Morpho-agronomic characterization and genetic divergence among pepper accessions[1]

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ABSTRACT

The germplasm characterization and the analysis of genetic divergence are fundamental requirements to determine the genetic diversity available and peppers have considerable variability not most explored and quantified. Thus, the aim of this study was to characterize morphological and agronomically pepper accessions and their genetic divergence. The experiment was conducted in a greenhouse of the Department of Agronomy of the Universidade Federal Rural de Pernambuco, Recife, between the months of December 2014 to June 2015. Thirteen pepper accessions in pots were sown, the experimental design was a randomized block with six replications, and the experimental unit consisted of three pots. Seedling, leaves, plant and fruit characteristics were evaluated. For the analysis of genetic divergence, the Mahalanobis distance and the UPGMA method were used. It was observed the formation of three distinct groups, being fruit width the trait that most contributed to the genetic discrimination of the accessions. Group I presented larger fruits, Group II showed heavier and wider fruits and Group III had mostly smaller, lighter and thinner fruits. There were differences for most of the evaluated characteristics, showing great morphological and agronomic variability among the accessions. The peppers have a high potential to be exploited in breeding.

Keywords: *Capsicum*; germoplasm; genetic breeding; phenotype; variability.

INTRODUCTION

Peppers of the genus *Capsicum* are vegetables with great diversity of colors, shapes and pungency, in addition to the aroma of the fruits (Martins *et al.*, 2020), characteristics responsible for the great popularity of peppers. The variability within this genus is considerable and little is known about its extent, so the full potential of this culture is not yet explored, as mentioned by Araújo *et al.* (2018).

Thus, according to Ferraz *et al.* (2016), morphological and agronomic characterization works of the genotypes available in germplasm banks aim to support the development of genetic improvement programs and contribute to the solution of culture challenges.

Characterization is a pre-breeding essential activity in the initial stage of improvement programs, which consists of describing, identifying and differentiating accessions of the same species, and allows to know the existing variability (Lopes *et al.*, 2016). This pre-breeding work, in addition to allowing the discovery of superior characteristics that bring the opportunity to use desirable germplasms for the development of new cultivars (Silva Neto *et al.*, 2014), still maintains the diversity and genetic purity of pepper species.

The characterization of genetic diversity among *Capsicum* genus has become essential for the breeding programs of pepper species, important as a vegetable and spice crop world-wide (Bianchi *et al.*, 2020). Research with a focus on diversity is essential for the conservation of genetic resources and should bring valuable information for its use in breeding programs (Martins *et al.*, 2020).

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The breeding of peppers has been performed via mass selection in landraces and, recently, some breeders have given emphasis to the use of hybridization, noting the need to carry out the characterization of promising materials in the search for genetic variability, high-yielding cultivars with fruit quality (Rêgo & Rêgo, 2016). Thus, the present study aimed to characterize and analyze the divergence among different accessions of pepper of the genus *Capsicum* through morphological and agronomic characteristics, in order to make this information available to the breeding programs of the genus.

MATERIAL AND METHODS

The experiment was conducted from December 2014 to June 2015 in a greenhouse of the Universidade Federal Rural de Pernambuco (UFRPE), *Campus* Dois Irmãos, in Recife-PE (08°01'01"S, 34°56'45"O, 10,3 m above sea level). The greenhouses used were covered with agricultural film with low density and transparent polyethylene and the sides covered with black screen (shade) with 50% shading.

The accessions of *Capsicum* peppers (Table 1) provided by a private collector were evaluated, and their exsiccates were incorporated into Herbário Dárdano de Andrade Lima´s collection, belonging to the Agronomic Institute of Pernambuco, in Recife-PE, under the numbers 90305 to 90317.

For seedling production, the sowing was carried out in trays of 200 cells, using the sieved coir dust substrate and previously autoclaved at 120 °C for two hours. Seedling production was carried out with 13 accessions (treatments) and four replications of 25 seeds each, placing only one seed per cell, 2 cm deep, with trays being placed on benches in the greenhouse.

