Morphoagronomic and molecular profiling of *Capsicum* spp from southwest Mato Grosso, Brazil

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ABSTRACT. The genus *Capsicum* ranks as the second most exported vegetable in Brazil, which is also considered to be a center of diversity for this genus. The aim of this study was to rescue genetic variability in the genus *Capsicum* in the southwest region of Mato Grosso, and to characterize and estimate the genetic diversity of accessions based on morphoagronomic descriptors and inter-simple sequence repeat molecular markers. Data were obtained following the criteria of the International Plant Genetic Resources Institute, renamed Bioversity International for *Capsicum*. Data were analyzed using different multivariate statistical techniques. An array of binary data was used to

analyze molecular data, and the arithmetic complement of the Jaccard index was used to estimate the genetic dissimilarity among accessions. Six well-defined groups were formed based on the morphological characterization. The most divergent accessions were 142 and 126, with 125 and 126 being the most similar. The groups formed following agronomic characterization differed from those formed by morphological characterization, and there was a need to subdivide the groups for better distinction of accessions. Based on molecular analysis, accessions were divided into two groups, and there was also a need to subdivide the groups. Based on joint analysis (morphological + agronomic + molecular), six groups were formed with no duplicates. For all groups, the cophenetic correlation coefficient was higher than 0.8. These results provide useful information for the better management of the work collection. All correlations between the combined distance matrix were significant by the Mantel test.

Key words: Pepper; Molecular analysis, ISSR; Multivariate analysis; Genetic variability; Brazil

INTRODUCTION

The genus *Capsicum*, which includes different varietal groups of peppers and chilies, has significant economic importance, and its fruits are used worldwide as fresh fruit, condiments, and spices. Their fruits also have ornamental/medicinal uses, lacrimogenic effects, and are an important source of vitamins A and C (Bosland and Yotava, 2000; Rêgo et al., 2012). The genus *Capsicum* is a member of the Solanoideae subfamily, which is believed to have its ancestral origins in the central tropical region of South America, in what is now Bolivia (Olmstead et al., 1999). *Capsicum* is important in defense against free radicals, and the consumption of these species can help in the prevention of chronic degenerative diseases, including cancer, cardiovascular disease, cataracts, and immune system dysfunction (Davis et al., 2007; Ogiso et al., 2008).

Currently, 38 species of *Capsicum* have been reported (USDA-ARS, 2011). The highest number of species (13) is concentrated in several regions of Brazil and the country is one of the largest centers of distribution of the genus, where representative forms of domesticated, semi-domesticated, and wild species can still be found (Rêgo et al., 2012).

In Brazil, peppers and chilies are grown by small farmers. The *Capsicum* production area is over 12,000 ha, of which 5000 ha contain peppers and the major producing States are Minas Gerais, São Paulo, Goiás, Ceará, and Rio de Janeiro. The annual yield is 75,000 ton/ha and productivity varies in the range of 10-ton/ha on small farms with an area ranging from 0.5 to 10 ha (Ribeiro et al., 2007). The *Capsicum* market in Brazil involves more than US$62.111 million per year, and the production of these species is growing. In 2008, Brazilian exports of *Capsicum* reached 9.22 tons, with a corresponding value of US$14.582,608, positioning itself as the second most exported vegetable in Brazil (Reifschneider and Ribeiro, 2008).

For these reasons, plant genetic resources are considered a world heritage site of incalculable value, and their loss is an irreversible process, which affects global food security (Gomes, 2009). Therefore, the collection and conservation of plant genetic resources is of high importance.

Genetic diversity can be assessed simultaneously based on various characteristics, and the use of measurements and dissimilarity is recommended (Cruz and Carneiro, 2003). A convenient and efficient way of obtaining such measurements is through cluster analysis, which aims to group individuals such that there is maximum homogeneity within the group and maximum heterogeneity between groups (Johnson and Wichern, 1992; Cruz and Regazzi, 2001). The most widely used clustering methods are those involving optimization and hierarchical clustering (Cruz et al., 2011).

This study aimed to rescue genetic variability in the genus *Capsicum* in the Mato Grosso southwest region through the implementation of a *Capsicum* working collection, and thus, to ensure the preservation of these accessions for future use. In this study, the accessions collected were characterized based on morphoagronomic descriptors and inter-simple sequence repeat (ISSR) molecular markers, and the genetic diversity of the accessions studied was estimated.

