Characterization and genetic diversity of pepper (*Capsicum* spp) parents and interspecific hybrids

M.P.S.D. Costa[1], M.M. do Rêgo[1], A.P.G. da Silva[2], E.R. do Rêgo[1] and P.A. Barroso[1]

[1]Universidade Federal da Paraíba, Centro de Ciências Agrárias, Campus II, Areia, PB, Brasil
[2]Universidade Estadual do Sudoeste da Bahia, Campus Juvino Oliveira, Itapetinga, BA, Brasil

Corresponding authors: M.P.S.D. Costa / M.M. Rêgo
E-mail: agro30costa@gmail.com / mailson@cca.ufpb.br

ABSTRACT. Pepper species exhibit broad genetic diversity, which enables their use in breeding programs. The objective of this study was to characterize the diversity between the parents of different species and their interspecific hybrids using morphological and molecular markers. The parents of *Capsicum annuum* (UFPB-01 and -137), *C. baccatum* (UFPB-72), and *C. chinense* (UFPB-128) and their interspecific hybrids (01x128, 72x128, and 137x128) were used for morphological and molecular characterization. Fruit length and seed yield per fruit (SYF) traits showed the highest variability, and three groups were formed based on these data. CVg/CVe ratio values (>1.0) were calculated for leaf length (1.67) and SYF (5.34). The trait that most contributed to divergence was the largest fruit diameter (26.42%), and the trait that least contributed was pericarp thickness (0.33%), which was subject to being discarded. The 17 primers produced 58 polymorphic bands that enabled the estimation of genetic diversity between parents and hybrids,

and these results confirmed the results of the morphological data analyses. The principal component analysis results also corroborated the morphological and random-amplified polymorphic DNA data, and three groups that contained the same individuals were identified. These results confirmed reports in the literature regarding the phylogenetic relationships of the species used as parents, which demonstrated that *C. annuum* was closer to *C. chinense* as compared to *C. baccatum*.

Key words: *Capsicum* spp; Genetic breeding; Genetic diversity; RAPD

INTRODUCTION

The study of genetic diversity in the genus *Capsicum* is necessary, because it provides parameters for the identification of parents that produce greater heterotic effects on progeny and increase the probability of obtaining superior genotypes in segregating generations (Sudré et al., 2005; Rêgo et al., 2009). Hybridization is a breeding method that is widely used in peppers (*Capsicum* spp), and it is able to generate new varietal sources (Rêgo et al., 2011a).

The genetic diversity of *Capsicum* species can be evaluated using several characteristics, including agronomic, morphological, and molecular traits (Rêgo et al., 2011a; Barroso et al., 2012; Nascimento et al., 2012). Using morpho-agronomic characterization, markers with high heritability, easy identification, and little genotype x environment interactions should be considered, and these characteristics allow the identification, preservation, and transfer of new sources of genetic variation (Bento et al., 2007; Rêgo et al., 2009; Nascimento et al., 2012). Molecular characterization has been a useful tool for the evaluation of genetic diversity in different plant species.

Moreover, molecular characterization is an efficient form used to study the partition of genetic variability between cultivated species and their wild relatives. This is because it clarifies phylogenetic relationships, providing rational strategies for breeding, germplasm collection, preservation, and the use of genetic resources (Rêgo et al., 2011b). Among the numerous molecular marker techniques, random-amplified polymorphic DNA (RAPD) is advantageous because of its simple use, low cost, and rapid generation of results, despite the low repeatability of results (Vieira et al., 2010; Rodrigues and Costa, 2011).

Total proteins represent another low-cost marker with easy access and rapid results, and they are also used to evaluate genetic variability. In addition, they have the potential to help to classify species, and they serve as markers for interspecific hybridization studies (Murphy et al., 1990; Lanham et al., 1994).

Therefore, the objective of this study was to characterize genetic diversity using morphological traits and molecular markers associated with the parents and interspecific hybrids of peppers (*Capsicum* spp). Moreover, the study aimed to utilize these data as a tool in programs focused on the breeding of parents and generations of more promising hybrids.

MATERIAL AND METHODS

Accessions belonging to the Active Germplasm Bank of *Capsicum* from UFPB, located in Areia, PB, Brazil, were used. Regarding the morphological characterization of plants, parents of *C. annuum* (UFPB-01 and -137), *C. baccatum* (UFPB-72), and *C. chinense*

(UFPB-128) and their interspecific hybrids (01x128; 72x128, and 137x128) were characterized based on descriptors proposed by IPGRI (1995).

Morphological characterization occurred in the field using a completely randomized design with five plants and three replicates. The following traits were evaluated: plant height (PH), crown width (CW), stem length (SL), stem diameter (SD), leaf length (LL), petiole length (PtL), leaf width (LW), fruit weight (FW), fruit length (FL), largest fruit diameter (LFD), smallest fruit diameter (SFD), peduncle length (PdL), pericarp thickness (PT), seed yield per fruit (SYF), and dry matter content (DMC).

