



## Karyotypic characterization of *Capsicum* sp. accessions

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**ABSTRACT.** This study aimed to determine the chromosome number and the karyotype of *Capsicum annum*, *Capsicum chinense*, *Capsicum frutescens* and *Capsicum baccatum* accessions in the active *Capsicum* sp. genebank at the Federal University of Piauí (BGC-UFPI). These species have great economic importance throughout the world, and their cytogenetic characterization can inform taxonomy and lead to improvement in the genus. Karyotypes were obtained from the rootlet meristems of the studied accessions using the squash method and Giemsa staining. The chromosome number  $2n=2x=24$  was verified for each of the four species. Chromosomal polymorphisms were observed for the *C. frutescens* accession BGC 37, which presented 12 pairs of metacentric chromosomes instead of 11 pairs of metacentric chromosomes and 1 submetacentric chromosome pair present in other accessions. In the accessions BGC 01 and BGC 37, secondary constrictions were observed in the 1 and 12 as well as in the 6 and 11 homologs, respectively. The karyotypes of the studied species were asymmetrical among themselves. The results obtained in this study confirm the high genetic diversity previously described in the literature for this genus.

**Keywords:** pepper, cytogenetics, genebank, morphometry.

### Caracterização cariotípica de acessos de *Capsicum* sp.

**RESUMO.** Este trabalho teve por objetivo caracterizar numérica e morfológicamente os cariótipos de acessos de *Capsicum annum*, *Capsicum chinense*, *Capsicum frutescens* e *Capsicum baccatum* pertencentes à coleção do Banco ativo de Germoplasma de *Capsicum* sp. da Universidade Federal do Piauí (BGC-UFPI). Estas espécies têm grande importância econômica em todo mundo, e a caracterização citogenética fornece informações importantes para estudos de taxonomia bem como para programas de melhoramento genético do gênero na qual estão inseridas. Os cariótipos foram obtidos pelo método de esmagamento e coloração com Giemsa de células dos meristemas das radículas dos acessos estudados. A partir dos resultados verificou-se número cromossômico  $2n = 2x = 24$  para todas as quatro espécies. Foi observado polimorfismo cromossômico para o acesso BGC 37 da espécie *C. frutescens*, que apresentou 12 pares de cromossomos metacêntricos, diferindo dos outros que apresentaram 11 pares de cromossomos metacêntricos e 1 par de cromossomo sub-metacêntrico. Visualizou-se nos acessos BGC 01 e BGC 37 desta espécie constrições secundárias nos homólogos 1 e 12, e 6 e 11, respectivamente. Os cariótipos das espécies estudadas foram assimétricos entre si. Os resultados obtidos neste estudo corroboram com a grande diversidade genética descrita na literatura para o gênero *Capsicum*.

**Palavras-chave:** pimenta, citogenética, banco de germoplasma, morfometria.

### Introduction

The *Capsicum* sp. peppers of the Solanaceae family, which are found in tropical and temperate regions worldwide, are valued as spices or vegetables by many different cultures. The genus has significant economic importance for the national and international condiment, seasoning and canning markets, and it is cultivated at scales ranging from family production to industrial systems (FONSECA et al., 2008). These peppers are also employed in phytotherapeutic medicine, primarily in South and Latin American countries. The genus contains high

concentrations of vitamins A and C,  $\beta$ -carotene and capsaicins, which have been shown to provide antioxidant, antimicrobial, anti-inflammatory and hypocholesterolemic action among other benefits (COSTA et al., 2008; ZENI; BOSIO, 2011).

Four domesticated species of *Capsicum* are widely grown and consumed in Brazil: *C. annum* L. (bell pepper, sweet pepper), *C. chinense* Jacq. (Yellow Lantern Chili), *C. frutescens* L. (Malagueta Pepper) and *C. baccatum* L. (Bishop's Crown) (LANNES et al., 2007). These species are highly adapted to tropical climate conditions and contain high biological diversity, which is presented by the variety of fruit

sizes, forms and coloration (HAVERROTH; NEGREIROS, 2011; ZENI; BOSIO, 2011).

Brazil is considered a natural habitat of these peppers, and the main pepper-producing states are Minas Gerais, São Paulo, Rio de Janeiro, Ceará and Bahia (ZENI; BOSIO, 2011). However, knowledge about the genetic diversity of these plants in Brazil is still rudimentary despite a significant research commitment to biochemical, molecular and cytogenetic studies of the species over the past two decades (HAVERROTH; NEGREIROS, 2011).