MATERIAL AND METHODS

Properties and morphoagronomic characterization of collections

Units of the *Capsicum* collection have been identified and cataloged through visits to agricultural shops, business premises, and by identifying other *Capsicum* growers in the southwest region of the State of Mato Grosso, in the cities of Cáceres, São José dos Quatro Marcos, Curvelândia, and Mirassol d'Oeste, and nearby Bolivia (Corixa). A total of 21 *Capsicum* accessions were studied for morphological, agronomic, and molecular characterization. In turn, a joint analysis (morphological + agronomic + molecular) of these 21 accessions was performed. Seventy descriptors were used, consisting of 24 for the vegetative part, 41 for the inflorescence and fruit, and 5 for the seed. The accessions were characterized by specific morphoagronomic descriptors for *Capsicum*, as proposed by Bioverstity International (IPGRI, 1995).

The field experiment was conducted in the area of UNEMAT (State University of Mato Grosso), Cáceres - MT. The study environment is characterized, according to the Köppen classification, by a hot and humid tropical climate, with a dry winter. The average annual temperature of Cáceres is 26.24°C. Here, the rainy season lasts for 4 months (December to March) and the dry season lasts for 8 months (April to November). The average annual water deficit is 400.30 mm and the water surplus is 147.03 mm. The city of Cáceres is located in the southwest region of Mato Grosso, between the latitudes 15° 27' and 17° 37' south, and the longitudes 57° 00' and 58° 48' west, with an area of 24,398.399 km^2 at an altitude of 118 m (Neves et al., 2011).

Seedlings were produced using protected cultivation and accessions were sowed in expanded polystyrene trays (128 cells) containing Plantmax® substrate. Plants were grown in a greenhouse and treatments were performed as recommended for the crop. One month from seedling emergence, the seedlings were planted in beds with spacing of 0.8 m between plants and 1.2 m between rows. The seedlings were placed in an experimental design of randomized blocks, with three replications, and three plants per plot. Cultural practices were performed and agricultural chemicals were applied as recommended for the crop. The drip irrigation system was adopted using auto-compensating drippers.

Molecular characterization

Molecular characterizations were performed in the Laboratory of Plant Breeding (LGMG)/Molecular Markers of the Universidade Estadual do Norte Fluminense Darcy Ribeiro, in Campos dos Goytacazes, RJ, Brazil.

Samples were prepared following the collection of young, healthy leaves in the active growth phase. Leaves from each accession were wrapped in aluminum foil, identified, and immediately plunged into liquid nitrogen to ensure that DNA was intact. Once in the laboratory, this material was macerated in liquid nitrogen to form a very fine powder.

About 100 mg macerated tissue was transferred to 1.5-mL tubes and immersed in liquid nitrogen for DNA extraction according to the protocol described by Sharma et al. (2008), with modifications as described below. Samples were placed in tubes and 1 mL preheated extraction buffer was added, containing 2% CTAB; 2.0 mM NaCl; 20 mM EDTA; 100 mM Tris-HCl, pH 8.0; 2% PVP; and 2.0% mercaptoethanol. The latter two components were needed to remove the phenolic compounds. Next, 5 µL proteinase K (10 mg/mL) was added to each sample. Samples were then incubated at 37°C for 30 min, stirred gently every 10 min, and incubated at 65°C for a further 30 min. Then, the samples were centrifuged at 8000 *g* for 10 min. The supernatant (about 800 µL) was transferred to a new tube and an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added for deproteinization. Samples were subjected to gentle inversions for 10 min until they became turbid. The organic phase was separated by centrifugation at 8000 *g* for 10 min.

The supernatant was transferred to a new tube and 200 µL NaCl 2.0 M containing 4% PEG was added to remove proteins and recover DNA; the samples were then incubated for 15 min at 4°C. The material was centrifuged at 8000 *g* for 10 min. Nucleic acids were precipitated by adding two-thirds (400 µL) of the cold isopropanol volume, and incubating for 20 min at -70°C. The precipitate was sedimented by centrifugation at 8000 *g* for 10 min. The supernatant was discarded and the precipitate was washed twice with 200 µL 75% ethanol containing ammonium acetate to remove salts (between each wash, the material was centrifuged at 8000 *g* for 5 min).

