GENETIC DIVERGENCE IN PEPPER GENOTYPES FROM SOUTHWEST GOIÁS

Divergência genética entre genótipos de pimenta coletados no sudoeste goiano

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ABSTRACT

Knowledge on the genetic diversity in genebanks is important for germplasm conservation and use in breeding programs, where it can reduce time and costs of breeding of new genotypes. The purpose of this study was to evaluate the genetic divergence among 137 genotypes of *Capsicum chinense* Jacq. by morphological descriptors and multivariate techniques, with a view to the identification of groups for promising crosses for breeding programs. The experiment was conducted in a greenhouse, arranged in a randomized complete block design with four replications, where each plot consisted of a pot with one plant. The 20 descriptors recommended by the *International Plant Genetic Resources Institute* - IPGRI were considered for the morphological characterization. By analysis of variance, significant differences between genotypes were detected for the studied descriptors. Clustering by the Tocher optimization method formed five groups, and by the hierarchical clustering method UPGMA, 11 groups. Based on larger distances intergroup, crosses are recommended among genotypes of the groups II x V, II x IV, and I x V for the Tocher method, and by UPGMA among genotypes of the groups VI x XI, II x XI, IV x XI,. The cophenetic correlation coefficient for the hierarchical clustering method UPGMA was 0.797 (p <0.01). The traits that contributed most to the total genetic diversity were number of days to flowering and plant height.

Index terms: *Capsicum chinense* Jacq., morphological evaluation, multivariate techniques, plant breeding.

RESUMO

O conhecimento da divergência genética em coleções de germoplasma é importante para fins de conservação e uso em programas de melhoramento genético, reduzindo tempo e custo na obtenção de novos genótipos. Neste trabalho, objetivou-se avaliar a divergência genética entre 137 genótipos de *Capsicum chinense* Jacq., por meio de descritores morfológicos, empregando-se técnicas multivariadas, para a identificação de grupos de intercruzamentos com potencial uso em programas de melhoramento. O experimento foi conduzido em casa de vegetação, sendo os genótipos dispostos no delineamento de blocos completos casualizados, com quatro repetições, cada parcela constituída de um vaso com uma planta. Para a caracterização morfológica foram considerados vinte descritores entre os recomendados pelo *International Plant Genetic Resources Institute – IPGRI*. Pela análise de variância, evidenciaram-se diferenças significativas entre os genótipos para os descritores estudados. O método de otimização de Tocher detectou a formação de cinco grupos e, o método de agrupamento hierárquico UPGMA, a formação de 11 grupos. Com base nas maiores distâncias intergrupos, recomenda-se cruzamentos, pelo método de otimização de Tocher, entre genótipos dos grupos II com V, II com IV, I com V, e, pelo método hierárquico UPGMA, entre genótipos dos grupos VI com XI, II com XI, IV com XI. O coeficiente de correlação cofenético para o agrupamento hierárquico UPGMA foi de 0,797 (p<0,01). Os descritores que mais contribuíram para a divergência genética total foram número de dias para florescimento e altura de plantas.

Termos para indexação: *Capsicum chinense* Jacq., avaliação morfológica, técnicas multivariadas, melhoramento de plantas.

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INTRODUCTION

Brazil is an important center of diversity of the genus *Capsicum* spp, where domesticated as well as semidomesticated and wild species are found (CARVALHO et al., 2003), allowing the exploration of this variability in breeding programs. According to Carvalho and Bianchetti (2008), the domesticated species in Brazil are *C. annuum* L. var. *annuum, C. baccatum* var. *pendulum* (Wild) Eshbaugh, *C. frutescens* L., and *C. chinense* Jacq.

The species *C. chinense* Jacq. is noteworthy for being considered the most Brazilian of the domesticated species, since the Amazon is its largest center of genetic diversity (CARVALHO; BIANCHETTI, 2008). According to Reifschneider (2000), the species is also found in the Midwest and Northeastern regions of Brazil. It comprises morphotypes known as pimenta de cheiro, pimenta bode, cumari do Pará, murupi, habanero, and biquinho. Also, according to Reifschneider (2000), in the states of midwestern Brazil, the most relevant morphotypes are pimenta bode and

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pimenta de cheiro, widely used in cooking as a seasoning, aside from being sold as canned and preserved food. For being remarkably adaptable to tropical and equatorial climate conditions and due to its great genetic variability, particularly evident in the fruits that can have different shapes, colors, sizes, and pungency levels, *Capsicum chinense* is among the most produced and consumed peppers in Brazil (CARVALHO et al., 2003; LANNES et al., 2007).

