MORPHO-AGRONOMIC CHARACTERIZATION AND GENETIC DIVERSITY IN PEPPERS (*Capsicum* spp.)[1]

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ABSTRACT – Peppers and bell peppers (*Capsicum* spp.) are vegetables that have wide genetic variability, as well as a wide range of applications. Studies on genetic diversity help to understand the existing variability and the conservation of genetic resources, allowing breeders to select superior genotypes that meet the needs of breeding programmes. This study aimed to evaluate the genetic diversity among 15 *Capsicum* genotypes based on morpho-agronomic descriptors and through the Ward-MLM (Modified Location Model) procedure, as well as to indicate the most promising genotypes for breeding programmes. The research was conducted in an experimental area using a randomized block design, with three replications, using nine qualitative descriptors and ten quantitative descriptors. Analysis of variance was performed, and means were subsequently grouped by Scott-Knott's method. There was a significant difference between genotypes for all traits evaluated, making it possible to select promising genotypes. According to the Scott-Knott grouping test, the number of seeds per fruit had the largest number of groups, with eight distinct groups, while plant height had only two groups. The genotypes GEN18 and GEN21 had the highest average values for fruit wall thickness and thus are indicated for the preparation of sauces. The genotypes GEN14 and GEN16 were the most promising for use for ornamental purposes. The Ward-MLM method made it possible to differentiate *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq.*,* and *C. frutescens* L. into different groups, and is thus a useful tool to detect genetic divergence.

Keywords: Pepper plants. Genetic resources. Ward-MLM. Plant breeding.

CARACTERIZAÇÃO MORFOAGRONÔMICA E DIVERSIDADE GENÉTICA em pimenteiras (*Capsicum* spp.)

RESUMO – Pimentas e Pimentões (*Capsicum* spp.) são hortaliças que possuem ampla variabilidade genética, bem como vasta versatilidade de aplicações. Estudos sobre diversidade genética auxiliam no conhecimento da variabilidade existente e na conservação dos recursos genéticos, permitindo aos melhoristas selecionar genótipos superiores que atendam às necessidades de programas de melhoramento genético. Objetivou-se analisar a diversidade genética entre 15 genótipos de *Capsicum* com base em descritores agromorfológicos e por intermédio do procedimento Ward-MLM (*Modified Location Model*), bem como indicar os mais promissores para programas de melhoramento. A pesquisa foi conduzida em campo experimental utilizando delineamento em blocos ao acaso, com três repetições, utilizando-se nove caracteres qualitativos e 10 caracteres quantitativos. Foi utilizada análise de variância e, em seguida, as médias foram agrupadas pelo método Skott-Knott. Houve diferença significativa entre os genótipos para todas as características avaliadas, sendo possível a seleção de genótipos promissores. Considerando agrupamento Scott-Knott, a característica número de sementes por fruto obteve o maior número de grupos com oito classes distintas, enquanto a característica altura da planta formou apenas duas classes. Os genótipos GEN18 e GEN21 possuem os maiores valores médios para a caraterística espessura da parede do fruto, e podem ser indicados para o preparo de molhos. Os genótipos GEN14 e GEN16 foram os mais promissores a serem utilizados para fins ornamentais. O método de Ward-MLM possibilitou a diferenciação das espécies *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., and *C. frutescens* L. em grupos distintos, sendo uma ferramenta útil para detectar divergência genética.

Palavras-chave: Pimenteiras. Recursos genéticos. Ward-MLM. Melhoramento genético.

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INTRODUCTION

The *Capsicum* genus belongs to the family Solanaceae (PICKERSGILL, 1971), and has about 38 described species, of which six are considered domesticated: *C. annuum*, *C. assamicum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens* (LEE, 2019). Brazil is a vital diversity centre of the *Capsicum* genus, and according to Rêgo, Finger and Rêgo (2011), this existing diversity has fomented breeding programmes.