DNA was quantified using a spectrophotometer (NanoDrop®). Subsequently, the DNA was diluted (5 ng/mL) for use in polymerase chain reactions (PCR).

Amplification reactions were completed in a final volume of 19 µL containing the following reagents: 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 2.4 mM $MgCl_2$; 100 µM each dNTP; 0.4 µM primer oligonucleotides; 5 ng genomic DNA; and 0.75 U Taq DNA polymerase. A total of 2 µL DNA was added to the rection mixture. PCR (GeneAmp PCR System 9700 Thermal cycler - Applied Biosystems) was conducted as follows: 3 min at 94°C for initial denaturation, followed by 40 cycles, each consisting of 94°C for 1 min, 40°-55°C for 1 min (depending on the primer used), 72°C for 3 min, and a final extension at 72°C for 7 min. The amplified fragments were then separated on 1.5% agarose gel, stained with Gel Red, and subjected to UV light to visualize the results using a Photodocumentor Minibis Pro - Bio-Imaging System). Gel images were captured for later analysis.

Statistical analysis

For analysis of the morphoagronomic data, the Genes software (Cruz, 2006) was used to obtain the dissimilarity matrix, and the R program (http://www.rproject.org) was

used to generate the dendrogram. Roots were grouped using the unweighted paired group method using arithmetic averages (UPGMA) and validation was provided by the cophenetic correlation coefficient.

To analyze molecular data, bands were visually assessed and the results were used to generate a matrix of binary data, which was subsequently used to calculate the dissimilarity matrix, wherein 1 corresponded to the presence of a band; 0, to the absence of a band; and 2, when it was not possible to determine the presence of a band or when it was not possible to amplify a given accession using a particular primer.

RESULTS

Collection

The fruits of *Capsicum* spp collected from farms had different shapes, colors, and sizes; therefore, large morphological variability was observed and is shown in Table 1.

Table 1. Identification of *Capsicum* spp accessions, their collection numbers, common name, and origin (Cáceres, MT, 2014).

Accession	Species	Common name	Origin	Locality
93	*C. chinense*	Frying pepper	Prop. Roberto	MT
117	*C. chinense*	Pepper	Prop. Antônio and Tereza	MT
119	*C. frutescens*	Pepper	Prop. Antônio and Tereza	MT
120	*C. chinense*	Pepper	Prop. Antônio and Tereza	MT
124	*C. Chinense*	Smelling pepper	Prop. Ecio Sampaio	MT
125	*C. chinense*	Red ('Goat') pepper	Prop. Esposa Cazuco	MT
126	*C. chinense*	Pepper	Prop. Antonio Boger	MT
127	*C. chinense*	Pepper	Prop. Antonio and Tereza	MT
129	*C. chinense*	Brave pepper	Prop. Adonias Ribeiro	MT
132	*C. chinense*	Smelling pepper	Prop. Antonio Boger	MT
133	*C. baccatum* var. *pendulum*	Girl Finger pepper	Prop. Pedro Camargo	MT
135	*C. chinense*	Malagueta pepper	Prop. Elcio S. Filho	MT
138	*C. baccatum* var. *pendulum*	Girl Finger pepper	Prop. Elcio Sampaio Filho	MT
140	*C. chinense*	Malagueta pepper	Prop. Roberto	MT
141	*C. chinense*	Bird pepper	Prop. Maria Menezes	MT
142	*C. chinense*	Smelling pepper	Prop. Antonio Boger	MT
144	*C. chinense*	Yellow ('Foolish') pepper	Prop. Arlindo Brasdenut	MT
145	*C. chinense*	Smelling pepper	Prop. Aparecido	MT
146	*C. baccatum* var. *pendulum*	Chilean pepper	Prop. Maria Menezes	MT
151	*C. chinense*	Sweet pepper	Prop. Valdemar G.	MT
152	*C. annuum* var. *glabriusculum*	*Capsicum* Ireasunes red		MT

Based on conversations with farmers, it was noted that, in all cases, the cultivated *Capsicum* accessions were provided by family members, or by neighboring farmers, representing the exchange of germplasm in this manner.