In view of the economic importance of the species, it is appropriate to study the genetic divergence as a useful and effective tool of separation of accessions in genebanks (REGO et al., 2003), as well as for the identification of suitable parents for hybrids with higher heterotic effect (CRUZ; CARNEIRO, 2006). For the species *Capsicum chinense* Jacq., significant heterosis has been found, making breeding for commercial exploitation viable (SOUSA; MALUF, 2000).

The success in breeding programs depends on divergent and promising plant material. To generate segregating populations for selection, the genetic complementation of the parents must be good, so the clustering techniques can assist in guiding the parent choice. In this sense, Benin et al. (2002) mentioned that genotypes in more distant clusters are possibly dissimilar, and can be considered promising for artificial crosses.

Few black pepper cultivars are available on the market, therefore, according to Bento et al. (2007), there is a great need for new cultivars that combine resistance to the major pepper pests and diseases with quality of taste and yield, especially to supply the agroindustry sector.

Within this context, numerous studies using multivariate analysis have been conducted to assess the genetic divergence, with the purpose of rationalizing breeding programs, by reducing the time required to develop promising accessions and consequently, spare costs. For the genus Capsicum, this technique was used by Sudré et al. (2005), Bento et al. (2007), Monteiro et al. (2010), Moura et al. (2010), among others.

The purpose of this study was to evaluate the genetic diversity of genotypes of the species *Capsicum chinense* Jacq., from the southwestern region of the State of Goiás, based on 20 descriptors, using multivariate analysis techniques to identify groups of promising intercrosses for pepper breeding programs.

MATERIALS AND METHODS

The experiment was conducted in Jataí, in the southwestern region of Goiás state (latitude 17°53'S, longitude 51°44'W, altitude 696 m asl) with a humid-tropical savanna, mesothermal climate, with rainy summers and dry winters, Awa according to the Köppen classification.

For the study, 137 genotypes of *Capsicum chinense* Jacq. were found on markets or donated by farmers in the southwestern region of Goiás State. At the time of sampling, about 20 mature fruits per plant were harvested for seed extraction. These were sown in polystyrene trays filled with commercial substrate, and left to grow in a greenhouse with anti-aphid mesh. Seedlings were transplanted 60 days after sowing to 8L plastic pots, filled with sifted soil and fertilized with yoorin Master®, at a rate of 2 g kg^{-1} soil.

The experiment was arranged in a randomized complete block design with 137 treatments (genotypes) and four replications. Each experimental unit consisted of a pot with one plant. Phosphorus (P) and potassium (K) were applied monthly and sprays of insecticides and fungicides biweekly during cultivation, as recommended by Filgueira (2000). At this stage, drip irrigation was automatically applied every three hours. The entire experiment was conducted in a greenhouse.

For the morphological characterization, 20 descriptors, which are part of the set recommended by the International Plant Genetic Resources Institute - IPGRI (1995), were used: plant height, height of first bifurcation, fruit peduncle length, fruit length, corolla length, mature leaf length, canopy diameter, stem diameter, fruit wall thickness, fruit width, width of the fruit peduncle, mature leaf width, 10-fruit weight, number of seeds, number of locules, number of days to flowering, days to fruit set, number of branches, 100-seed weight, and seed size. All evaluations were performed as recommended by Ipgri (1995).

Univariate statistical analysis and significance by the F test were performed for the 20 descriptors. Multivariate analysis was used to study the genetic divergence. The genetic distance matrix was estimated by Mahalanobis´ distance, as proposed by Cruz and Carneiro (2006), which was used as dissimilarity measure and applied for cluster analyses based on the Tocher optimization method described by Rao (1952) and the unweighted pair grouping method with arithmetic means (UPGMA), according to Sneath and Sokal (1973).

To evaluate the consistency of the dendrogram obtained by UPGMA, the cophenetic correlation coefficient (CCC) was calculated (SOKAL; ROHLF, 1962) with 1000 simulations and analyzed by the "t" test. After grouping, the intra and inter distances were computed and the means of each characteristic for the genotypes of each group estimated. The relative contribution of traits to divergence was obtained by the method of Singh (1981). All statistical analyses were performed using software GENES (CRUZ, 2008).

RESULTS AND DISCUSSION

Analysis of variance showed significant differences for all traits, evidencing the existence of variability and the possibility of selection among the genotypes studied.