Tocher's clustering method was used to analyze genetic divergence based on the generalized Mahalanobis distance and canonical variable analysis. The relative importance of the traits for divergence was calculated using the method proposed by Singh (1981) and the canonical variable analysis.

Regarding the molecular characterization based on RAPD, the *C. annuum* parent (UFPB-01) was not examined in the analysis because of the death of the plant. Therefore, two new interspecific hybrid plants (01x128.1 and 01x128.2), derived from crosses between *C. annuum* (UFPB-01) and *C. chinense* (UFPB-128), were included.

The methodology described by Ferreira and Grattapaglia (1998), with some modifications, was employed to extract genomic DNA. Samples were subjected to polymerase chain reaction analyses, which used 17 RAPD primers (Table 1).

Table 1. Primer sequences utilized for the DNA amplification of *Capsicum* spp using the RAPD technique.

Primer	Sequence (5'-3')
UB - 01	AGACGGCTCC
UB - 02	GTTCGGAACC
UB - 03	GGGCGACTAC
UB - 04	GTGCGCAATG
UB - 05	TCGCATCCAG
UB - 06	CAGAAGCGGA
UB - 07	CACAGCGACA
UB - 08	CAAAGCGCTC
UB - 09	TCCCCATCAC
UB - 10	TGCGGGTCCT
UB - 11	CAGGATTCCC
UB - 12	GTGGAGTCAG
UB - 13	AAGTCCGCTC
UB - 14	CAGCACTGAC
UB - 15	GACAGGAGGT
UB - 16	GGCTGCAGAA
UB - 17	CACACTCCAG

The similarity matrix was obtained by utilizing the complement (1-C) of Nei and Li's (1979) similarity coefficient, and the unweighted pair group method with arithmetic average (UPGMA) hierarchical clustering method was used. All analyses were performed using the GENES program (Cruz, 2006), and a principal component analysis (PCA) was conducted based on molecular data.

RESULTS

With the exception of PH, SL, and DMC, significant differences were found for

most of the evaluated quantitative traits (based on the F-test at a 5% significance level). After analyzing the heritability (h^2) values obtained in this study, the results indicated that of the plant size-related traits, PtL exhibited the highest h^2 value (92.93%) (Table 2).

Table 2. Summary of the variance (SV) analyses for quantitative plant- and fruit-related traits in *Capsicum* parents and interspecific hybrids.

SV	PH	CW	SL	SD	LL	PtL	LW	FW
Treatment	598.43ns	823.83*	118.83ns	0.52*	6.28*	1.29*	6.76*	105.53*
h^2 (%)	50.52	68.94	39.57	73.74	89.40	92.93	91.46	97.68
CVg/CVe	0.58	0.86	0.46	0.96	1.67	2.09	1.88	3.75
CV	26.14	22.20	45.39	29.85	11.42	14.51	20.20	44.61
SV	FL	LFD	SFD	PdL	PT	SYF	DMC	
Treatment	2.22*	2.64*	0.11*	0.92*	0.036*	1752.93*	155.62ns	
h^2 (%)	98.64	97.79	88.18	96.25	96.94	98.84	65.01	
CVg/CVe	4.92	3.84	1.57	2.92	3.25	5.34	0.78	
CV	10.16	14.44	12.19	8.46	14.96	20.00	43.88	

*Significant at 5% probability based on the F test. Plant height (PH); crown width (CW); stem length (SL); stem diameter (SD); leaf length (LL); petiole length (PtL); leaf width (LW); fruit weight (FW); fruit length (FL); largest fruit diameter (LFD); smallest fruit diameter (SFD); peduncle length (PdL); pericarp thickness (PT); seed yield per fruit (SYF); dry matter content (DM).

Regarding the CVg/CVe ratio, values greater than 1.0 were found for the following variables: LL, 1.67; PtL, 2.09; LW, 1.88; FW, 3.75; FL, 4.92; LFD, 3.84; SFD, 1.57; PdL, 2.92, PT, 3.25; and SYF, 5.34 (Table 2).

Significant differences were observed in SD, which exhibited different classes (Table 3). Hybrid 01 x 128 showed a higher average (1.83 cm), however, not differing from hybrids 72 x 128, 137 x 128 and parent 128. While the parents 01 and 137 had the lowest average values (0.52 and 0.55), respectively (Table 3).

Table 3. Mean values for seven plant- and fruit size-related traits in *Capsicum* parents and interspecific hybrids.

