducted in the following manner: 3 min at 94 ºC for initial denaturation, followed by 35 cycles, each consisting of 94 ºC for 1 min, 52-58 °C for 1 min (depending on the primer), 72 ºC for 1 min and one final extension at 72 ºC for 7 min, afterwards, temperature was reduced to l0 °C.

The amplified fragments were then separated in agarose gel at 3.5% in 1x TAE buffer and stained with ethidium bromide (10 mg/mL), at the rate of 3 µL of ethidium bromide/200 mL of TAE buffer. After the amplification, 2 µL 1x of DNA Loading Blue I 10x was added to each 10 µL of the amplified sample, then homogenizing and applying 10 µL in each gel well, and 5µL of the molecular weight standard of 1Kb Plus DNA Ladder - 1 µg/µL were utilized. The running was performed in an electrophoresis chamber at 70 v for approximately 1 hour. The gels were visualized in a Geo-Logic 212 Pro-Carestream® imaging system.

Gel images were taken for later analysis. The 1Kb Plus DNA ladder marker was used as a molecular weight reference for estimating the sizes of the amplification products. In the analyzed SSR loci, the allelic frequency in each category in all samples was classified as either present (1) or absent (0).

Joint and individual analyses of the quantitative, qualitative, and molecular variables were performed using the clustering of Ward's algorithm. Tocher's method was used based on distance matrixes. Furthermore, the non-metric multidimensional scaling (nMDS) was applied for the graphic representation in the bidimensional space of the distance matrixes. All analyses were performed with the R software, version 3.0.3 (R Development Core Team, 2014).

Table 1: Description of the qualitative traits of the parental generation of the ornamental pepper species (*Capsicum annuum* L.) used in this study

Parents	CDC	CAN	CDF	CFL	CFM
UFPB134	Green	Dark purple	Green	White	Orange
UFPB77.2	Green with purple strips	Purple	Variegated	Purple	Red

CDC – Stem color, CAN – Anthocyanin color in the stem, CDF – Leaf color, CFL – Flower color, and CFM – Color of the mature fruit.

Table 2: Microsatellite loci (SSR) and primers (forward and reverse)

Loci	SSR	Primer (Forward/Reserse)
EPMS-596	$(A)_{19}$	CTCGTGCCGTATTTCTGTCA/AAGGGCGTGTTTGGTATGAA
EPMS-642	(AT)8	CAACTTCGCGTTATTGTCCA/AGGGCGGACAAAGAAGATTT
EPMS-643	(CT)17	CCAAGATCAACTCTTACGCTAT/CCCCTCAAGAATTCCCTCCAT
EPMS-649	$(TA)_{12}$	AAGGGTTCTCGAGGAAATGC/TCAATCCCAAAACCATGTGA
EPMS-650	$(TA)_{19}$	CATGGGTGAGGGTACATGGT/AGAGGGAAGGGTTATTTGCC
EPMS-654	$(AAC)_5$	TTCCACTCTTCGAAGCACCT/GGTAGGGTTTAACACCGCCT
EPMS-657	$(AAG)_5$	CTGATCGTGGATGTGGATTG/TAGAATTGCTGTGAGTGCGG
EPMS-658	$(AAG)_5$	CCTTGAGTAGGCGCACAAAT/TTCCTCATTGCTTTTCCCAC
EPMS-677	$(ATA)_8$	ATCTGCCCTTATCGATGCAC/CCGAATTGTGGAGGAAACAT
EPMS-680	$(ATT)_6$	TGGAATTCACATGGTGAAAAA/TGAAACTTTGTGGGCTATGG
EPMS-683	$(CAA)_7$	AAATGGATCCCAACAACCAA/GGAGTTGAAAACGGTGGAGA
EPMS-694	$(CCA)_8$	CTAGTACGAGGCAGGGGAGG/CCAGATCCCGCTTTTGACTA
EPMS-703	$(GAA)_5$	AAGATTTGGCGGAGACTTCA/TGCACCAACTTTGTCTCTGC
EPMS-705	$(GAA)_5$	TCAACTAGATCCACCACGCA/TAACCCGTTGCTCACACTCA
EPMS-709	$(GAG)_6$	ACGCCGAGGACTATGATGAC/TTCTTCATCCTCAGCGTGTG
EPMS-712	$(GCA)_6$	CCACAAAGGGTTTAAGCAGC/AAGGCAGGAGCAGAGTTCAA
EPMS-924	$(CT)_6..(TA)_9..(GTA)_5$	GCCGTCGTCAGAAAAGGTAG/TGCATTTCTGTCAGAGGCTG
EPMS-925	$(AT)_8$	CTCACAAGCAGAAGTGGACC/CCCAGTAAAACTTAACCGCAC