The pepper market ranges from the sale of fresh and processed fruits in the form of paprika, sauces, and preserves, to the manufacture of medicines and cosmetics (PINTO; PINTO; DONZELES, 2013). Pepper fruits have important pharmacological effects, such as anti-inflammatory and antioxidant effects (ZIMMER et al., 2012). They are also used as ornamental plants due to the aesthetic attributes they exhibit (NEITZKE et al., 2016).

In Brazil, pepper production is of great importance either because of its profitability characteristics, especially when the producer adds value to the product, or because of its social significance, by employing labour (RUFINO; PENTEADO, 2006). The growing demand of the consumer markets, both internal and external, has led to a significant increase in the area cultivated with peppers (GUERINO et al., 2017). According to Nascimento et al. (2014) there is an increasing demand for new cultivars with higher quality, yield and pest resistance, all of which can be achieved through breeding programmes.

In the municipality of Floriano - PI, there is a demand for peppers adapted to the soil and climate conditions of the region, mainly from small farmers. This emphasizes the importance of conducting work aimed at evaluating and selecting the most promising genotypes for production and marketing in the region. The Universidade Federal do Piauí, Campus Amílcar Ferreira Sobral, hosts a collection of germplasm with almost 100 genotypes; their characterization and evaluation are of fundamental importance in order to determine the genetic diversity and allow the identification of useful genotypes in improvement programmes.

Characterization of germplasm is fundamental in order to provide information for plant breeding programmes (SHEORAN et al., 2019). With the use of appropriate genetic and statistical methodologies, it is possible to assess the variability of different genotypes and their potential for use (MARIM et al., 2009).

Genetic variation can be studied through specific methods or combinations of methods, which can include the use of quantitative or qualitative characteristics (BARBÉ et al., 2010), and these characteristics can be combined using multivariate analysis techniques (FERRÃO et al., 2011). Among the multivariate methodologies, the Ward-MLM procedure proposed by Franco et al. (1998) is a technique used to assess variability using both quantitative and qualitative variables simultaneously (CAMPOS et al., 2013). This method has been applied in studies of genetic divergence, providing support for the selection of superior genotypes for breeding programmes (PESTANANA et al., 2011).

Given the above, this study aimed to evaluate the genetic diversity among 15 *Capsicum* genotypes based on morpho-agronomic descriptors and through the Ward-MLM procedure, as well as to identify the most promising genotypes for breeding programmes.

MATERIAL AND METHODS

The experiment was carried out in an area of the experimental farm of the Technical College of Floriano, Piauí, Brazil, latitude 6°45'45.7"S and longitude 43°03'31.5"W, from January to August 2016. Fifteen genotypes of *Capsicum* spp. (Table 1) from the Seed Collection of the Universidade Federal do Piauí, Campus Amílcar Ferreira Sobral (UFPI/CAFS), were evaluated.

Sowing was carried out in disposable 150 mL containers drilled at the base and filled with Plus® Garden soil. At 35 days after sowing, when the seedlings had from four to six pairs of definitive leaves, they were transplanted into the experimental field. Transplants were placed in holes, using a spacing of 1.0 m between rows and 0.50 m between plants. The experimental design used was a randomized block with three replications and four plants per plot, the useful plot consisting of the two central plants. Mineral fertilizer (NPK - 5:30:15) and goat manure were applied in each planting hole. A drip irrigation system was used, and crop management was carried out according to the recommendations for the crop (FILGUEIRA, 2008).

The characterization of the genotypes was based on a list of ten quantitative and nine qualitative descriptors suggested by the International Plant Genetic Resources Institute (IPGRI, 1995). The following quantitative descriptors were evaluated: mature leaf length (LFL – mm), mature leaf width (LFW – mm), stem diameter (SDM – mm), plant height (PHT – cm), days to flowering (DTF – days), fruit pedicel length (FPL – mm), fruit length (FLT – mm), fruit width (FWL – mm), number of seeds per fruit (NSF) and fruit wall thickness (FWT – mm). Measurements of plant height and stem diameter were made with the aid of a tape measure graduated in centimetres. The leaf and fruit measurements were made with the aid of a digital caliper (Pantec® digital caliper) graduated in millimetres, measuring five leaves and five fruits per plant.