Accession	PH	CW	SL	SD	LL	PtL	LW	FW
01	28.00ab	30.20b	14.30a	0.52c	6.61bc	1.70bc	3.12b	0.22c
72x128	68.66a	68.66a	20.66a	1.21ab	6.77bc	1.77bc	3.35b	0.93b
137x128	72.33a	86.33a	27.33a	1.46ab	7.62ab	2.51ab	4.07b	0.83b
01x128	82.66a	79.33a	20.00a	1.83a	5.32c	1.64c	2.26b	0.45b
128	82.66a	44.66a	11.00 a	0.98ab	6.73bc	1.47c	2.93b	1.21b
72	45.00ab	81.00a	14.33a	0.75b	9.27a	3.02a	6.17a	14.10a
137	29.00ab	19.60c	10.00a	0.55c	6.00c	1.69bc	2.66b	1.18b
Accession	FL	LFD	SFD	PdL	PT	SYF	DMC	
01	0.87c	0.66c	0.45c	1.59c	0.11b	17.60b	13.00a	
72x128	1.14c	1.18b	0.89b	2.14b	0.20b	2.66c	12.89a	
137x128	1.15c	1.08b	0.86b	1.55c	0.16b	3.33c	23.98a	
01x128	1.13c	1.16b	0.72b	2.04bc	0.15b	10.33c	14.85a	
128	2.02b	1.42b	0.95ab	2.18b	0.19b	40.00b	24.41a	
72	3.09a	3.22a	1.24a	3.09a	0.42a	56.00a	7.91a	
137	2.18b	0.94b	0.55c	2.02bc	0.12b	32.00b	24.20a	

Mean values followed by the same letter do not differ statistically according to the Tukey test at 5% probability. Plant height (PH); crown width (CW); stem length (SL); stem diameter (SD); leaf length (LL); petiole length (PtL); leaf width (LW); fruit weight (FW); fruit length (FL); largest fruit diameter (LFD); smallest fruit diameter (SFD); peduncle length (PdL); pericarp thickness (PT); seed yield per fruit (SYF); dry matter content (DMC).

The PT trait formed two classes, and the parent 72 stood out the most with an average value of 0.42, thus forming a separate class (Table 3).

Based on SYF, variation occurred between the accessions, and three classes were formed, which varied (on average) from three (hybrid 72x128) to 56 seeds (parent 72).

In the study of diversity, the accessions were grouped using Tocher's method, which is based on the generalized Mahalanobis distance. The parents and hybrids were gathered into three groups (Table 4), and group 1 comprised most of the evaluated genotypes, including hybrids 72x128, 137x128, and 01x128 and parent 01.

Table 4. Clustering of *Capsicum* parents and hybrids based on Tocher's method.

Group	Accession
1	72x128, 137x128, 01x128
2	128, 137
3	72

The results of the canonical variable analysis indicated that phenotypic diversity was detected between the analyzed *Capsicum* accessions, and the first three canonical variables explained 99.97% of the total variance (Table 5).

The method of Singh (1981) was used to evaluate the relative importance of the 15 quantitative traits, and only six traits contributed approximately 80.00% of the genetic diversity (Table 6). The traits that contributed the most were LFD (26.42%), SYF (15.69%), LL (15.42%), SD (6.38%), LW (5.98%), and PdL (5.43%) (Table 6).

Regarding the molecular characterization, the 17 primers utilized for DNA amplification of the seven *Capsicum* accessions resulted in 58 polymorphic bands, and each primer produced an average of 3.41 bands. Some primers did not amplify some individuals, and the UB-6 and 7 primers showed the highest amplification number. The clustering analysis, based on the RAPD polymorphic loci, revealed genetic dissimilarity, which varied from 9.62% (72x128 with 01x128.1) to 58.33% (72 with 01x128.2).

The 72x128 hybrid was only amplified by primers 7, 14, and 15. However, genotypes 137x128, 128, 72, 01x128.1, and 01x128.2 were amplified by most of the primers. According to Table 7, the smallest genetic distance (0.0962) was recorded for the pair of interspecific hybrids 72x128 and 01x128. Three additional genotypic pairs (combinations of hybrid 72x128 with hybrid 137x128, hybrid 01x128.2, and parent 128) displayed the same genetic distance (0.1633) based on data obtained using the 17 primers, which indicated that they were very close genetically. The only differences between these two pairs of genotypes were the contributions of parents 137 (*C. annuum*) and 72 (*C. baccatum*), which both contributed 50.0% of their genes in the formation of hybrids 137x128 and 72x128, respectively. However, considering the 72x128 and 137x128 hybrid pairs, it was observed that parent 128 (*C. chinense*) contributed 50.0% of its genes to the genetic distance, whereas parents 72 and 137 only contributed 25.0%. Concerning the other pair of genotypes, 72x128 with 128, parent 128 contributed 75.0% of its genes to genetic diversity, while parent 72 only contributed 25.0%. Thus, their influence on the genetic distance was even more marked, thereby increasing the similarity between the two genotypic pairs.