RESULTS AND DISCUSSION

The dendrograms obtained from the dissimilarity matrix by Ward's algorithm for the quantitative, qualitative, and molecular descriptors and joint data analysis allowed to group the 44 individuals of the F_3 population into distinct groups (Figure 1), suggesting the presence of variability within the population based on the different evaluated traits. Variability is essential in the selection of individuals for generation advancement.

In the analysis of the quantitative data, the formation of a larger number of groups (five) was observed in comparison with the groups formed by using qualitative and molecular data (Figure 1a). Group I, formed by individuals 1, 3, 4, 2, and 5. Group II, formed by only one individual, 27, which possesses distinct characteristics from the remaining individuals of the population. Group III, formed by individuals 13 (41, 42, 34, 12, 13, 14, 23, 10, 9, 22, 6, 7, and 8). Groups IV (38, 39, 40, 35, 37, 21, 15, 31, 32, 30, and 33) and V (25, 26, 29, 24, 28, 11, 43, 44, 18, 19, 17, 20, 16, and 36) were formed by 11 and 14 individuals, respectively. These results suggest the existence of variability within the F_3 population, and that the genes continue segregating for the evaluated quantitative traits. The quantitative traits are, mostly, the result of the joint action of several genes, what implies in a strong environmental influence on the phenotypic expression, being of utter importance since they shall reflect the real potential of the individuals and their possibility of use in the breeding (Vieira *et al*., 2013). Detecting the variability within the population, for the quantitative traits, allows obtaining gains when practicing selection in an early generation (Silva Neto *et al*., 2014).

For the qualitative traits (Figure 1b), the formation of four groups of individuals was verified: group I formed by 11 individuals (1, 36, 29, 9, 15, 26, 34, 27, 25, 11, and 23), group II by seven (28, 10, 24, 12, 14, 37, and 39), group III formed by individuals 30, 20, 22, 19, 16, 2, 6, 21, and 31, and group IV with 17 individuals (43, 42, 40, 41, 44, 38, 13, 3, 4, 17, 8, 5, 7, 35, 33, 18, and 32). The fact that the quantitative traits formed the largest number of groups and are more discriminating in this work does not invalidate the need for the use of qualitative descriptors in studies of genetic divergence. However, a higher homogeneity of the individuals was verified for the qualitative traits of the studied F_3 population. Qualitative descriptors are important in the evaluation of plants with ornamental potential, since they are related to the visual aspect of the plant, such as the color of the stem, flowers, fruits, both mature and immature, growth habit of the plant, branching density, leaf density and color, and fruit shape (Moura *et al*., 2010), besides being of easy measurement