By the Tocher method it was possible to cluster five groups (Table 1), with the largest number of genotypes in group I. The greatest distance between the minima was 72.42, for genotype 46 allocated in group V, with some characteristics that made it the most divergent of all (fruit and peduncle length, canopy diameter, fruit and peduncle width and 100- seed weight) (Table 2). It is also noteworthy that although most of the genotypes were assigned to group I, the six genotypes in the groups II, III, IV, and V have important traits that are interesting with a view to the choice of parents for a breeding program with specific breeding objectives, e.g.: plant height, canopy diameter, cycle, fruit size, branching, and number of seeds per fruit.

Table 1 – Grouping by the Tocher optimization method using 137 genotypes of *Capsicum chinense* Jacq., evaluated for 20 morphological descriptors.

Groups	Genotypes
I	94 100 97 102 103 98 85 93 105 89 104 88 87 99 86 95 101 75 96 71 84 92 81 127 91 66 76 79 4 38 37 68 78 21 35 80 70 8 73 20 69 82 51 28 43 57 49 64 72 30 107 13 9 136 10 62 83 134 17 27 128 6 41 137 74 63 65 36 53 61 14 25 19 124 106 33 34 132 15 59 90 12 111 44 55 60 126 113 130 2 110 109 40 47 118 23 135 1 77 39 117 122 125 67 115 116 120 108 7 119 16 5 42 24 3 114 133 56 48 121 123 58 11 29 18 52 54 112 22 129 50
II	31 32 26
III	45
IV	131
V	46

Table 2 – Averages for the descriptors in the groups formed by the Tocher optimization method.

Variables	Tocher groups				
	I	II	III	IV	V
Plant height (cm)	89.00a	63.96a	97.25a	128.25a	91.75a
Height of the 1st bifurcation (cm)	29.59a	19.67a	27.38a	70.50a	48.75a
Fruit length (mm)	23.05d	53.17a	30.18c	12.62e	43.04b
Peduncle length (mm)	22.54a	23.82a	24.97a	24.13a	35.45a
Canopy diameter (cm)	111.54a	112.00a	122.00a	94.25a	50.13a
Corolla length (mm)	11.51a	11.91a	14.47a	11.51a	12.46a
Leaf length (mm)	70.26a	77.64a	88.35a	98.56a	91.48a
Stem diameter (mm)	10.77a	11.39a	14.76a	10.74a	12.03a
Fruit wall thickness (mm)	1.69a	1.65a	2.50a	1.87a	1.99a
Fruit width (mm)	13.71a	13.61a	20.44a	15.71a	28.10a
Peduncle width (mm)	1.43c	1.59b	1.37c	1.56b	1.90a
Leaf width (mm)	31.14a	29.44a	33.04a	47.74a	41.71a
10-fruit weight (g)	14.15a	29.54a	56.01a	13.76a	69.09a
100-grain weight (g)	0.60a	0.63a	0.82a	0.54a	0.92a
Number of locules	2.83a	3.22a	3.38a	2.83a	3.00a
Number of days until fruit set	108.93a	97.17a	120.00a	123.00a	134.25a
Number of branches	4.33a	14.08a	1.00a	0.00a	2.25a
Number of days until fruit set	156.35a	152.33a	168.75a	180.50a	173.67a
Number of seeds per fruit	22.61a	28.35a	35.47a	9.43a	29.00a
Seed size (mm)	3.59a	3.62a	4.24a	3.58a	4.24a

Means followed by the same letter in the horizontal do not differ among themselves by the Tukey test at the level of 5% probability.

Of the genotypes studied, 96% were assigned to group I, by the Tocher optimization method. However, the genotypes of this group were very heterogeneous. The reason was, as previously reported, the large genetic divergence between genotype 46 and the others, since, as reported by Cruz and Carneiro (2006) the criterion for belonging to a group by this technique is based on a pre-set value; in this work, the greatest distance between the minima was used. Therefore, if a plant is very divergent, the minimum distance from it is high and it is used as intra-group measure. Consequently, genetically different plants can be grouped in the same group and, as in this study, a regrouping of Group I may be indicated for a better discrimination of the genotypes of this group.

It was observed (Table 3) that the intragroup distances were shortest, as determined by the grouping method. It was also observed that the largest distances between groups were between II and V, indicating that these groups are most divergent from the others. As reported by Miranda; Cruz and Costa (1988), the genetic distance of parents, based on a satisfactory set of traits, is potentially promising for an efficient and economic identification of advantageous hybrid combinations, which may contribute to the choice of the parents to be intercrossed to increase segregation and have a more successful breeding program.