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Table 1. Identification and origin of 15 pepper genotypes from the Seed Collection of the Universidade Federal do Piauí, Campus Amílcar Ferreira Sobral (UFPI/CAFS).

Genotype	Identification	Popular name	Source
GEN02	*Capsicum baccatum*	Pimenta dedo de moça	Brasília-DF
GEN05	*Capsicum frutescens*	Pimenta Malagueta	Natal-RN
GEN06	*Capsicum frutescens*	Pimenta Malagueta	União-PI
GEN09	*Capsicum baccatum*	Pimenta dedo de moça	Teresina-PI
GEN10	*Capsicum chinense*	Pimenta de Cheiro	Teresina-PI
GEN12	*Capsicum chinense*	Pimenta Murici	Teresina-PI
GEN14	*Capsicum annuum*	Pimenta Ornamental	Teresina-PI
GEN15	*Capsicum frutescens*	Pimenta Malagueta	Teresina-PI
GEN16	*Capsicum annuum*	Pimenta Ornamental	Teresina-PI
GEN17	*Capsicum chinense*	Pimenta Bode	Brasília-DF
GEN18	*Capsicum annuum*	Pimentão	Ribeirão Preto-SP
GEN21	*Capsicum annuum*	Pimentão	Ribeirão Preto-SP
GEN26	*Capsicum frutescens*	Pimenta Malagueta	Floriano-PI
GEN28	*Capsicum annuum*	Pimentão Magna Super	Floriano-PI
GEN30	*Capsicum annuum*	Pimentão Amarelo Sf* 134	Floriano-PI

*Sf – Self-fertilization.

The following qualitative descriptors were also evaluated: stem colour (STC), leaf shape (LFS), number of flowers per axil (NFA), flower position (FPS), corolla colour (CCL), corolla spot colour (CSC), anther colour (ACL), fruit colour at intermediate stage (FCI), and fruit colour at mature stage (FCM).

The data were subjected to analysis of variance, and the Scott-Knott test grouped the means at 5% probability. The joint analysis of the quantitative and qualitative characters was performed using the Ward-MLM method, proposed by Franco et al. (1998), using the Gower dissimilarity matrix. The ideal number of groups was defined based on the likelihood function criterion, maximized according to the MLM method, and differences between them were analysed using canonical variables. Analysis of variance and Scott Knott grouping were performed using the Genes program (CRUZ, 2013). The quantitative and qualitative variables were analysed simultaneously based on the Ward-MLM method using SAS program version 9.0 (SAS Institute, Cary, North Carolina, USA). Figures were made using the SigmaPlot® software (version 10.0).

RESULTS AND DISCUSSION

There was a significant difference ($p < 0.01$) among the genotypes for all characters evaluated (Table 2), demonstrating the existence of genetic variability, and thus making it possible to select superior individuals. It is worth mentioning that, according to Cruz, Carneiro and Regazzi (2014), the existence of genetic variability in a population is a determining factor for success in any breeding programme.

The values of the coefficients of experimental variation (CVs) ranged between 6.80% (DTF) and 25.18% (SDM). According to Pimentel-Gomes (1990), most CVs were considered low (below 10%) and moderate (from 10 to 20%), except for SDM, PHT and FWL. This classification (from low to moderate) for most of the characters evaluated demonstrates little influence of the environment, conferring credibility to the results obtained and indicating good experimental precision. In a study of peppers, Silva et al. (2011) determined that the CV varied according to trait, genotype and species under study.

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Table 2. Mean squares and coefficient of variation (CV) obtained in the analysis of variance of ten characters evaluated in 15 pepper genotypes from the Seed Collection of the Universidade Federal do Piauí, Campus Amílcar Ferreira Sobral (UFPI/CAFS).