The transplant was carried out 60 days after sowing, when the seedlings presented three to four pairs of true leaves. The most vigorous seedlings were transplanted to another greenhouse, in pots with a capacity of five liters, using the coir dust substrate. Fertigation was performed with daily applications of nutrient solution in an open hydroponics system, by dripping.

For the morphological and agronomic characterization of the plants, the experimental design used was in randomized blocks, with 13 accessions (treatments) and six replications, with the experimental part consisting of three pots. The pots were arranged in six lines with a spacing of 1 m between the lines and 0.5 m between the pots.

The morpho-agronomic characterization of the plants was carried out based on descriptors proposed by the International Plant Genetic Resources Institute (IPGRI *et al.*, 1995). The observations were made respecting each phenological phase of the culture, being evaluated 13 qualitative and 13 quantitative descriptors:

1) On the leaf: density of leaves; color; form; leaf blade margin; pubescence; leaf length (cm), using an average of 30 ripe leaves when half of the plants had maturing fruits; leaf width (cm), measured at the widest part, using an average of 30 mature leaves when half of the plants had maturing fruits.

2) On the plant: color of the stem, evaluated during transplanting; stem shape, seen in mature plants; pubescence of the stem, observed in mature plants; plant height (m), when half of the plants showed maturing fruits; stem diameter (cm), measured at harvest; growth habit and; branching, evaluated when half of the plants showed ripe fruits.

3**)** In fruiting: days to fruiting, from sowing to half the plants sustain ripe fruit in the first and second forks; number of fruits per plant; fruit color, obtained through the pulp of the ripe fruit, with a digital colorimeter (Konica Minolta® CR-400, Tokyo, Japan), according to the CIELAB system (L*, a*, b*), where L* indicates light (0 =

Table 1: Accession, species and origin of 13 pepper *Capsicum* spp. accessions.

Accession	Species	Origin
JM 9909	*Capsicum chinense* Jacq.	Bahia, Brazil
JM 9910	*Capsicum chinense* Jacq.	Bahia, Brazil
JM 9911	*Capsicum chinense* Jacq.	Pernambuco, Brazil
JM 9912	*Capsicum chinense* Jacq.	Pernambuco, Brazil
JM 9913	*Capsicum chinense* Jacq.	Bahia, Brazil
JM 9914	*Capsicum baccatum* var. *pendulum* (Willd.) Eshbaugh	Pernambuco, Brazil
JM 9915	*Capsicum chinense* Jacq.	Bahia, Brazil
JM 9916	*Capsicum chinense* Jacq.	Pernambuco, Brazil
JM 9917	*Capsicum chinense* Jacq.	Paraíba, Brazil
JM 9918	*Capsicum chinense* Jacq.	Pernambuco, Brazil
JM 9919	*Capsicum chinense* Jacq.	Pernambuco, Brazil
JM 9920	*Capsicum annuum* L.	Brazil
JM 9921	*Capsicum annuum* L.	Brazil

black and 100 = white), a* and b* are coordinates for green (-a*) / red (+ a*) and blue (-b*) / yellow (+ b*); anthocyanin stains on immature fruits; fruit shape; fruit surface; fruit length (cm), average of 30 ripe fruits; fruit width (cm), measured at the widest point, average of 30 ripe fruits; fruit mass (g), average of 30 ripe fruits, using an analytical balance (accuracy 0.001 g); pulp thickness (mm), obtained with an average of 30 ripe fruits; number of seeds per fruit, evaluated with the average of ten fruits and; fruit production (kg / plant), using an average of 18 plants.

Data analysis was performed using statistical software (Sisvar, v. 5.0) for grouping averages using the Scott-Knott test, at 5% probability. In the clustering analysis, generalized Mahalanobis distance was used as a measure of genetic dissimilarity and the analysis of genetic divergence between genotypes was performed by the unweighted pair-group method (UPGMA) for quantitative characteristics, except fruit color. The relative importance of the evaluated traits was calculated by Singh's method (Singh, 1981). Dissimilarity analysis was performed with (Genes, v. 5.1) program (Cruz, 2013).