Table 3 – Intra-and inter-group averages of Mahalanobis´ distance in *Capsicum chinense* Jacq., grouped by the Tocher method.

Group	I	II	III	IV	V
I	40.8	105.3	91.9	73.2	167.4
II		60.9	143.5	194.2	195.3
III			0.0	122.4	95.1
IV				0.0	156.7
V					0.0

By the UPGMA clustering method, it was possible to observe the distribution of genotypes in a larger number of groups, compared to the Tocher method. This was due to the different algorithms used in the methods. Eleven groups were formed by the UPGMA method, considering the cutting on the coordinate axis at a relative distance of 24% between the genotypes (Figure 1). This cutoff, although subjective, was determined from the observation of the point of high variation level, considered a threshold of the number of genotypes in the group (CRUZ; CARNEIRO, 2006). Sudré et al. (2005) used a cutoff at a distance of 30% in the dendrogram, and emphasized the importance of using more than one clustering method.

For the grouping of the genotypes considered most divergent (26, 31, 32, 45, 46, and 131) coincidence was observed between the groups formed by the Tocher and by the UPGMA analysis. This fact shows the great influence of these genotypes on determining the distance established by the Tocher method for the inclusion of a plant in the same group.

The cophenetic correlation coefficient for the hierarchical clustering method UPGMA was 0.797 and significant at 1% probability. This coefficient can be used to assess the consistency of the grouping pattern by hierarchical clustering methods, where values close to unity indicate better representativeness (CRUZ; CARNEIRO, 2006). Therefore, the dendrogram expressed a strong relation with the original data.

The intra and inter-group distances for the hierarchical clustering method UPGMA. (Table 4) show the shortest mean distances between the groups V and X (0.1), II and VI (0.3) and I and III (0.7), showing that the intercrossing of plants of these pairs of groups is not indicated when the heterotic effect is the target. The average distances were highest between the group pairs VI - XI (153.5), II - XI (153.2), and IV- XI (146.7). Therefore, these group pairs are considered the most divergent, with differences for most traits (see Table 4). Intercrossing of the genotypes of these groups is indicated to increase the variability and the possibility of expression of the heterotic effect. Genotype 46 of group V (Tocher) and of group XI (UPGMA) was the most divergent of all, as indicated by the greatest distances between groups (Table 3 and table 4), by both grouping methods.

Fruit-related traits are highly important for pepper breeding, since the consumer market determines the form of consumption and acceptance. Ripe, small and round fruits are preferentially consumed in the Northeast, and thin and elongated peppers in the Southeast (REIFSCHNEIDER, 2000). In this study, variability among the genotypes under study was verified for fruit-related variables such as length, width, 10-fruit weight, and fruit wall thickness, allowing the selection of parents for crosses with a view to the market demand.