SV	DF	Mean square				
		LFL	MLW	SDM	PHT	DTF
Replication	2	38.84	35.57	15.59	65.69	4.29
Genotypes	14	349.9**	183.56**	20.01**	401.26**	118.04**
Error	28	33.81	6.87	6.25	88.78	10.07
Mean		64.24	29.85	9.93	44.58	46.71
CV (%)		9.05	8.78	25.18	21.14	6.80

SV	DF	Mean square				
		FPL	FLT	FWL	NSF	FWT
Replication	2	2.18	33.28	28.06	2.37	0.01
Genotypes	14	98.46**	1229.83**	624.99**	1605.09**	3.94**
Error	28	10.75	41.29	19.40	18.23	0.07
Mean		26.98	37.16	19.48	28.4	2.02
CV (%)		12.15	17.29	22.62	15.04	13.53

**Significant at 1% probability by the F test.
SV: source of variation; DF: degrees of freedom; Characteristics: mature leaf length (LFL), mature leaf width (MLW), stem diameter (SDM), plant height (PHT), days to flowering (DTF), fruit pedicel length (FPL), fruit length (FLT), fruit width (FWL), number of seeds per fruit (NSF), and fruit wall thickness (FWT).

Regarding the average values grouped by the Scott-Knott test, the genotypes for NSF were grouped into eight distinct groups (Table 3), demonstrating the high variability of genotypes for this characteristic. Rufino and Penteado (2006) affirmed that the NSF characteristic is relevant for commercialization, given that the farmer can obtain profits if they choose to sell the seeds.

The LFL, SDM, DTF and FLP formed three distinct groups, while for MLW four distinct groups were observed (Table 3), demonstrating the variability of these traits. The highest mean values for length were observed for genotypes GEN06, GEN15, GEN18 and GEN21 (Table 3), while for leaf width the genotype GEN02 had the highest mean value. According to Taiz and Zeiger (2013) leaf length and width are very important characteristics, given the direct relationship between leaf area, photosynthesis and plant growth and development. The genotypes GEN14 and GEN16 had the lowest average values in terms of leaf length and width. These characters are relevant due to their ornamental traits, with Barroso et al. (2012) affirming that pepper genotypes with smaller leaves are interesting for this purpose.

The characteristic with the lowest number of groups was PHT (with two distinct groups),

indicating greater uniformity among individuals for this attribute. The genotypes GEN16 and GEN14 had the lowest averages for PHT, at 20.33 mm and 23.33 mm, respectively. According to Costa et al. (2019), smaller pepper genotypes (heights of 6 to 32 cm) are ideal for growing in pots, while larger ones can be grown in gardens.

For FLT the genotypes GEN09, GEN10 and GEN18 stood out, while GEN18 and GEN21 stood out for the FWT (Table 3). In the species *C. annuum* the GEN18 genotype stood out for both FLT (63.34 mm) and FWL (46.81 mm), with the highest average values for both characters (Table 3). These attributes indicate that this genotype is promising for fresh use and the production of sauces. The length and width of the fruit are essential attributes for commercialization, since according to Blat, Braz and Arruda (2007) the trade values large fruits. The highest average values for fruit wall thickness were observed in genotypes GEN18, GEN21, GEN28, and GEN30. These genotypes are promising as important gene sources for breeding programmes since, according to Charlo et al. (2009), fruits that have thicker walls are more resistant to transport, have a longer postharvest duration, and a greater mass yield, besides being preferred by the market.

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Table 3. Means of ten quantitative descriptors referring to 15 pepper genotypes from the Seed Collection of the Universidade Federal do Piauí, Campus Amílcar Ferreira Sobral (UFPI/CAFS).