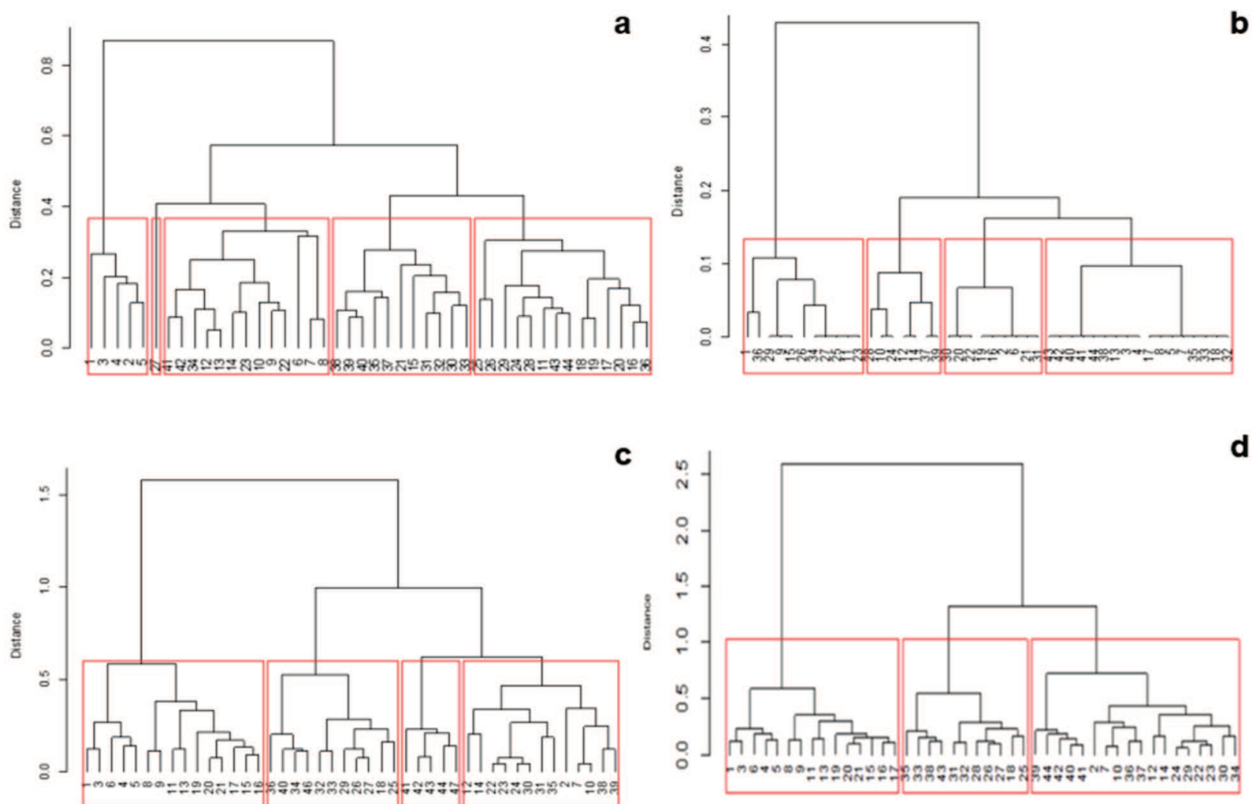

Figure 1: Dendrogram obtained by Ward's algorithm, based on the generalized distance of Mahalanobis. Quantitative (a), qualitative (b) and molecular data (c) and joint analysis (d) in an F_3 population of ornamental peppers (*Capsicum annuum* L.).

and less influenced by the environment. These descriptors should be considered in the identification of plants with greater ornamental potential, as well as in crossings for the production of ornamental pepper cultivars (Neitzke *et al*., 2010).

The dendrogram obtained with the dissimilarity matrix generated from the molecular descriptors allowed the formation of four distinct groups (Figure 1c). Group I was formed by 15 individuals (1, 3, 6, 4, 5, 8, 9, 11, 13, 19, 20, 21, 17, 15, and 16), group II was formed by 11 plants (36, 40, 34, 46, 32, 33, 29, 26, 27, 18 e 25), group III by five individuals (41, 42, 43, 44 e 47), and group IV by 13 plants (12, 14, 22, 23, 24, 30, 31, 35, 2, 7, 10, 38, and 39). This grouping resulted in the same number of groups as with the qualitative data, as well as the grouping manner of some individuals, emphasizing the importance of qualitative data analysis in ornamental peppers. Villela *et al*. (2014) reported the formation of divergent groups using microsatellite molecular markers. Ulhoa *et al*. (2014) in a study with the molecular characterization of 26 pepper lineages using microsatellite loci, identified the formation of three distinct groups.