RESULTS AND DISCUSSION

Morpho-agronomic characterization

A greater variation was observed in the peppers evaluated in terms of leaf shape, with the majority of accessions having oval leaves, accessions JM 9914 and JM 9919 having deltoid leaves and accessions JM 9913, JM 9920 and JM 9921, lanceolate leaves. As for leaf density, they varied between dense, sparse and intermediate, with dense foliage predominating (Table 2). Melo *et al.* (2014) mention that the leaf shape, among other characteristics, is significant in the ornamental pepper trade, drawing consumer attention.

The evaluated accessions presented stems varying their coloration between green and green with the presence of stripes or nodes of purplish pigmentation (Table 2). The pubescence of the stem was intermediate in the accessions JM 9920 and JM 9921, highlighting these

Table 2: Qualitative characteristics evaluated in leaf and plant of 13 accessions of pepper *Capsicum* spp..

Accession	Leaf				
	Color	**Margin**	**Pubescence**	**Form**	**Leaf density**
JM 9909	Green	Wavy	Sparse	Oval	Dense
JM 9910	Green	Wavy	Intermediate	Oval	Intermediate
JM 9911	Light green	Wavy	Sparse	Oval	Intermediate
JM 9912	Green	Wavy	Sparse	Oval	Sparse
JM 9913	Green	Wavy	Sparse	Lanceolate	Sparse
JM 9914	Light green	Wavy	Sparse	Deltoid	Dense
JM 9915	Green	Wavy	Sparse	Oval	Dense
JM 9916	Green	Wavy	Sparse	Oval	Intermediate
JM 9917	Green	Wavy	Intermediate	Oval	Sparse
JM 9918	Green	Wavy	Sparse	Oval	Sparse
JM 9919	Green	Wavy	Sparse	Deltoid	Dense
JM 9920	Dark green	Wavy	Sparse	Lanceolate	Dense
JM 9921	Dark green	Wavy	Sparse	Lanceolate	Dense
	Plant				
	Stem color	**Stem Pubescence**		**Growth Habit**	**Branching**
JM 9909	Green with purple nodes	Sparse		Intermediate	Intermediate
JM 9910	Green with purple stripes	Sparse		Upright	Intermediate
JM 9911	Green	Sparse		Upright	Intermediate
JM 9912	Green with purple stripes	Sparse		Upright	Sparse
JM 9913	Green	Sparse		Upright	Sparse
JM 9914	Green	Sparse		Prostrate	Intermediate
JM 9915	Green with purple nodes	Sparse		Intermediate	Dense
JM 9916	Green with purple stripes	Sparse		Upright	Intermediate
JM 9917	Green with purple nodes	Sparse		Upright	Sparse
JM 9918	Green	Sparse		Upright	Sparse
JM 9919	Green with purple nodes	Sparse		Upright	Intermediate
JM 9920	Green with purple nodes	Intermediate		Upright	Dense
JM 9921	Green with purple nodes	Intermediate		Upright	Dense

genotypes as of possible interest for future crosses and evaluations in obtaining strains resistant to viruses, since the presence of hair on leaves and stems can have a direct effect on access to plants by insect vectors of diseases (Ferraz *et al.*, 2016).

The attributes related to fruits present variability among the accessions. It was noticed a shorter time for fruiting in the accessions JM 9914, JM 9920 and JM 9921 (Table 3), showing fruits in the first and second bifurcations between 90 and 104 days. The search for early materials has guided the research with *Capsicum* peppers, as confirmed by Costa *et al.* (2019). Early accession is better for breeders and producers, reducing crop maintenance and optimizing the production process.