Genotype	LFL (mm)	MLW (mm)	SDM (mm)	PHT (cm)	DTF (days)	FPL (mm)	FLT (mm)	FWL (mm)	NSF	FWT (mm)
GEN02	67.36b	43.09a	6.78c	33.66b	49.66b	34.84a	47.30b	16.65c	73.29b	1.60c
GEN05	68.40 b	27.02c	9.83c	49.00a	49.33b	22.89c	20.62d	4.17d	16.14g	0.60d
GEN06	78.37a	32.49b	11.16b	46.00a	48.66c	24.50c	17.94d	4.10d	13.11g	0.45d
GEN09	65.61b	34.40b	11.64b	52.00a	44.00c	38.45a	73.10a	20.01c	42.95c	2.13b
GEN10	62.85b	36.38b	8.75c	39.33a	56.66a	27.72b	63.41a	12.39c	14.18g	1.75c
GEN12	59.52b	27.94c	6.35c	49.66a	55.00a	21.15c	18.22d	16.70c	23.05f	1.69c
GEN14	46.34c	14.73d	8.13c	23.33b	40.33c	25.68c	27.99c	19.36c	26.42e	2.31b
GEN15	72.41a	34.53b	11.68b	55.33a	48.66b	23.32c	16.47d	4.10d	6.64h	0.68d
GEN16	40.65c	13.96d	9.48c	20.33b	39.66c	28.63b	30.96c	20.52c	27.31e	2.11b
GEN17	57.51b	36.12b	7.27c	44.66a	56.66a	18.98c	12.88d	14.54c	35.15d	2.18b
GEN18	82.04a	35.92b	9.77c	46.66a	41.33c	28.60b	63.34a	46.81a	86.06a	3.55a
GEN21	70.89a	27.70c	9.14c	51.00a	44.33c	19.28c	38.76c	46.95a	18.33f	3.68a
GEN26	67.69b	27.07c	16.51a	56.66a	48.66b	25.71c	19.71d	4.77d	6.72h	0.54d
GEN28	59.99b	27.14c	12.63b	40.33a	38.33c	33.66a	53.47b	21.69c	21.58f	3.40a
GEN30	63.86b	29.14b	9.73c	60.66a	39.33c	31.31b	53.14b	39.34b	14.94g	3.58a

Means followed by the same letter, in each column, belong to the same group, according to the Scott-Knott test (p ≤ 0.05). Descriptors: Mature leaf length (LFL), mature leaf width (MLW), stem diameter (SDM), plant height (PHT), days to flowering (DTF), fruit pedicel length (FPL), fruit length (FLT), fruit width (FWL), number of seeds per fruit (NSF), and fruit wall thickness (FWT) of 15 pepper genotypes.

The Log-Likelihood function showed that the optimal number of groups was four, with a maximum increase of 41.47%, according to the pseudo-F and pseudo-t2 statistics score, as obtained using the Ward-MLM strategy (Figure 1).

The first two canonical variables obtained by the Ward-MLM method explained 99.73% of the total variation (Figure 2), illustrating the genetic variability between the genotypes evaluated by dispersion graphical analysis. Ferrão et al. (2011) also used canonical variable techniques in research on genetic divergence among pepper genotypes involving dispersion graphs, demonstrating efficiency in the separation of the genotypes. According to Cruz, Carneiro and Regazzi (2014), when values exceed 80% of the total variation for the first two canonical variables, a satisfactory description of the variability between the genotypes is possible, allowing their representation in a two-dimensional scatter plot.
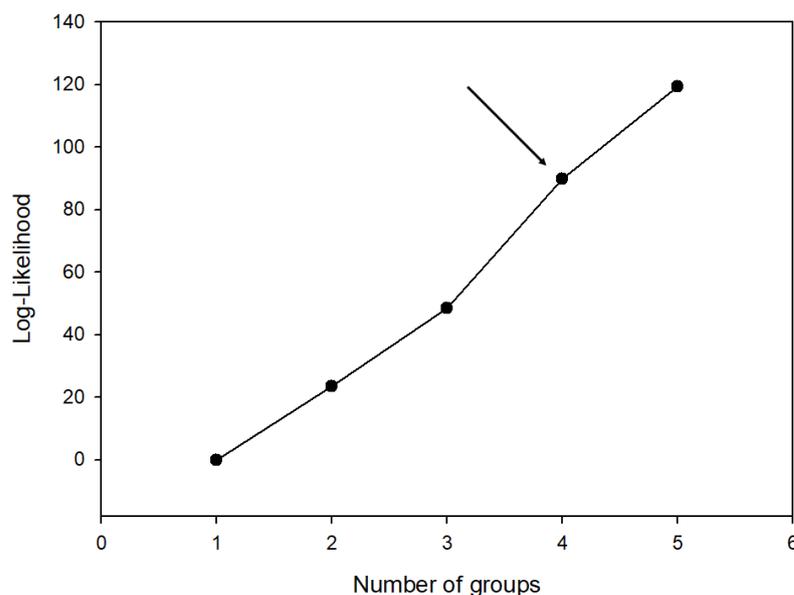

Figure 1. Graph of the logarithmic likelihood function (Log-Likelihood) showing the optimum number of groups formed using the Ward MLM strategy in pepper genotypes.