The joint data analysis involving quantitative, qualitative, and molecular traits revealed the formation of three distinct groups (Figure 1d), presenting efficiency in quantifying the differences between individuals of the population. Group I was formed by 15 individuals (1, 3, 6, 4, 5, 8, 9, 11, 13, 19, 20, 21, 15, 16, and 17), group II by 11 (35, 33, 38, 43, 31, 32, 28, 26, 27, 18, and 25), and group III was formed by the largest number of individuals, totaling 18 plants (39, 44, 42, 40, 41, 2, 7, 10, 36, 37, 12, 14, 24, 29, 22, 23, 30, and 34) (Figure 1d); this group revealed a wide variability for the evaluated traits. These results evidence the presence of genetic variability within the population, as well as the possibility of genetic gain in later selections. The selection should be preferably applied to individuals belonging to distinct groups since those belonging to the same group are more similar than those that belong to different groups (Correa & Gonçalves, 2012).

In this population, the individuals continued to segregate, being necessary to select the plants of interest and advance the generation. Individuals 1, 23, 25, 27, 39, and 41 were the most divergent among the evaluated analyses and may be indicated for selection. Mesquita *et al*. (2016), working with an F_3 population of ornamental pepper plants, and using only quantitative characters, identified the formation of only two groups among the populations. In the present work, the formation of more groups was observed when using the joint analysis of qualitative, quantitative, and molecular data.

According to Moura *et al*. (2010), the joint data analysis allows allocating individuals in a single dendrogram, providing better data analysis and being efficient in quantifying the differences between individuals. Machado *et al*. (2015) also reported the importance of the joint data analysis, which may allow greater efficiency in the knowledge of the divergence between accessions in germplasm banks.

According to Tocher's method, distinct groups were formed among the individuals according to the evaluated traits (Table 3). For the quantitative data, the formation of seven groups was registered (Table 3a). Group I was formed by a larger number of individuals, 77, or 28% of the total of evaluated individuals. Group II was formed by only two plants, 7 and 8 (4.54 %). Group III was formed by plants 2, 5, 4, and 3 (9.09 %), and groups IV, V, VI, and VII were formed by only one genotype each: 1, 6, 21, and 27, respectively (Table 3a). This method provided a different grouping from the previous method (Ward's algorithm), with a larger number of groups, presenting the characteristic of forming groups with only one genotype each, in case of genotypes with greater dissimilarity (Vasconcelos *et al*., 2007). According to Ramalho *et al*. (2016), this method is employed along with other methods to complement the results and aid in the better distinction of the formed groups (Ramalho *et al*., 2016).

Tocher's grouping method for qualitative data allowed the formation of eight distinct groups (Table 3b). Group 1 was formed by 15 individuals (34.09% of the total of individuals in the population). Group II (22.72%). Group III (18.18%). Groups IV and V were formed by three individuals each, corresponding to 6.81% of the total of individuals evaluated for each group. Groups VI and VII were each formed by two plants, or 4.55% of the total of evaluated plants. Group VIII was formed by one individual (2.7%). According to Cruz & Regazzi (1997), the higher is the number of formed groups, the more heterogeneous is the analyzed population for the evaluated characteristics.