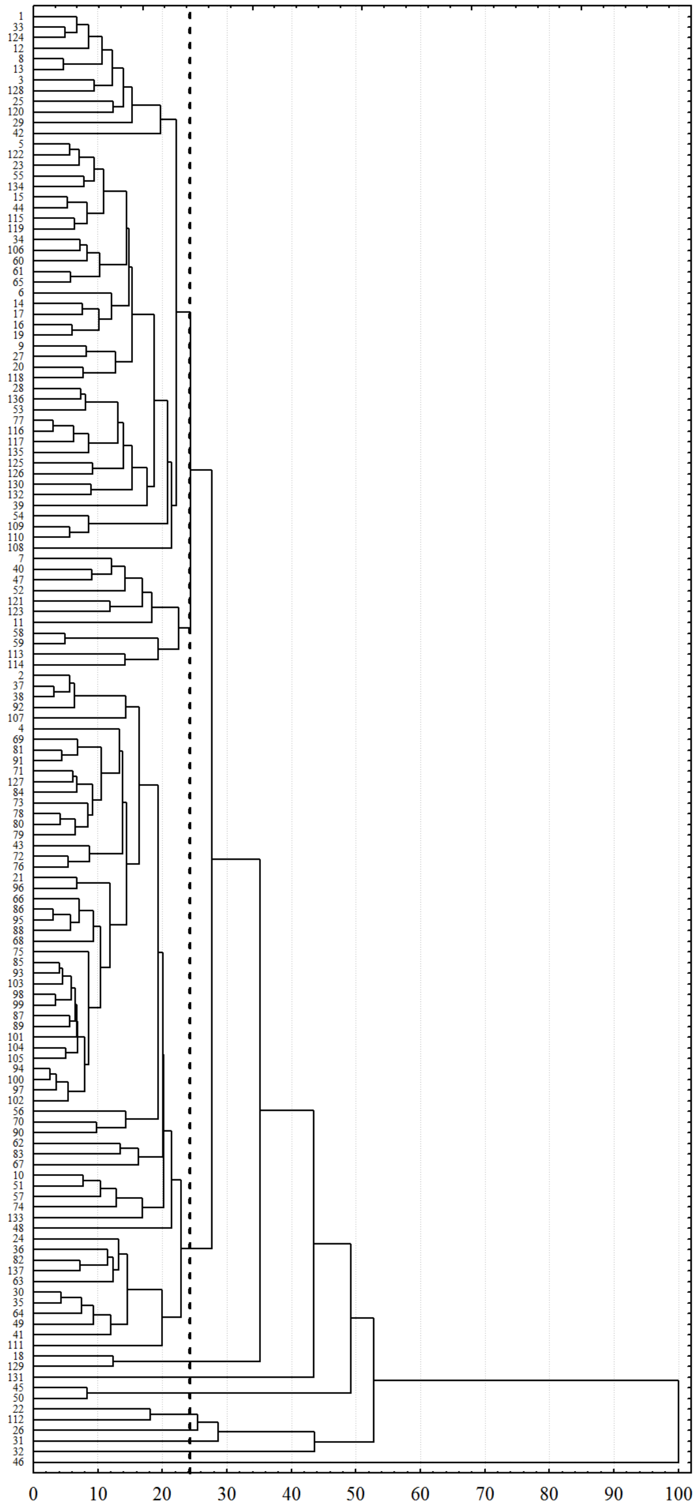

Figure 1 – UPGMA dendrogram of 137 genotypes of *Capsicum chinense* Jacq. using Mahalanobis´ distance for the studied variables.

Table 4 – Intra-and inter-group averages of Mahalanobis´ distance in *Capsicum chinense* Jacq., clustered by the UPGMA method.

Group	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	16.5	24.5	0.7	18.0	33.9	24.8	8.6	3.9	9.1	34.0	128.7
II		11.9	23.8	6.5	58.4	0.3	15.9	28.4	33.6	58.5	153.2
III			18.9	17.3	34.6	24.1	7.9	4.6	9.8	34.7	129.4
IV				20.7	51.9	6.8	9.4	21.9	27.1	52.0	146.7
V					0.0	58.7	42.5	30.0	24.8	0.1	94.8
VI						14.0	16.2	28.7	33.9	58.8	153.5
VII							30.3	12.5	17.7	42.6	137.3
VIII								0.0	5.2	30.1	124.8
IX									0.0	24.9	119.6
X										0.0	94.7
XI											0.0

Plant height and cycle are highly important in crop management, making group VIII, IX and X (UPGMA) interesting, for the traits of genotypes 26, 31 e 32 which could be used in breeding programs for short and early plants. The averages of the variables of the other groups are shown in table 5.

The two clustering methods were partially concordant in the separation of genotypes, allowing an improvement of group I, discriminated by Tocher, by the UPGMA method, and to keep the most divergent genotypes in separate groups.

By the method of Singh (1981), used to evaluate the relative importance of the 20 descriptors, it was found that 10 descriptors contributed with 97.07% to the total variation. The characteristics that most contributed by the method to total variation was the number of days to flowering (22.26%), followed by plant height (19.44%), leaf length (12.18%), diameter canopy (12.10%), number of seeds per fruit (9.54%), 10-fruit weight (6.41%) and height of the first bifurcation (6.41%). Number of days to flowering, plant height and canopy diameter are traits associated with the early reproductive stage or plant size, and therefore have a high agronomic value. The other traits contributed little to the total divergence, so in future studies they may serve as a reference in the choice of descriptors that should be measured.

These results disagree with results of Bento et al. (2007), who found that the two variables that contributed most to genetic diversity were number of fruits per plant (45.99%)and number of seeds per fruit (21.88%), and of Sudré et al. (2005), who identified fruit length (32%) and fruit diameter (32%) as the most divergent. However, both studies addressed the genus *Capsicum* spp, not only the species *Capsicum chinense* Jacq, as in this work. In a study of Ferrão et al. (2011) on *C. baccatum*, the trait that contributed most to the differentiation was fruit length (40.9%), and the value was lowest (0.5%) for the number of locules.