Table 5. Variance estimates for the canonical variables and the relative importance (eigenvectors, EV) for 15 traits evaluated in *Capsicum*.

	EV	EV (%)	Accumulated (%)	PH	CW	SL	SD	LL	PtL	LW
1	2,654,773.869	96.287	96.287	8.750	458.043	185.273	-229.594	-521.095	-107.192	113.358
2	71,613.867	2.597	96.287	-76.217	8.042	183.056	-170.353	135.926	-58.012	-3.375
3	29,983.861	1.087	99.971	-20.986	-355.637	-127.801	275.528	328.193	-29.445	-136.559
4	774.456	0.028	100.000	-34.908	96.665	77.002	-97.026	-46.869	6.007	54.670
5	0.00	0.00	100.000	0.300	-5.224	-4.668	6.954	2.124	0.3990	-1.358
6	0.00	0.00	100.000	47.468	0.682	-21.346	-60.502	-26.120	20.111	27.082
7	0.00	0.00	100.000	1.194	-2.898	-3.269	4.004	1.536	-0.581	-2.451
8	0.00	0.00	100.000	-1.849	-1.320	1.192	-0.847	0.832	0.855	2.999
9	0.00	0.00	100.000	-1.012	1.608	1.102	-0.844	-0.541	-0.629	1.277
10	0.00	0.00	100.000	0.996	0.101	-0.932	0.302	0.560	-0.522	-1.536
11	0.00	0.00	100.000	0.733	-0.022	0.366	-0.187	0.307	-0.129	0.377
12	0.00	0.00	100.000	-0.721	-1.535	0.366	1.720	0.307	-0.129	0.037
13	0.00	0.00	100.000	0.359	-7.232	-3.574	7.004	3.662	1.844	-1.949
14	0.00	0.00	100.000	-0.216	-3.862	-0.404	2.612	1.546	1.103	3.168
15	0.00	0.00	100.000	0.356	3.511	-1.425	-0.647	-4.186	-0.311	3.281

	EV	EV (%)	Accumulated (%)	FW	FL	LFD	SFD	PdL	PT	SYF	DMC
1	2,654,773.869	96.287	96.287	0.923	7.756	397.044	51.578	27.862	-7.872	90.876	-65.223
2	71,613.867	2.597	96.287	-26.092	-75.840	39.179	2.518	3.732	0.843	-7.647	15.936
3	29,983.861	1.087	99.971	17.483	129.05	-9.607	-7.334	56.219	4.303	141.897	101.684
4	774.456	0.028	100.000	1.908	-16.517	-82.135	-26.516	-18.453	-5.365	56.198	-25.596
5	0.00	0.00	100.000	-0.345	-0.544	3.815	1.436	-1.686	0.243	-1.321	0.123
6	0.00	0.00	100.000	7.312	44.896	-0.084	22.052	27.126	-4.666	15.460	52.155
7	0.00	0.00	100.000	-0.374	0.025	4.686	0.480	-2.597	0.473	-1.438	-0.006
8	0.00	0.00	100.000	0.756	-0.163	4.087	-1.924	2.766	-0.287	0.679	0.868
9	0.00	0.00	100.000	0.071	-1.231	0.571	-0.367	0.235	-0.102	1.244	-0.536
10	0.00	0.00	100.000	0.709	-0.123	0.850	0.731	-1.253	0.131	-0.193	-0.458
11	0.00	0.00	100.000	0.474	0.0958	-0.180	0.108	0.179	-0.018	0.020	0.061
12	0.00	0.00	100.000	0.047	0.095	-0.180	0.108	0.179	0.018	0.020	0.613
13	0.00	0.00	100.000	0.274	0.880	1.205	0.154	0.584	0.882	-2.091	1.517
14	0.00	0.00	100.000	0.962	4.242	5.953	-2.182	3.868	-0.182	-2.470	2.869
15	0.00	0.00	100.000	-0.298	0.959	5.017	-0.495	-1.211	4.211	1.027	-1.252

Table 6. Relative contribution of traits to diversity (Singh, 1981) based on the generalized Mahalanobis distance.

Variable	Value (%)
Plant height	0.91
Crown width	2.09
Stem length	3.48
Stem diameter	6.38
Leaf length	15.42
Petiole length	3.92
Leaf width	5.98
Fruit weight	1.53
Fruit length	6.54
Largest fruit diameter	26.42
Smallest fruit diameter	0.71
Peduncle length	5.43
Pericarp thickness	0.33
Seed yield per fruit	15.69
Dry matter content	2.67

Table 7. Similarity matrix obtained based on the complement (1-C) of Nei and Li's (1979) coefficient from RAPD data between *Capsicum* parents and specific hybrids.