The largest number of individuals observed by Tocher's method corresponded to the molecular data, with the formation of 11 distinct groups (Table 3c). Group I was formed by the largest number of plants, 8 individuals (18.18%). Groups II and III grouped seven individuals each (15.91%). Group IV was formed by five individuals (11.36%). Groups V and VII were formed by four individuals, corresponding to 9.09% of the total population. Group VII was formed by individuals 1, 3, and 4, which presented genotypic similarities between each other, corresponding to 6.81% of the total of evaluated plants. Groups VIII and IX were formed by two individuals each, 31 and 35, and 12 and 14, respectively, representing 4.55% of the total of evaluated plants, whereas groups X (2) and XI (11) presented only one individual each (2.27%). There was no concordance in the separation of the individual by groups in the individual analysis of the quantitative, qualitative, and molecular data by Tocher's

method, with the molecular traits being more efficient in the separation of individuals. Microsatellites or simple sequence repeats markers may currently be considered as the most used markers for genetic studies due to their simplicity of execution and for being able to detect a high degree of genetic polymorphism, with greater consistency in the results concerning genetic similarity or divergence (Dutra Filho *et al*., 2013).

Grouping analysis by Tocher's optimization method for joint data analysis allowed to gather the 44 genotypes into ten distinct groups (Table 3d). Group I was formed by ten individuals: 24, 29, 22, 23, 36, 30, 34, 7, 10, and 37, what corresponds to 22.72% of the total of evaluated individuals. Group II was formed by five individuals: 40, 41, 42, 44, and 39, formed by 11.36% of the plants in the population. Group III was formed by seven individuals: 26, 27, 28, 25, 31, 32, and 18, representing 15.90% of the evaluated individuals. Group IV was formed by eight individuals: 20, 21, 16, 17, 15, 19, 13, and 9 (18.18%). Individuals 1, 3, 4, 5, and 6 formed group V, with 11.36% of the plants evaluated in the population. Group VI allowed to group four individuals: 38, 43, 33, and 35, corresponding to 9.09% of the evaluated plants. These individuals presented similar traits, allowing them to remain in the same group. This characteristic of this method becomes interesting since it allows to identify genetically similar individuals, and not only distinct groups (Silva *et al*., 2016). Individuals 12 and 14 formed group VII, which corresponds to 4.58% of the total of individuals. Groups VIII, IX and X were formed by only one individual each: 2, 8 and 11, respectively, corresponding to 2.27% of the total of evaluated plants. Genetic variability is the raw material for genetic breeding, being necessary for the practice of selection in the advancement of segregating generations. Therefore, the selection of individuals 1, 2, 7, 9, 12, 26, 34, 37, and 44 is recommended for representing divergences by the evaluated methods.

With the use of Tocher's method, it was possible to form a larger number of groups when compared to a dendrogram obtained by Ward's algorithm. Faria *et al*. (2012) reported that when a method is more sensible to determine the number of groups, it can verify with more efficiency the existence of discrepant individuals. This method was applied in ornamental peppers plants in the identification of the parental individuals (Neitzke *et al*., 2010; Pessoa *et al*., 2018), in an F_2 population (Silva Neto *et al*., 2014; Pessoa *et al*., 2015) and in F_3 populations (Mesquita *et al*., 2016) only for quantitative data. Few works used this method for mixed data in pepper plants.

According to the non-metric multidimensional scaling, it was observed that the individuals remained dispersed (Figure 2). For the quantitative data, individuals 1, 2, 5, 4, 3, 7, 8, 6, and 27 were distant in relation to most plants evaluated in the population. Therefore, such as in the remaining evaluated methods (Ward's Algorithm and

Table 3: Clustering of 44 individuals for the quantitative (a), qualitative (b), and molecular (c) traits and joint analysis (d) in F_3 populations of ornamental peppers, according to Tocher's method