Morphoagronomic characterization

Twenty-one accessions were used to estimate genetic diversity based on the morphological descriptors of *Capsicum*. Variation was found for all studied characters and a weighted index was used for this analysis. A dendrogram was generated based on morphological descriptors (Figure 1), and a cut was performed at a distance of 0.31, whereas a point of abrupt change provided the formation of five groups.

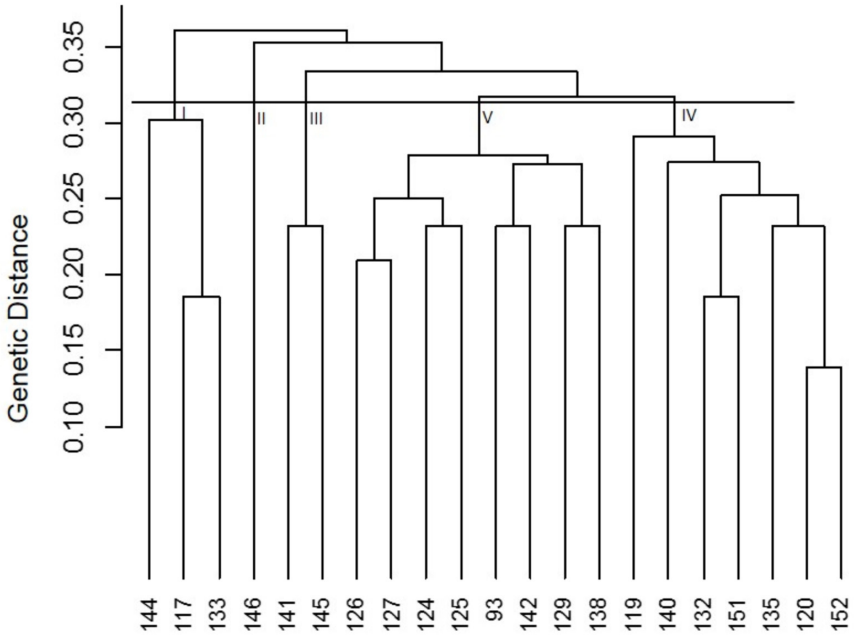

Figure 1. Dendrogram obtained by the unweighted pair group method with arithmetic (UPGMA) from the dissimilarity matrix expressed by the weighted index between 21 *Capsicum* accessions, by morphological descriptors. Cáceres, MT, 2013.

The data were grouped by the UPGMA method. The cophenetic correlation between data on the matrix of distance and grouping was 0.81. Group I consists of three accessions, which are accessions 144, 117, and 133, and despite being from different species, this group is represented by broad leaves and large fruit. Group II consists of only one accession (accession 146), which belongs to the species *C. baccatum* var. *pendulum*, and was grouped alone owing to the presence of yellow-colored anthers, white-colored corolla, and small fruit. Group III consists of the accessions 141 and 145, which have small fruits, yellow coloration, and erect flower position. Group IV contained eight accessions (126, 127, 124, 125, 93, 142, 129, and 138), which possessed different characteristics such as leaf size, flower color, and fruit shape. Group V was characterized by the accessions 119, 140, 132, 151, 135, 120, and 152, which each bore red fruit.

The most divergent accessions are 142 and 126; however, there were from the same locality, and the absence of crossing was noted. In turn, accessions 125 and 126 are the most similar, even though they were obtained from distinct properties.

Agronomic characterization

The UPGMA method was used to analyze the agronomic data, and clustering is shown in the dendrogram in Figure 2. A cophenetic correlation of 0.82 was obtained, providing the formation of three groups, with a cut of 1.5 being used.