The accessions JM 9910, JM 9912, JM 9913 and JM 9917 showed purple spots on the ripening fruit (Table 3), determining that these accessions have a medicinal potential to be studied, since the purple color is attributed to anthocyanins (Neitzke *et al.*, 2015). Anthocyanins are accumulated at different levels in plants and have various biological functions, such as attracting pollinating agents and preventing photo-oxidative damage in plants (Aza-Gonzáles *et al.*, 2012).

The shape and surface of the pepper fruits varied considerably (Table 3), the elongated shape being the most common, present in seven of the thirteen accessions evaluated, followed by the block formats, triangular and only one round access. The shape, together with the vibrant colors of the peppers increases its commercial value, making preserves more beautiful and attracting consumers.

The lowest number of seeds (16 seeds) was found in the accessions JM 9912 and JM 9913, while in the accessions JM 9909, JM 9914, JM 9917, JM 9918 and JM 9920 more than 40 seeds were obtained per fruit (Table 3). The commercialization of seeds by farmers can generate profits (Nascimento *et al.*, 2019a). In addition, peppers with less seeds are ideal for jams, sauces and dehydration of the pulp, where the seed is discarded (Guillen *et al.*, 2018).

Regarding the color analysis (Table 4), explained by Carvalho *et al.* (2014), it should be noted that in the genotypes JM 9920, JM 9913, JM 9912, JM 9918, JM 9914, JM 9909 and JM 9921 colors of more intense red were observed, in the order of the most intense to the least intense, based on the average. In addition, the JM 9921 genotype showed an intermediate value of b *, showing a strong orange hue. The genotypes JM 9910, JM 9911, JM 9916 and JM 9917 showed little reddish

Table 4: Pulp color of ripe fruits evaluated at 13 genotypes of pepper *Capsicum*. L* brightness; a* color intensity; b* hue angle.

Accession	L*	a*	b*
JM 9909	32,34 c	25,43 b	22,43 f
JM 9910	51,84 b	5,78 d	57,56 b
JM 9911	54,04 b	8,95 c	60,01 b
JM 9912	34,87 c	31,30 a	31,30 e
JM 9913	33,81 c	31,95 a	28,82 f
JM 9914	32,60 c	26,40 b	24,95 f
JM 9915	60,30 a	-4,33 e	49,18 c
JM 9916	54,29 b	11,47 c	67,37 a
JM 9917	50,70 b	10,08 c	61,42 b
JM 9918	31,61 c	29,63 a	26,19 f
JM 9919	53,53 b	7,03 d	37,77 d
JM 9920	33,37 c	34,68 a	28,19 f
JM 9921	48,34 b	24,43 b	46,01 c

Means followed by the same letter in the column belong to the same group, at 5% significance by Scott-Knott test.

Table 3: Qualitative and quantitative characteristics evaluated in fruits of 13 accessions of pepper *Capsicum* spp..

Accession	Days to bear fruit	Stain anthocyanin	Form	Surface	Number of seeds per fruit
JM 9909	114 b	Absent	Round	Flat	58 a
JM 9910	162 a	Present	Triangular	Rough	26 b
JM 9911	162 a	Absent	Block	Semi-rough	27 b
JM 9912	133 b	Present	Elongated	Rough	16 c
JM 9913	162 a	Present	Elongated	Rough	16 c
JM 9914	104 c	Absent	Elongated	Semi-rough	55 a
JM 9915	112 b	Absent	Triangular	Flat	35 b
JM 9916	162 a	Absent	Elongated	Rough	35 b
JM 9917	114 b	Present	Block	Semi-rough	50 a
JM 9918	162 a	Absent	Elongated	Flat	47 a
JM 9919	114 b	Absent	Block	Semi-rough	33 b
JM 9920	92 c	Absent	Elongated	Flat	40 a
JM 9921	90 c	Absent	Elongated	Semi-rough	36 b
CV (%)	13,45	-	-	-	40,68

Means followed by the same letter in the column belong to the same group, at 5% significance by Scott-Knott test.

color and the b * coordinate with high values, predominantly yellow-orange color. The genotype JM 9915 showed a light yellow color and the genotype JM 9919 showed a little predominant red and a weak yellow, with a salmon color.