Groups	Data / Individuals			
	Quantitativetraits (a)	**Qualitative traits (b)**	**Molecular traits (c)**	**Joint analysis (d)**
1	12, 13, 34, 11, 44, 28, 43, 24, 16, 36, 38, 32, 22, 14, 42, 41, 9, 10, 23, 37, 35, 31, 29, 39, 33, 17, 40, 18, 15, 25, 20, 19, 30, 26	2, 6, 16, 19, 21, 31, 3, 4, 13, 38, 40, 41,42, 43, 44	7, 10, 38, 39, 23, 22, 24, 30	24, 29, 22, 23, 36, 30, 34, 7, 10, 37
2	7, 8	5, 7, 8, 17, 18, 32, 33, 35, 12, 14	20, 21, 15, 16, 17, 13, 19	40, 41, 42, 44, 39
3	2, 5, 4, 3	9, 15, 29, 11, 23, 25, 27, 34	26, 27, 29, 32, 25, 33, 18	26, 27, 28, 25, 31, 32, 18
4	1	10, 24, 28	42, 43, 44, 47, 41	20, 21, 16, 17, 15, 19, 13, 9
5	6	20, 22, 30	8, 9, 5, 6	1, 3, 4, 5, 6
6	21	37, 39	34, 46, 40, 36	38, 43, 33, 35
7	27	1, 36	1, 3, 4	12, 14
8		26	31, 35	2
9			12, 14	8
10			2	11
11			11	