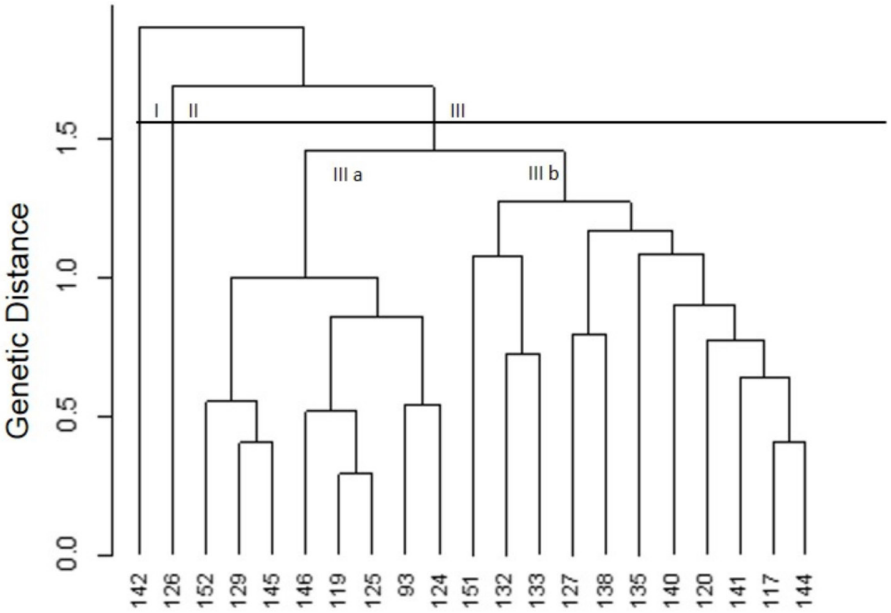

Figure 2. Dendrogram obtained by the UPGMA from the dissimilarity matrix expressed by the average weighted Euclidean distance between 21 *Capsicum* accessions, by agronomic descriptors. Cáceres, MT, 2013.

Group I consisted of one accession, which belongs to the species *C. chinense*. Accession 126 was allocated to Group II. The remaining 19 accessions were allocated to the third group, which accounted for approximately 93% of all accessions. Hence, it is difficult to analyze divergence between accessions because most of them formed a single group. Therefore, there was a need to cluster the third group into subgroups to better discriminate between accessions. In this way, accessions 152, 129, 145, 146, 119, 125, 93, and 124 make up subgroup III a, and subgroup III b contains the accessions 151, 132, 133, 127, 138, 135, 140, 120, 141, 117, and 144.

Molecular analysis

Primers were selected and evaluated based on the number of bands generated and the polymorphism observed for these bands. The most polymorphic primer was UENF 43, which generated eight bands, followed by the primers UENF 07 and UENF 13, which generated six polymorphic bands each (Figure 3).

Based on the Jaccard index, the most distant genotypes were found to be 142 and 126, while accessions 125 and 126 were identified as the most similar.

A dendrogram based on data generated from the ISSR marker (Figure 4) was obtained, with a cut being made at a distance of 0.50, taking into consideration the point of abrupt change, which resulted in the formation of two groups. This made it possible to discriminate between accessions. Similarly, accession 142 remained isolated in Group I, and the other accessions were placed in Group II.

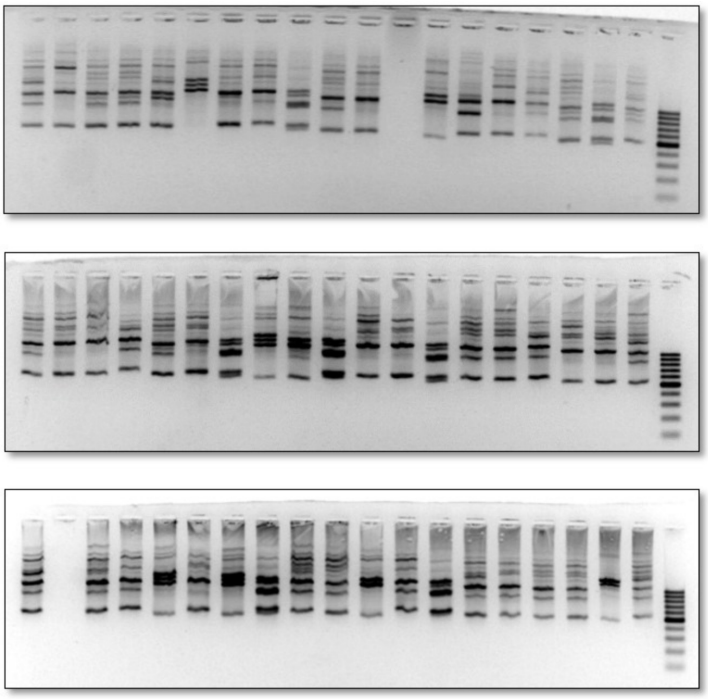

Figure 3. Profile of an ISSR (inter-simple sequence repeat) gel using the UENF 43 primer for 21 accessions of *Capsicum* spp. Cáceres, MT, 2013.