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The joint analysis of the quantitative and qualitative characters yielded four groups (Figure 1). Group I consisted of genotypes GEN05, GEN06, GEN15, and GEN26, which belong to the species *C. frutescens*. The individuals in this group have floral structures with a yellow and greenish-yellow colour, with two flowers per axil, lanceolate leaves and red-coloured fruits at the final stage of maturation (Table 4). The genotypes GEN05, GEN15 and GEN26 are characterized by the presence of erect flowers. The genotypes of this group, represented by chilli peppers, have the lowest average values for the characteristics of fruit width, fruit length, and fruit wall thickness. According to Bento et al. (2007), chilli pepper fruits have greater potential to be marketed for the production of industrialized sauces.
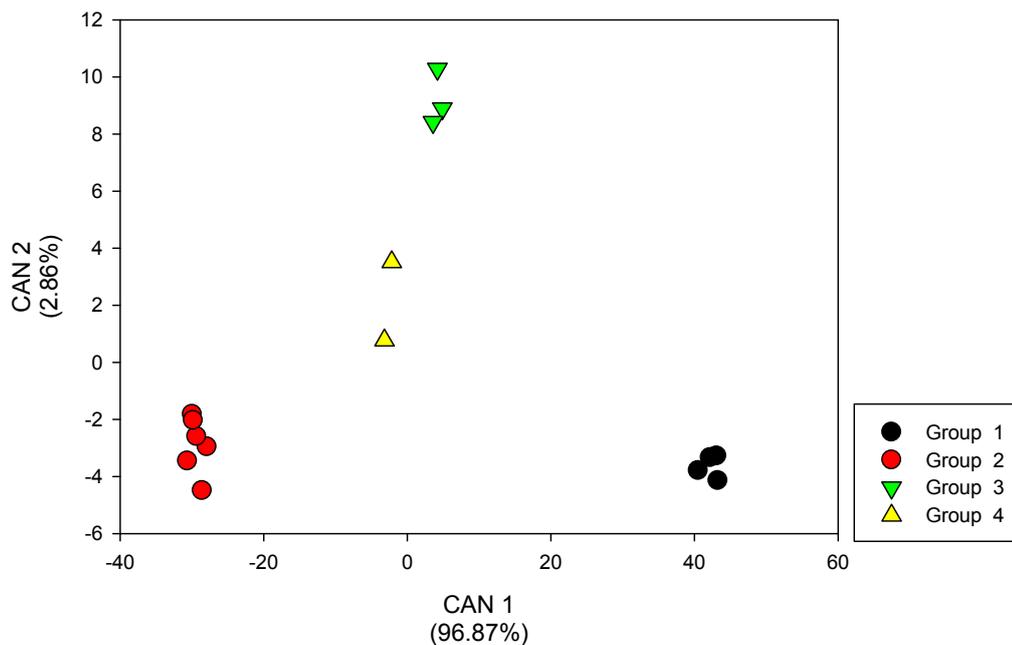

Figure 2. Graphic dispersion for the first two canonical variables (CAN 1 and CAN 2) representing the formation of four groups using the Ward-MLM method for the quantitative and qualitative characteristics in *Capsicum* spp. Group 1: *Capsicum frutescens*; Group 2: *Capsicum annuum*; Group 3: *Capsicum chinense*, and Group 4: *Capsicum baccatum*.

Group II consisted of the genotypes GEN14, GEN16, GEN18, GEN21, GEN28, and GEN30, belonging to the species *C. annuum*. This group includes the genotypes with the lowest average values for the DTF characteristic (Table 3). Genotypes with these attributes are of interest for breeding programmes since precocity is a characteristic of great importance for the development of superior genotypes and should be explored.