Individual	72x128	137x128	128	72	137	01x128.1	01x128.2
72x128	0.0000	0.1633	0.1633	0.5405	0.2258	0.0962	0.1633
137x128		0.0000	0.2128	0.4615	0.3103	0.1875	0.2128
128			0.0000	0.5000	0.2528	0.2128	0.2667
72				0.0000	0.3412	0.4250	0.5833
137					0.0000	0.1753	0.2000
01x128.1						0.0000	0.2128
01x128.2							0.0000

The genotypic pair that showed the largest genetic distance was 72 and 01x128.1, with a distance of 0.5833. Parent 72 is *C. baccatum*, while hybrid 01x128.1 is formed from the parents of two different species (*C. annuum* and *C. chinense*, respectively).

A UPGMA clustering analysis was performed to group individuals based on the genetic diversity between the parents and hybrids using RAPD markers, and two groups were formed (Figure 1). The first group included parents 128 and 137 as well as all hybrid combinations (72x128, 01x128.1, 01x128.2, and 137x128). The second group contained a single element, parent 72.
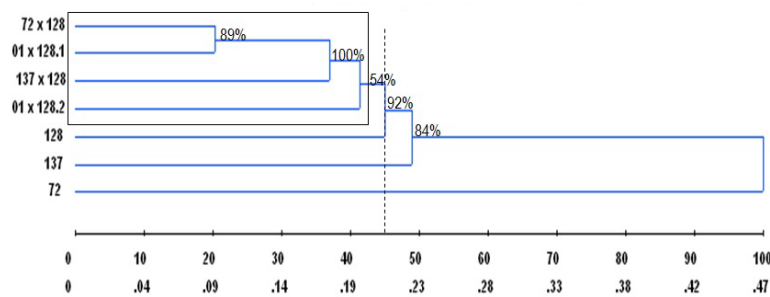
Figure 1. Genetic similarity (Nei and Li's coefficient) dendrogram that is based on the RAPD band patterns. Genetic relationships between parents and interspecific hybrids of *Capsicum* spp are shown.

The PCA results grouped the parents and interspecific hybrids according to their parentage relationship, which indicated the clustering of parents and hybrids of closer species into a larger group [e.g., parents 128 (*C. chinense*), 137, and 01 (*C. annuum*) and the hybrids originating from these parents]. However, the second group only contained parent 72 (*C. baccatum*) (Table 7).

DISCUSSION

High heritability values found in this study indicate high genetic control in the selection trait, because it reflects the genetic proportion of the phenotypic values, making it possible to obtain gains via selection. Working with *C. baccatum*, Rêgo et al. (2011b) observed traits with high h^2 values, which demonstrated high genetic control compared to the environmental effect.

Greater CVg/CVe ratio results indicated that variables could be selected, and that they could provide genetic gain. Moreover, this result agreed with the h^2 values described in Rêgo et al. (2011c) and Nascimento et al. (2012), which examined *C. baccatum* and *C. annuum*, respectively, and found values >1 for some of the traits evaluated in this study. Nascimento et al. (2012) reported that CVg/CVe values <1 indicated unfavorable conditions for selection, because the genetic variation surpassed the environmental variation.

Regarding the characteristics SD, the hybrids were superior to the parents. Furthermore, similar data were found by Geleta et al. (2005) and Nascimento et al. (2012) for this trait in studies of genetic diversity in the hybrids and parents of *C. annuum*. These authors concluded that this trait was efficient for the discrimination of hybrids from parents.

Analysis of these values indicated that the differences observed in parent 72, in relation to those characteristics LL, PtL, LW, FW, FL, LFD, SFD, and PdL were pronounced because its species (*C. baccatum*) had superior traits compared to the other species. These results corroborated those found by Rêgo et al. (2011c), which examined different *C. baccatum* accessions and found large differences in the morphological traits of mature fruits.

According to Lannes et al. (2007) and Rêgo et al. (2011a), this trait PT is important for the selection of more adequate varieties for the sale of fresh products, because they are resistant to post-harvest damage.

The lower number of seeds observed in the hybrids relative to the parents is probably due the unviable pollen of the interspecific hybrids, which contributes to a lower seed yield. It is important to note that it is extremely difficult to obtain good interspecific hybrids with regard to this trait (Rêgo et al., 2009). According to Nascimento et al. (2011), hybrid seeds do not always exhibit good vigor and germination, particularly when they originate from interspecific crosses.

In the adoption of Tocher's optimization method based on Mahalanobis's distance, the results show that although in the group 1 are included interspecific crosses, it showed that the species belonging to these groups were close, especially regarding traits related to plant and fruit size. Group 2 consisted of parents 128 (*C. chinense*) and 137 (*C. annuum*), which are two species that belong to the same phylogenetic group, while the Group 3 only included parent 72 (*C. baccatum*), which is distantly related to *C. annuum* and *C. chinense*. Depending on the objectives of the breeding program, the traits associated with the divergence of parent 72 can be improved.