Studies of pepper chromosome number and morphology generate important data for *Capsicum* taxonomy (SOUZA et al., 2011), contribute to the understanding of the genetic variations involved in the evolution of the genus (MOSCONE et al., 2007), aid in the delineation of the cultivated, semi-cultivated and wild species (CARVALHO; BIANCHETTI, 2008; PEREIRA et al., 2006) and further plant diversity conservation by supplying information to aid genetic improvement programs for this genus (SOUZA et al., 2011). Rohani et al. (2010) mentioned that cytogenetic studies of different *Capsicum* genebank accessions comprise an important data source for breeders, which allows for better gene pool administration and a more efficient selection of genetic resources.

Because of the economic relevance and genetic variability described in the literature for *C. annuum*, *C. chinense*, *C. frutescens* and *C. baccatum* along with the relative lack of data for these species, this study aimed to characterize and analyze the karyotypes of accessions from the collection of the active *Capsicum* sp. genebank of the Federal University of Piauí (BGC-UFPI). In addition, the data on the agricultural production of peppers in the state of Piauí are imprecise and irregular as small producers dominate production in the region. Commercialization of this product involves small homemade or artisanal operations for sauces, preserves, jellies and powdered pepper, as well as small companies that directly sell their products to street markets and small supermarkets. However, Piauí possesses environmental conditions, such as temperature and soil type, that are quite favorable for the large-scale production of these four *Capsicum* species. Cytogenetic studies of these species may aid in the genetic enhancement and expansion of production of this genus in Piauí through better characterizing their variability.

## Material and methods

### Material

The accessions of the studied species were obtained from the active *Capsicum* sp. genebank at the Federal University of Piauí, which is located in

the city of Teresina. Four accessions of the species *C. annuum* (BGC 34, BGC 36, BGC 39 and BGC 59), two accessions of the species *C. chinense* (BGC 07 and BGC 49), two accessions of the species *C. frutescens* (BGC 01 and BGC 37) and four accessions of the species *C. baccatum* (BGC 21, BGC 26, BGC 27 and BGC 54) were cytogenetically characterized.

The geographical distances between the species were also taken into consideration. The accessions BGC 39, BGC 36, BGC 01, BGC 37, BGC 21 and BGC 26 were collected in Teresina, Piauí State; the accessions BGC 34, BGC 54 and BGC 07 were acquired in São Raimundo Nonato, Piauí State, Pedro II, Piauí State and Piripiri, respectively; the accession BGC 49 was collected in the city of São Paulo (SP); and the accessions BGC 59 and BGC 27 were collected in São Luís do Maranhão, Maranhão State and São Francisco, Maranhão State, respectively.

Seeds of these accessions were germinated in the Biochemical Genetics and DNA Sequencing laboratory (LGBS) of the Biology Department of the Pernambuco Rural Federal University (UFRPE) in January 2011. The seeds were placed in a growth chamber under room temperature and controlled lighting (12-hour days) in Petri dishes covered with filter paper and moistened daily with distilled water for five days.

### Cytogenetic evaluation

The cytogenetic analyses were conducted in the same laboratory in the first semester (January-July) of 2011. The rootlets (approximately 0.5 cm) of the different accessions were collected and pre-treated with 8-hydroxyquinoline (2 mM) for four hours at room temperature. The rootlets were washed in distilled water, fixed in a solution of Carnoy-absolute ethanol and glacial acetic acid (3:1) for 24 hours and preserved at -20°C. For the preparation of the slides, the fixed rootlets were washed in distilled water and hydrolyzed in 1 N HCl at room temperature for 10 minutes.

After hydrolysis, the rootlets were washed again in distilled water and slides for each accession were prepared. For the preparation of each sheet, a rootlet tip was removed with a scalpel, macerated in a drop of 45% glacial acetic acid and covered with a coverslip. The coverslips were removed with nitrogen, and the slides were air-dried and stained with 2% Giemsa stain for 10 to 15 minutes and mounted with Entellan (Merck®).

### Karyotypic analysis

The slides were analyzed at 100X magnification using a *Leica DM 2500* microscope equipped with a *DC 345 FX* digital microcamera coupled to a

microcomputer for direct capture of the images of interest. The images were obtained using the Image-Pro Plus program (version 5.1).