The color is a quality attribute for fruits destined for processing, as evaluated by Andrade *et al.* (2019). The exuberance of the colors of the pepper fruits and their great variability allow the insertion of these accessions in the production of ornamental preserves and red and yellow liquid sauces (Ohara & Pinto, 2012).

For the quantitative characters evaluated in the plants and fruits (Table 5), it was possible to detect a high variability between accessions for all characteristics, except stem diameter, where there was no significant difference. This variability is essential, mainly, in the identification of genitors to carry out crosses of interest in the genetic improvement of the crop (Costa *et al.*, 2015).

The height of the plants (Table 5) is a factor of interest in breeding and production, providing greater facility of harvest and is also, the smaller size, a characteristic of ornamental interest (Pessoa *et al.*, 2018). In this case, because they are taller, all the accessions evaluated can be grown in functional gardens (Fortunato *et al.*, 2019), or they can develop cultivars adapted to mechanical harvesting as mentioned by Gomes *et al.* (2019).

The average length of the fruits (Table 5) ranged from 1.61 to 6.34 cm and the width of the fruits from 0.97 to 2.61 cm. The accession JM 9914 stands out, in view of the size of its fruits, which have quality for in natura and canned commercialization (Paulus *et al*., 2015).

Table 5: Mean of ten quantitative characteristics evaluated in plants and fruits of 13 accessions of pepper *Capsicum* spp.. Federal Rural University of Pernambuco, Recife, Pernambuco, Brazil. 2015. PH: plant height; SD: stem diameter; LL: leaf length; LW: leaf width; FL: fruit length; FWi: fruit width; FWe: fruit weight; NFP: number of fruits per plant; PT: pulp thickness; Pro: production

Acession	Plant			
	PH (m)	SD (cm)	LL (cm)	LW (cm)
JM 9909	1,31 b	1,63 a	12,00 c	7,03 b
JM 9910	1,66 a	1,74 a	13,20 b	6,85 b
JM 9911	1,52 a	1,64 a	14,17 b	7,55 b
JM 9912	1,67 a	1,71 a	13,55 b	6,72 b
JM 9913	1,64 a	1,78 a	12,42 c	6,00 c
JM 9914	1,16 b	1,43 a	14,02 b	8,40 a
JM 9915	1,27 b	1,97 a	8,90 d	4,70 d
JM 9916	1,63 a	1,69 a	14,37 b	6,97 b
JM 9917	1,73 a	1,60 a	17,72 a	8,63 a
JM 9918	1,53 a	1,83 a	13,57 b	7,42 b
JM 9919	1,34 b	1,68 a	13,00 b	8,00 a
JM 9920	1,00 c	1,88 a	11,00 c	4,62 d
JM 9921	0,80 d	1,51 a	11,30 c	4,92 d
CV(%)	9,96	14,35	9,11	9,62

	Fruit					
	FL (cm)	FWi (cm)	FWe (g)	NFP	PT (mm)	Pro (kg/plant)
JM 9909	1,61 g	2,61 a	5,401 c	104 b	2,15 b	0,560 a
JM 9910	4,41 c	1,62 d	4,357 d	152 b	1,81 c	0,662 a
JM 9911	3,91 d	1,95 c	5,496 c	103 b	2,37 a	0,564 a
JM 9912	5,39 b	1,41 e	3,514 e	224 b	1,68 c	0,788 a
JM 9913	5,69 b	1,40 e	4,202 d	158 b	1,39 d	0,664 a
JM 9914	6,34 a	1,42 e	4,530 d	103 b	1,43 d	0,468 b
JM 9915	1,78 g	1,26 e	1,220 f	509 a	1,32 d	0,621 a
JM 9916	4,55 c	1,86 c	5,548 c	123 b	2,12 b	0,684 a
JM 9917	3,74 d	2,20 b	6,404 b	103 b	2,36 a	0,659 a
JM 9918	4,20 d	1,49 d	3,969 e	98 b	1,67 c	0,388 b
JM 9919	4,76 c	2,51 a	7,995 a	110 b	1,63 c	0,880 a
JM 9920	2,54 f	0,97 f	1,213 f	253 b	1,05 e	0,307 b
JM 9921	3,36 e	1,05 f	1,455 f	169 b	1,06 e	0,247 b
CV(%)	11,39	8,32	13,72	55,19	15,48	43,69

Means followed by the same letter in the column belong to the same group, at 5% significance by Scott-Knott test.