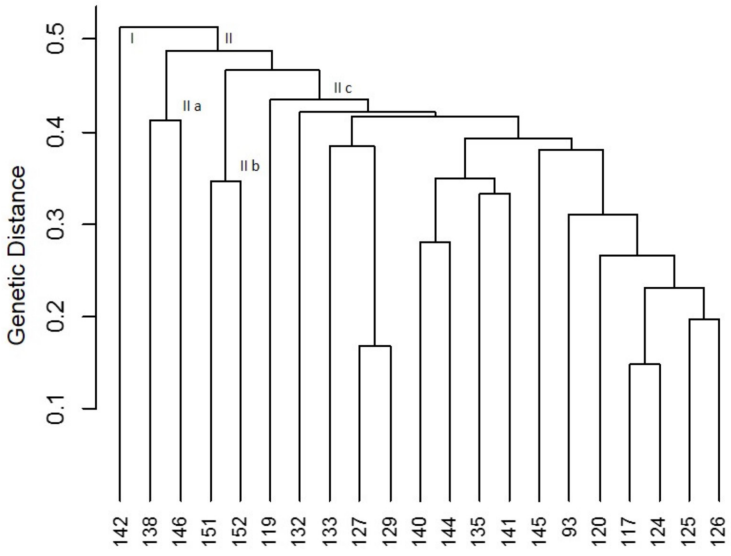

Figure 4. Dendrogram obtained by the UPGMA method from the dissimilarity matrix expressed by the arithmetic complement of Jaccard between 21 *Capsicum* accessions, by ISSR marker. Cáceres, MT, 2013.

To improve analysis, the second group was divided into three subgroups. Subgroup II a was formed by accessions 138 and 146. Accessions 151 and 152, despite belonging to different species, were grouped in subgroup II b. A further subgroup (II c) containing 16 accessions was formed and included accessions 119, 132, 133, 127, 129, 140, 144, 93, 120, 117, 124, 125, and 126.

Accession 142 was found to separate from the others and was allocated to a specific group both for ISSR markers and for the agronomic descriptor, which demonstrates a good correlation between analyses.

All 17 molecular markers showed polymorphism. These data show that there was genetic diversity between accessions studied.

Joint analysis

A dendrogram was obtained based on the joint analysis of data generated by morphological, agronomic, and molecular traits (Figure 5), which revealed a cophenetic correlation coefficient of 0.81.

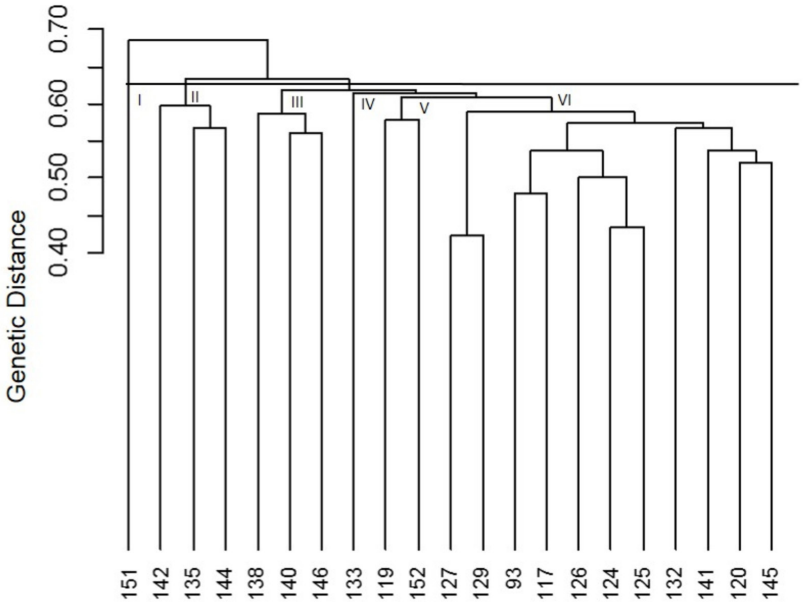

Figure 5. Dendrogram obtained by the UPGMA method from the dissimilarity matrix expressed by Gower distance, among 21 *Capsicum* accessions, by agronomic, morphological, and molecular descriptors. Cáceres, MT, 2013.