Five cells in metaphase from each accession, each corresponding to one repetition, were used for the chromosome measurements, which were conducted with the aid of the MicroMeasure 3.3 Program (REEVES; TEAR, 2000). The following average values were obtained: a) length of the long arm (LA); b) length of the short arm (SL); c) total length of the chromosome (TCL = LA + SA); d) total length of the haploid batch (TLHB =  $\sum$  TCL); and e) relative length ( $r = (TCL/TLHB) \times 100$ ).

The identification of the homologs was conducted using the selected parameters described above. The chromosomes were classified as metacentric, submetacentric, acrocentric or telocentric based on the ratio between the arms ( $r$ ) and centromere index ( $ic$ ), which was proposed by Guerra (1986). The accessions were also analyzed using the Asymmetry Index (AI) according to Romero-Zarco (1986).

#### Data analysis

The accessions were compared for two parameters: TLHB and TCL. Analysis was conducted as a randomized design with five repetitions using the following model:

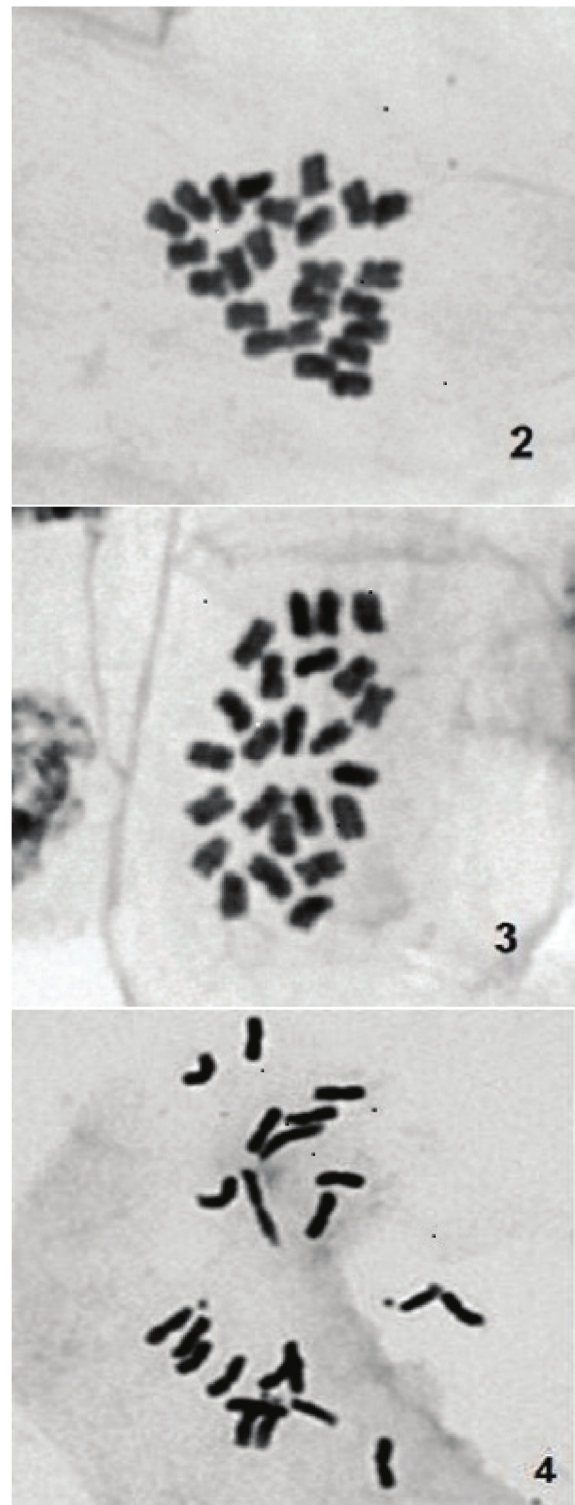
Variance analysis was conducted using Tukey's test for the comparison of averages (1949) at a 5% level of significance. All analyses were calculated using the Genes software (CRUZ, 2006).

#### Results and discussion

Giemsa staining of the *C. annuum*, *C. chinense*, *C. frutescens* and *C. baccatum* accessions allowed for accurate descriptions of the number, morphology and size of the chromosomes, type of interphase nucleus and chromosomal condensation pattern. The majority of the chromosomes did not display differentiated areas along their length aside from the centromeric region and visualization of secondary constrictions in the accessions of one species.