The highest fruit mass was observed in the accession JM 9919 (7,995 g), while the lightest fruits were observed in the accessions JM 9915, JM 9920 and JM 9921 (1,220 g, 1,213 g and 1,455 g, respectively), which presented small fruits and with thinner pulp thickness (Table 5). The accessions with greater pulp thickness were JM 9911 and JM 9917 (2.37 mm and 2.36 mm, in that order), followed by the accessions JM 9909 and JM 9916 (2.15 mm and 2.12 mm, respectively), which also presented heavy fruits, below only the access JM 9919. Bianchi *et al.* (2020) and Gomes *et al.* (2019) agree that the weight of the fruit and the thickness of the pulp are important for the commercialization of fruits, thus, it can be suggested that the accessions JM 9909, JM 9911, JM 9916, JM 9917 and JM 9919 are indicated for the purpose of commercialization of fruits in natura or in the industry.

As for the production, the accessions JM 9914, JM 9918, JM 9920 and JM 9921 presented production between 0.247 and 0.468 kg / plant and the other accessions presented production between 0.560 and 0.880 kg / plant (Table 5). The accessions JM 9919 and JM 9912 stand out, with expressive production, that being an important characteristic for cultivars selection (Rêgo & Rêgo, 2016).

Despite having an average of 253 and 169 fruits per plant (Table 5), respectively, the accessions JM 9920 and JM 9921 obtained low fruit masses, which explains the low production, expressed in kilograms per plant. Pessoa *et al.* (2018) consider that accessions that exhibit small and numerous fruits are recommended for ornamental use.

Genetic divergence

The dendogram obtained shows the distinction of three groups (Figure 1 and 2), demonstrating that there is variability among the accessions for the evaluated traits. The species was also a major factor in the formation of these groups.

Group I was the major group, composed of seven accessions, mainly *C. chinense*, which presented larger fruits with intermediate weight and width, taking up to 162 days to bear fruits. Group II was composed of three accessions of the species *C. chinense* and characterized by clustering plants with green leaves, heavier and wider fruits and taking 114 days to fruiting. Group III comprised three accessions, mostly *C. annuum*, in which predominated sparse leaf pubescence, dense leaf density, dense branching, absence of anthocyanin stain