By the method of canonical variables, similar data were found by Rêgo et al. (2011c), in which the first three canonical variables associated with *Capsicum* explained approximately 81.94% of the total variance. When the first three canonical variables explain more than 70.0,

there is strong evidence that the data fit a tridimensional graphic representation (Rêgo et al., 2003; Bento et al., 2007). The graphic dispersion enabled the separation of the accessions into groups, so this method could be used as a strategy to select diverging genotypes for utilization in artificial crosses (Neitzke et al., 2010). In the dispersion graph (Figure 2), the clustering was compatible with the groups composed using Tocher's method, because three groups were formed in both cases. According the canonical variables method, the traits that showed the highest eigenvectors at the last eigenvalues (which were subject to being discarded) were LL, LFD, CW, SD, PH, and LW (Table 5). However, additional methods were required to determine if these traits were actually subject to being discarded.
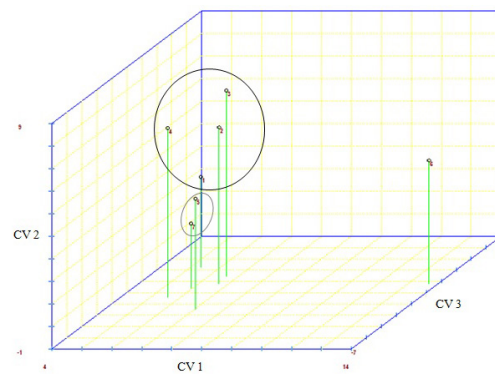
Figure 2. Graphic dispersion of scores relative to the axes, which represent the first three canonical variables (CV) that refer to the 15 examined *Capsicum* traits. 1 = 01; 2 = 72x128; 3 = 137x128; 4 = 01x128; 5 = 128; 6 = 72; and 7 = 137.

Based on studies conducted by Sudré et al. (2005), using the method of Singh, only two traits assessed in this study coincided with those described, by which examined 56 *Capsicum* accessions. The same authors found that the traits that most contributed to divergence were LFD (31.06%) and SYF (13.35%), and the traits that contributed least to genetic diversity were SFD (0.71%), PH (0.91%), CW (2.09%), PtL (3.93%), FW (1.53%), DMC (2.67%), and SL (3.48%) (Table 6), resulting in minimal importance for the clustering of the evaluated hybrids and parents. Variables that contributed a very low percentage or those that did not contribute at all to the detected variability may be discarded in future studies of genetic diversity (Rêgo et al., 2003).

Regarding the determination of divergence, the traits of least importance included PH and CW, and these results corresponded with those obtained using the canonical variables and Singh (1981) methods. Considering the examination of these hybrids and parents, these traits could be expendable in future genetic diversity studies.

Regarding the molecular characterization, the amplified bands were monomorphic, and this result likely stems from the fact that several *Capsicum* species were involved. Williams et al. (1990) reported that a single change in one nucleotide could prevent the entire amplification.

The RAPD markers revealed that the parents 137 and 72 had little influence on the genetic diversity of hybrid pair 72x128 and 137x128. This result was corroborated by the fact that a distance of 0.3103 was found when we considered the genetic diversity between the genotypic pair 137x128 and parent 137 in the distance matrix. This distance was higher than 0.1633, which indicated that the influence of parent 128 increased the similarity between parents and hybrids. Likewise, the distance was even greater (0.5405) in the genotypic pair

72x128 and 72. In their *C. annuum* study, Paran et al. (1998) found an average distance of 0.7 between the genotypic pairs belonging to this species. Genetic distances of a great magnitude allow the selection of genotypes with a broad genetic base.

The pair that showed the largest genetic distance is formed from parent 72 (*C. baccatum*) with a simple hybrid (01x128) derived of two different species (*C. annuum* and *C. chinense*, respectively). This distance was expected, since it represents the distance between a pair of parents derived from three different species (i.e., three different genomes). Similar results were observed by Thul et al. (2012) who used RAPD and SRR markers to examine 22 *Capsicum* accessions that were native to India, and the results indicated that the data obtained with these markers revealed genetic similarity values of approximately 23.0 to 99.0% and 11.0 to 96.0%, respectively, between the evaluated accessions.

Regarding a UPGMA clustering analysis, these data were consistent with the results of Ince et al. (2010) who examined 24 different *Capsicum* accessions, and the results indicated that *C. chinense* and *C. annuum* grouped together and separately from *C. baccatum*.