Considering a cut-off of 0.61, six groups were formed. Group I consisted of one accession, which was 151. Accessions 142, 135, and 144 composed Group II. Group III contained the accessions 138, 140, and 146, and Group IV contained only accession 133. Group V contained accessions 119 and 152, and Group VI was formed by 11 accessions, which were 127, 129, 93, 117, 126, 124, 125, 132, 141, 120, and 145.

Comparison of matrices

The four distance matrices were compared using the correlation of matrices (Mantel, 1967). The correlation values between matrices were as follows: joint with molecular, 0.81; joint with agronomic, 0.21; joint with morphological, 0.21; molecular with agronomic, 0.12; molecular with morphological, -0.04; agronomic with morphological, -0.02 (Table 2). All correlations between the combined distance matrix were significant by the Mantel test (1967). No other correlations were significant, highlighting the need to analyze genetic divergence by studying different types of characters.

Table 2. Correlation between matrices of joint distance.

Matrices	Joint	Molecular	Agronomic	Morphological
Joint	-			
Molecular	0.81**(++)	-		
Agronomic	0.21**(++)	0.12	-	
Morphological	0.21**(++)	-0.04	-0.02	-

++: Significant at 1% probability by the Mantel test based on 1000 simulations.

DISCUSSION

Using the Tocher and UPGMA methods of grouping when studying the genetic diversity among 23 accessions of cultivated *Capsicum* spp, Monteiro et al. (2010) reported that *C. chinense* was the most divergent of all groups, with the largest intragroup distances. This is consistent with the results obtained in the present study, which showed a high number of accessions in the same group.

Generally, fruit size and pungency descriptors were found to be the characteristics with the greatest variability; therefore, these can be considered important descriptors for the clustering of accessions, which contribute to the differentiation of groups. All traits studied contributed to the genetic dissimilarity of the accessions. Fruit size and flower color were important for the differentiation of groups, while the leave shape contributed the least to the observed divergence.

In a study on genetic dissimilarity among 17 accessions of *Capsicum* spp peppers, Neitzke et al. (2010) found similar results when verified the formation of four distinct groups by Tocher method for quantitative data of *Capsicum* spp.

The results of the present study show that considerable genetic diversity between the pepper hybrids studied could be detected by ISSR markers, which could be used for the conservation of genetic resources for new hybrids and crop breeding through assisted selection markers. The high variability of *Capsicum* accessions is maintained by farmers in the southwest region of Mato Grosso State. In the molecular characterization, the ISSR marker showed marked differences and polymorphism among the studied accessions, and no duplicates were detected.

For joint analysis, the value generated by the combination of traits (morphological, agronomic, and molecular) was greater than that found for the correlation between matrices generated individually. Thus, joint analysis of the data was more efficient at determining the genetic relationships among *Capsicum* accessions.

Corroborating the present findings, Vieira et al. (2007) obtained high Mantel

correlation values when studying 19 wheat genotypes and using data from molecular and quantitative morphological markers. Those authors observed moderate-to-high correlation between the matrices of the joint analysis (molecular and quantitative markers) and the quantitative and molecular arrays. Nonetheless, unlike this study, Silva et al. (2009) showed significant correlations only in the joint distance matrix, with the molecular array (0.55). Silva et al. (2009) also noted that when considering the association between morphological and molecular markers, the latter provides a much larger sample of the genome than analysis with morphological characters. Therefore, the ISSR marker makes it possible to evaluate different parts of the accession when evaluating the genetic diversity of *Capsicum*. According to Lefebvre et al. (2001) and Máric et al. (2004), the different number of morphological and molecular markers used also hinders the association. In all analyses, one can observe the absence of duplicates in the UNEMAT work collections, a result that allows the use of these materials in breeding programs of *Capsicum*.

The morphological and agronomic characterizations, as well as the molecular analysis through ISSR markers, were efficient to estimate the diversity and the genetic variability among the *Capsicum* spp accessions studied.

Conflicts of interest

The authors declare no conflict of interest.

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