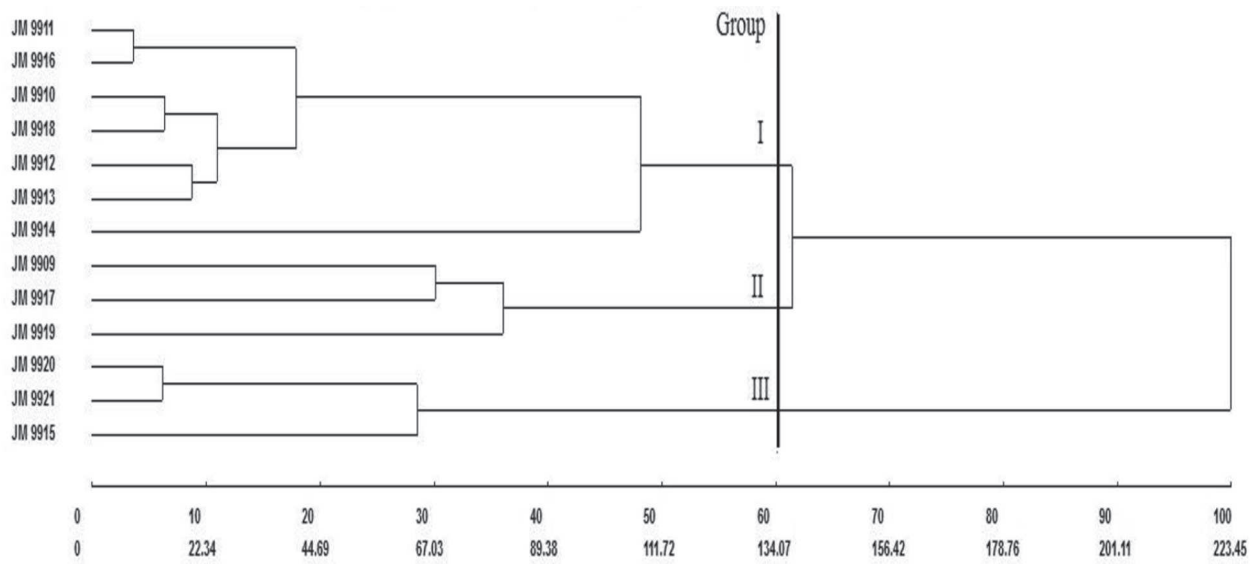

Figure 1: Representative dendrogram of genetic dissimilarity between 13 accessions of pepper *Capsicum* spp., obtained by the unhweighted pair-group method, using the generalized Mahalanobis distance.

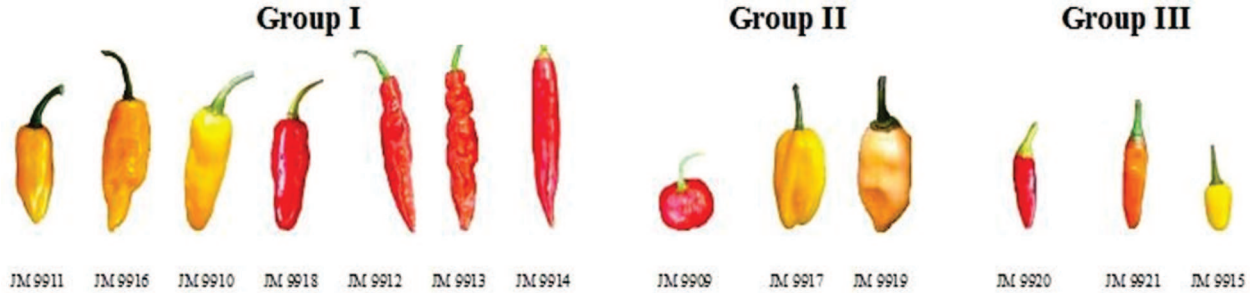

Figure 2: Phenotypic representation of fruits allocated in the dendogram groups referring to the morpho-agronomic characterization.

on the fruit, shorter leaves, smaller, lighter and thinner fruits, being more precocious, between 90 and 112 days to bear fruits.

In general, the fruit width (18.86%) was the most effective attribute to the discrimination of the accessions, followed by fruit weight (16.28%), fruit length (15.35%) and days for fruiting (15.32%). These traits can help in the selection of superior genotypes (Nascimento *et al*., 2019b). These results are similar to those found by Bianchi *et al*. (2020). In contrast, stem diameter (0.46%) was the characteristic that presented less relative importance for the phenotypic divergence. That evidences low genetic variability for this character, thus, little expectation of gain with the selection (Pessoa *et al*., 2018).

CONCLUSIONS

Great variability was observed for most of the characteristics in the evaluated accessions, constituting important material for the genetic improvement of plants.

The analyzed descriptors allowed us to distinguish between the accessions of pepper, highlighting the high diversity within the species evaluated, due to the variety of sizes, shapes and colors of plants and fruits.

The results obtained in the present work can be explored both in breeding and directly in the vegetable market, where the variety of types of peppers draws the consumer's attention with preference from preserves to ornamental interest.

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