The fruits of the genotypes GEN14, GEN18, and GEN28, when ripe, are red (Table 4). According to Lannes et al. (2007), red-coloured fruit is one of the desired attributes for use as paprika. The individuals GEN16, GEN21, and GEN30 produce orange fruits, being quite attractive for ornamental purposes. It is worth mentioning that, according to Neitzke et al. (2016), the colour of the fruits is the most relevant factor when purchasing ornamental peppers.

The genotypes GEN02, GEN14 and GEN16 had the lowest averages for plant height (Table 3).

Belonging to the species *C. annuum*, the genotypes GEN14 and GEN16 therefore have potential for ornamental purposes, since according to Costa et al. (2019), plants with smaller heights are more suitable for cultivation in pots. The genotypes GEN18 and GEN21 had the highest mean values for fruit wall thickness. This is attractive for the commercialization of fresh fruit or to produce sauces. According to Blat, Braz and Arruda (2007), the thickness of the fruit wall is linked to fruit quality and yield. Thicker fruits are more resistant to damage during transport, besides being used in sauce production.

Group III is represented by *C. chinense* genotypes: GEN10, GEN12, and GEN 17. These have two flowers per axil, which are erect and pendant, with oval-shaped leaves. Fruit colour at the intermediate stage is orange and yellow (Table 4). The fruits varied in length and width, demonstrating intraspecific diversity.

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Table 4. Variables and number of genotypes per group of qualitative characteristics, in each of the four groups (G1, G2, G3, and G4) formed using the Ward-MLM strategy in 15 pepper genotypes from the Seed Collection of the Universidade Federal do Piauí, Campus Amílcar Ferreira Sobral (UFPI/CAFS).

Variables	Groups			
	G1(04)	G2(06)	G3(03)	G4(02)
Stem colour (STC)				
Green	1	5	1	1
Green with purple stripes	3	1	2	1
Leaf shape (LFS)				
Deltoid	-	-	-	2
Ovate	-	-	3	-
Lanceolate	4	6	-	-
Number of flowers per axil (NFA)				
One	-	6	-	2
Two	4	-	3	-
Flower position (FPS)				
Pendant	-	2	2	-
Intermediate	1	-	-	2
Erect	3	4	1	-
Corolla colour (CCL)				
White	-	6	-	2
Light-yellow	-	-	1	-
Yellow	1	-	1	-
Yellow-green	3	-	1	-
Corolla spot colour (CSC)				
White	2	6	3	-
Yellow	-	-	-	2
Green-yellow	2	-	-	-
Anther colour (ACL)				
White	-	-	1	-
Yellow	-	1	-	2
Blue	1	-	-	-
Purple	3	5	2	-
Fruit colour at intermediate stage (FCI)				
Yellow	-	1	2	-
Green	-	3	-	-
Orange	3	-	1	2
Purple	1	2	-	-
Fruit colour at mature stage (FCM)				
Orange	-	3	2	-
Red	4	3	1	2

Group IV comprised the genotypes GEN02 and GEN09, belonging to the species *C. baccatum*. These genotypes have a white corolla with yellow spots and anthers. According to Vasconcelos et al. (2012), flower characters can be used as morphological markers to determine genetic diversity and discriminate species. Fruits at the intermediate stage are orange, while in the final maturation stage they are red.

High genetic variability is essential for germplasm collections when we consider the source of alleles present in those plant genetic resources. According to Moreira et al. (2018) the preservation of such resources is of extreme importance for plant breeding, as well as for society as a whole.

CONCLUSIONS

There is genetic diversity among the studied

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genotypes.

The Ward-MLM strategy was very useful to detect genetic divergence and to cluster the genotypes by species.

The genotypes GEN18 and GEN21 are indicated for fresh consumption and processing in the form of sauces and paprika.

GEN14 and GEN16 can be used as ornamental peppers.

GEN14, GEN16, GEN18, GEN21 and GEN30 genotypes can be used in genetic breeding programmes.

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