Table 5 – Averages of the 20 descriptors for the groups formed by the hierarchical clustering method UPGMA.

Variables	UPGMA Groups										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Plant height (cm)	85.46de	86.13de	92.34cd	113.66b	128.25a	100.50c	79.50e	66.50f	63.38f	62.00f	91.75cd
Height of the 1st bifurcation (cm)	26.07d	22.38de	33.10c	44.38b	70.50a	34.31c	32.50c	18.50e	21.50e	19.00e	48.75b
Fruit length (mm)	29.75c	14.34e	15.26e	19.73d	12.62e	31.98c	42.53b	54.25a	53.20a	52.05a	43.04b
Pdeuncle length (mm)	22.89f	22.08fg	21.64fg	31.71b	24.13e	25.93d	26.56d	28.54c	22.29f	20.63g	35.45a
Canopy diameter (cm)	107.76cd	109.65cd	115.24bc	111.25cd	94.25e	107.63cd	139.13a	105.25d	121.75b	109.00cd	50.13f
Corolla length (mm)	11.96cd	11.21de	10.93e	12.69bc	11.51de	14.38a	11.47de	13.01ab	12.45bc	10.27f	12.46bc
Leaf length (mm)	67.13g	76.09ef	72.25fg	77.08ef	98.56a	97.69ab	80.30de	86.08cd	71.51fg	75.32ef	91.48bc
Stem diameter (mm)	10.33f	11.55cde	11.18def	10.38f	10.74ef	14.54a	10.88ef	8.95g	12.22bc	13.00b	12.03cd
Fruit wall thickness (mm)	1.71ef	2.25b	1.63f	1.62f	1.87cd	2.46a	1.79de	1.56f	1.82de	1.58f	1.99c
Fruit width (mm)	14.30f	13.21f	12.96fg	9.98g	15.71i	20.97d	16.77b	18.53d	11.19c	11.11hi	28.10a
Peduncle width (mm)	1.44de	1.64b	1.40e	1.29f	1.56bc	1.49cde	1.84a	1.52cd	1.67b	1.59bc	1.90a
Leaf width (mm)	30.03g	33.54ef	31.72fg	34.77de	47.74a	36.95cd	38.43c	36.22cde	26.19h	25.90h	41.71b
10-fruit weight (g)	17.81f	10.53h	9.38hi	7.08i	13.76g	52.23b	29.19d	36.51c	28.85d	23.24e	69.09a
100-grain weight (g)	0.62a	0.44a	0.60a	0.54a	0.54a	0.77a	0.59a	0.57a	0.73a	0.57a	0.92a
Number of locules	2.82ef	2.45h	2.85de	2.68f	2.83ef	3.41b	3.20c	3.80a	3.20c	2.67g	3.00d
Number of days to flowering	106.71d	102.25e	111.27cd	112.25c	123.00b	127.13b	107.38cd	98.50ef	97.25ef	95.75f	134.25a
Number of branches	4.27bc	3.28bcd	4.55b	4.29bc	0.00e	0.63e	3.38cd	8.25a	4.50bc	9.50a	2.25d
Number of days to fruiting	153.89ef	149.83fg	158.88de	170.13bc	180.50a	172.38bc	149.13fg	143.25g	165.50cd	148.25fg	173.67b
Number of seeds per fruit	23.88ef	25.51de	21.12f	5.13g	9.43g	33.33ab	33.38a	32.05abc	25.00def	28.01cde	29.00bcd
Seed size (mm)	3.68c	3.00g	3.52d	3.13f	3.58cd	4.18a	3.48d	3.97b	3.32e	3.57cd	4.24a

Means followed by the same letter in the horizontal do not differ among themselves by the Tukey test at the level of 5% probability.

CONCLUSIONS

There is genetic divergence among the accessions of *C. chinense* for all the descriptors, indicating a potential use in breeding programs. The traits that contributed most to the divergence are number of days to flowering, plant height, leaf length, and canopy diameter.

The groups formed by Tocher and UPGMA methods are partially coincident in the separation of the genotypes, improving the discrimination by Tocher of Group I with UPGMA.

The most promising crosses between genotypes of groups clustered by the Tocher optimization method were II x V, II x IV, and V x I, and by UPGMA crosses between VI x XI, II x XI, and IV x XI.

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