By multivariate analysis and using PCA, results grouped the parents and interspecific hybrids according to their parentage relationship, in addition to revealing the consistency of the PCA, which accumulated 97.13% of the diversity analyzed in the three components; these data also confirmed the morphological data that grouped the parents and hybrids into three groups (Figure 3), with parent 72 forming a single isolated group. The results also confirmed the data from the clustering analysis, which also formed the three groups. Therefore, these results provided evidence that parent 72 (*C. baccatum*) was genetically distant from the other parents and interspecific hybrids.
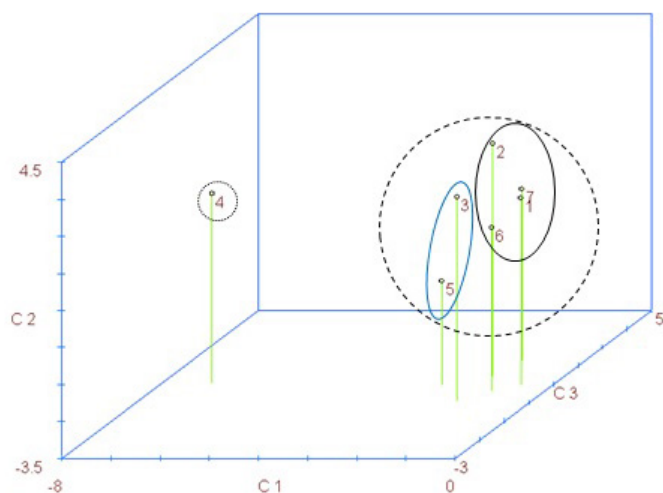
Figure 3. Graphic dispersion of the parents and interspecific hybrids based on the principal component analysis (PCA) of the genetic similarity matrix containing RAPD data. Numbers 1, 2, 6, 7, 3, and 5 form one group and correspond to hybrids 72x128, 137x128, 01x128.1, and 01x128.2 and parents 128 and 137, respectively. Parent 72 was isolated, forming the second group.

After analyzing the genetic variability of 22 pepper accessions belonging to six different species using RAPD and inter-simple sequence repeat (ISSR) molecular markers and PCA, Thul et al. (2012) observed that *C. annuum* was genetically closer to *C. chinense*,

followed in order by *C. frutescens*, *C. baccatum*, *C. luteum*, and *C. eximium*. The results obtained from the taxonomy study, the evaluation of genetic diversity of domesticated species in the Andean region, and the genetic diversity between different *Capsicum* species based on amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), and RAPD molecular markers also indicated that *C. annuum* and *C. chinense* were more closely related, and that *C. baccatum* was the most distant (Ince et al., 2010; Ibiza et al., 2012). These results confirmed the data found in the present study, since parent 72 (*C. baccatum*) formed a separate group that was isolated from the larger group containing parents 128 and 137 and hybrids 72x128, 01x128.1, 01x128.2, and 137x128.

Despite sharing the same parents (i.e., full siblings), hybrids 01x128.1 and 01x128.2 were distinct. These two hybrids, which resulted from the crossing between parents UFPB-01 (*C. annuum*) and UFPB-128 (*C. chinense*), bear fruits of different colors (red and yellow fruit, respectively). These morphological results also confirmed the data obtained from the RAPD marker using the primer UB-6, which was used to differentiate the two hybrids (data not shown).

In conclusion, the morphological and molecular data efficiently determined the genetic diversity between parents and interspecific hybrids. Moreover, the UPGMA clustering and PCA analyses separated the parents and interspecific hybrids into three groups.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

REFERENCES

Barroso PA, Rêgo ER, Rêgo MM, Nascimento KS, et al. (2012). Analysis of segregating generation for components of seedling and plant height of pepper (*Capsicum annuum* L.) for medicinal and ornamental purposes. In: XXIV International Eucarpia Symposium Section Ornamentals: Ornamental Breeding Worldwide, 953: 269-275.

Bento CS, Sudré CP, Rodrigues R, Riva EM, et al. (2007). Descritores qualitativos e multicategóricos na estimativa da variabilidade fenotípica entre acessos de pimentas. *Sci. Agraria* 8: 149-156. http://dx.doi.org/10.5380/rsa.v8i2.8379

Cruz CD (2006). Programa Genes: versão Windows; aplicativo computacional em genética e estatística. *Viçosa*: *UFV*.

Ferreira ME and Grattapaglia D (1998). Introdução ao uso de marcadores moleculares em análise genética. Brasília.

Geleta LF, Labuschagne MT and Viljoen CD (2005). Genetic variability in pepper (*Capsicum annuum* L.) estimated by morphological data and amplified fragment length polymorphism markers. *Biodivers. Conserv.* 14: 2361-2375. http://dx.doi.org/10.1007/s10531-004-1669-9

Ibiza VP, Blanca J, Cañizares J and Nuez F (2012). Taxonomy and genetic diversity of domesticated *Capsicum* species in the Andean region. *Genet. Resour. Crop Evol.* 59: 1077-1088. http://dx.doi.org/10.1007/s10722-011-9744-z

Ince AG, Karaca M and Onus AN (2010). Genetic relationships within and between *Capsicum* species. *Biochem. Genet.* 48: 83-95. http://dx.doi.org/10.1007/s10528-009-9297-4

International Plant Genetic Resources Institute - IPGRI (1995). Descritores para Capsicum *(Capsicum spp)*. Roma, IPGRI.