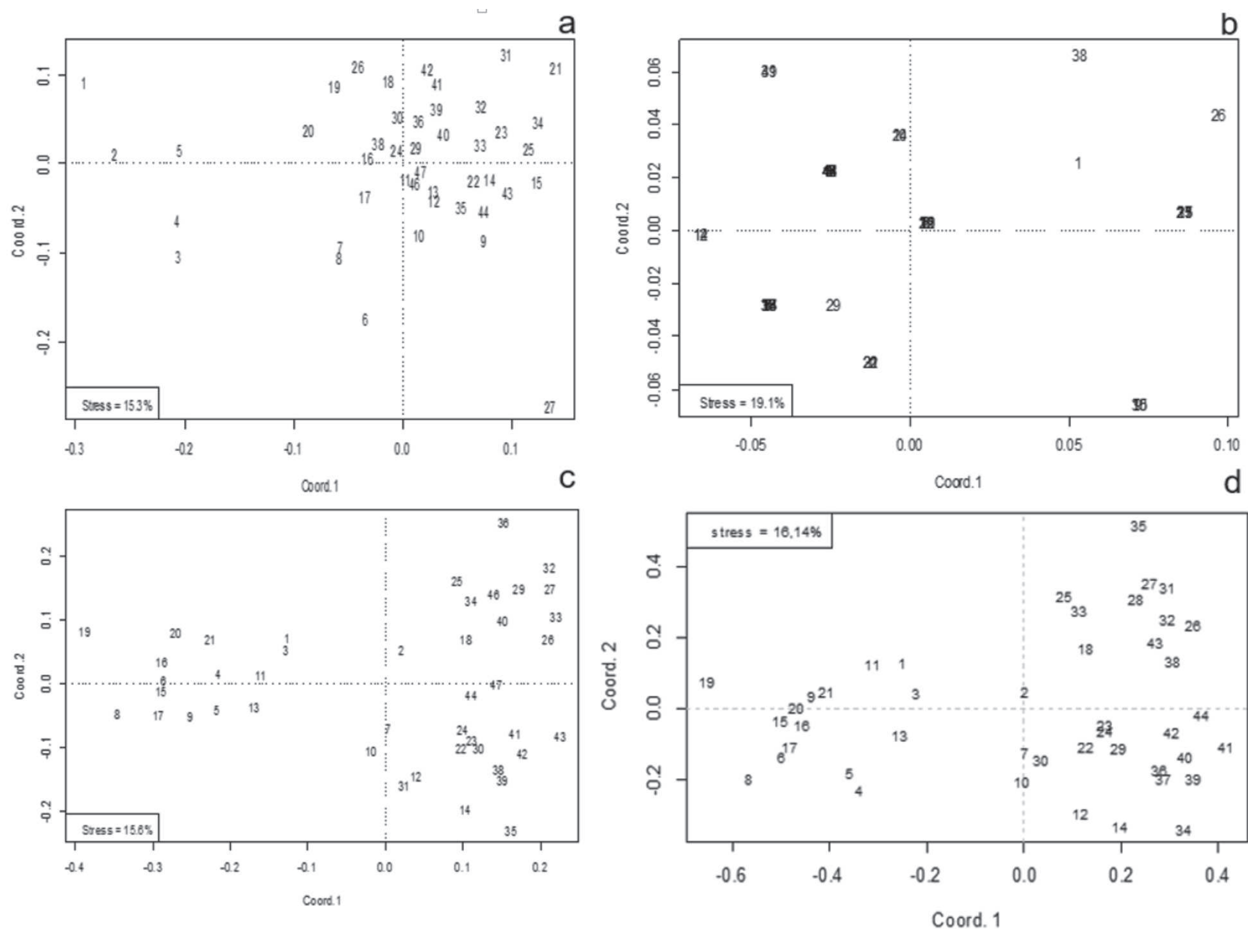

Figure 2: Graphic representation of the non-metric multidimensional scaling of the quantitative (a), qualitative (b), and molecular (c) traits and joint analysis (d) in an F_3 population of ornamental peppers (*Capsicum annuum* L.).

Tocher's method), individual 27 was separated from the remaining individuals of the population (Figure 2a), being recommended for selection to open the line in the next generation. In the qualitative analysis of the data, it was verified that individuals 29, 1, 38, and 26 were distant from the remainder, ant the 40 remaining plants in the population were together in dispersed groups in the graph (Figure 2b). When using molecular data (Figure 2c), a greater dispersion of plants 19, 36, 8, 10, and 2 was verified in the population.

The non-metric multidimensional scaling (nMDS) separated individuals 19, 8, 11, 1, 3, 13, 5, 4, 35, and 2, through joint data analysis, presenting a greater genetic divergence. Individuals 2, 8, and 11 remained isolated in this scaling, as well is through Tocher's method (Figure 2d), being recommended for selection.

By calculating Kruskal's stress for the mapping adjustment level of the non-metric multidimensional scaling for the evaluated characteristics it was possible to observe a low stress value (Figure 2). Stress values up to 20% are low and acceptable (Kruskal *et al.*, 1964). The stress values were 15.3%, 19.1%, 15.6 %, and 16.4% for the quantitative, qualitative, molecular, and joint analyses, respectively (Figure 2). These values are considered acceptable for the representation of the distances of the individuals in the graph (Sturrock & Ocha, 2000), what indicates a desired and efficient ordination in the distances of the individuals of the population using this type of analysis. The lower the stress value, the truer the position of the points in the generated image, representing the calculated distances (Clarke & Warwick, 2001).

Based on these analyses it was possible to verify a genetic divergence in the F_3 population, with variability for the quantitative, qualitative, and molecular traits, probably because the genes continued to segregate for the evaluated traits, in this generation. These results allow selecting contrasting individuals to advance generations. Thus, individuals 1, 2, 7, 8, 10, 21, 27, 29, 35, and 38 were the most divergent through this analysis, and some that coincided among the evaluated traits are indicated for selection and continuation of the breeding program of ornamental pepper plants.

CONCLUSION

There is genetic variability among the individuals of the F_3 population of *C. annuum*, verified by the individual and joint analysis of the quantitative, qualitative, and molecular traits.

The three groping methods used in this work, Ward's algorithm, Tocher's algorithm, and the non-metric multidimensional scaling (nMDS) were efficient in representing the genetic distance between ornamental pepper individuals in the population.

Tocher's grouping method is recommended to evaluate the diversity in an F_3 population of ornamental peppers for presenting a greater variability between the data for the different evaluated traits.

Individuals 1, 2, 7, 27, and 29 are indicated to open the line in an F_4 generation and continue the program of genetic breeding.

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