Lanham PG, Forster BP, McNicol P, Moss JP, et al. (1994). Seed storage protein variation in Arachis species. *Genome* 37: 487-496. http://dx.doi.org/10.1139/g94-068

Lannes SD, Finger FL, Schuelter AR and Casali VWD (2007). Growth and quality of Brazilian accessions of *Capsicum chinense* fruits. *Sci. Hortic. (Amsterdam)* 112: 266-270. http://dx.doi.org/10.1016/j.scienta.2006.12.029

Murphy RW, Sites JR and Buth DG (1990). Proteins I: isozyme electroforesis. In: Hillis DM, Moritz C. *Molecular Systematics*. Sunderland: Sinauer Associates. 45-26.

Nascimento MF, Rêgo ER, Rêgo MM, Nascimento NFF, et al. (2011). Vigor e germinação de sementes híbridas de pimenteiras ornamentais. *Rev. Bras. Hortic. Ornam.* 17: 51-56.

Nascimento NFF, Rego ER, Nascimento MF, Finger FL, et al. (2012). Heritability and variability of morphological traits in a segregating generation of ornamental pepper. In: XXIV International Eucarpia Symposium Section Ornamentals: Ornamental Breeding Worldwide, 953: 299-304.

Nei M and Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* 76: 5269-5273. http://dx.doi.org/10.1073/pnas.76.10.5269

Neitzke RS, Barbieri RL, Rodrigues WF, Corrêa IV, et al. (2010). Dissimilaridade genética entre acessos de pimenta com potencial ornamental. *Hortic. Bras.* 28: 47-53. http://dx.doi.org/10.1590/S0102-05362010000100009

Paran I, Aftergoot E and Shifriss C (1998). Variation in *Capsicum annuum* revealed by RAPD and AFLP markers. *Euphytica* 99: 167-173. http://dx.doi.org/10.1023/A:1018301215945

Rêgo ER, Rêgo MM, Cruz CD, Finger FL, et al. (2003). Genetic diversity analysis of peppers: a comparison of discarding variables methods. *Crop Breed. Appl. Biotechnol.* 3: 19-26. http://dx.doi.org/10.12702/1984-7033.v03n01a03

Rêgo ER, Rêgo MM, Cruz CD, Finger FL, et al. (2009). Diallel study of yield components and fruit quality in chilli pepper (*Capsicum baccatum*). *Euphytica* 168: 275-287. http://dx.doi.org/10.1007/s10681-009-9947-y

Rêgo ER, Rêgo MM, Matos IWF and Barbosa LA (2011a). Morphological and chemical characterization of fruits of *Capsicum* spp. accessions. *Hortic. Bras.* 29: 364-371. http://dx.doi.org/10.1590/S0102-05362011000300018

Rêgo ER, Rego MM and Farias-Filho LP (2011b). Genetic diversity in pepper (*Capsicum* spp.) by RAPD marker. In: XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2011): III International Symposium 918: 341-347.

Rêgo ER, Rêgo MM, Cruz CD, Finger FL, et al. (2011c). Phenotypic diversity, correlation and importance of variables for fruit quality and yield traits in Brazilian peppers (Capsicum baccatum). *Genet. Resour. Crop Evol.* 58: 909-918. http://dx.doi.org/10.1007/s10722-010-9628-7

Rodrigues R and Costa FRB (2011). Marcadores moleculares em pimenteira. In: Produção, genética e melhoramento de pimenta (*Capisicum* ssp) (Rêgo ER, Finger FL and Rêgo MM, eds.). Recife, 137-163.

Singh D (1981). The relative importance of characters affecting genetic divergence. *Indian J. Genet. Plant Breed.* 41: 237-245.

Sudré CP, Rodrigues R, Riva EM, Karasawa M, et al. (2005). Divergência genética entre acessos de pimenta e pimentão utilizando técnicas multivariadas. *Hortic. Bras.* 23: 22-27. http://dx.doi.org/10.1590/S0102-05362005000100005

Thul ST, Darokar MP, Shasany AK and Khanuja SP (2012). Molecular profiling for genetic variability in *Capsicum* species based on ISSR and RAPD markers. *Mol. Biotechnol.* 51: 137-147. http://dx.doi.org/10.1007/s12033-011-9446-y

Vieira EA, Freitas Filho IJF, Bellon GF, Fonseca GKG, et al. (2010). Caracterização molecular e variabilidade genética de acessos elite de mandioca para fins industriais. *Cienc. Rural* 40: 2467-2471. http://dx.doi.org/10.1590/S0103-84782010001200005

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, et al. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535. http://dx.doi.org/10.1093/nar/18.22.6531