For each of the 12 accessions, a chromosome number of  $2n = 2x = 24$  was confirmed (Figure 1); this ploidy level is widely described in the literature for a number of *Capsicum* species (GUERRA, 2001; MOSCONE et al., 2007; PICKERSGILL, 1997; POZZOBON, WITTMANN, 2006; SOUZA et al., 2011; TEODORO-PARDO et al., 2007;) and is also common in the Solanaceae family (PICKERSGILL, 2007). However, in some wild *Capsicum*, such as *C. buforum*, *C. capylopodium* and *C. cornutum*, a ploidy level of  $2n = 2x = 26$  has been reported (POZZOBON; WITTMANN, 2006). Pozzobon and Wittmann (2006) suggest that two different evolutionary lines emerged in the diversification of this genus, which is shown by a clear separation between the wild species

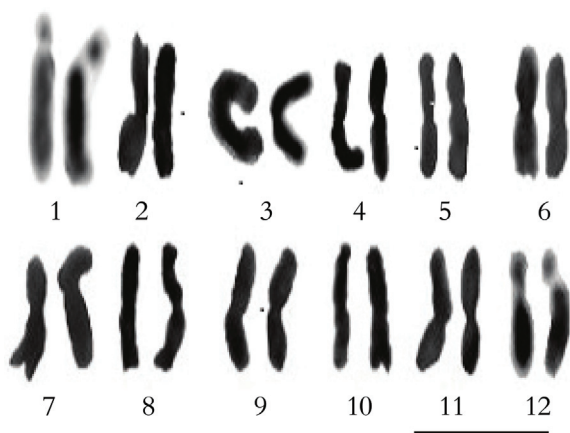
(base number  $x = 13$ ) and domesticated species ( $x = 12$ ). These authors also hypothesized that the  $x = 13$  lines are ancestral to the  $x = 12$  plants.



**Figure 1.** (1) Metaphasic chromosomes of *C. annuum* (BGC 39) (2) Metaphasic chromosome of *C. chinense* (BGC 49), (3) Metaphasic chromosomes of *C. frutescens* (BGC 01), and (4) Metaphasic chromosomes of *C. baccatum* (BGC 21). Bar = 10 mm.

Of the studied accessions, 11 presented the karyotypic formula 11M + 1SM (Figure 2), in which chromosome 12 was classified as submetacentric. The *C. frutescens* accession BGC 37 presented the karyotypic formula 12M (Table 1, Figure 3), which demonstrates chromosomal polymorphisms in relation to the other accessions.

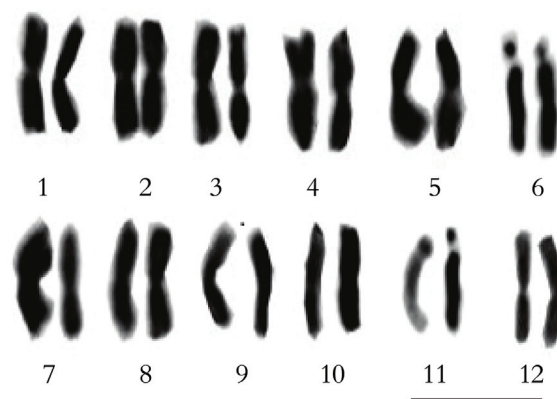
However, the karyotypic formulas reported in the literature conflict with those observed in the present study. Guerra (2001) reported the karyotypic formula 11M + 1A for a number of Venezuelan accessions of *C. chinense* using Giemsa stain. Souza et al. (2011) also observed the formula 11M + 1A through conventional cytogenetics in *C. chinense* accessions from different states of Brazil. Sousa et al. (2011) and Moscone et al. (1996) analyzed the evolutionary patterns across species of *Capsicum* by chromosome banding and observed the karyotypic formulas of 11M + 1SM + 1A and 11M + 1A for *C. frutescens*, respectively.



**Figure 2.** Diploid karyogram representative of the *Capsicum frutescens* (BGC 01) karyotype with karyotypic formula 12M. (bar = 10  $\mu$ m).

Teodoro-Pardo et al. (2007) explained how different karyotypic formulas in a single species can

occur due to genetic variations among populations, which are generated from the genomic response to different environments. The same authors discuss how the appearance of polymorphisms at the chromosomal level in individuals of the same population can alter the karyotypic pattern of those specimens and originate distinct chromosomal races.



**Figure 3.** Diploid karyogram representative of the *Capsicum frutescens* (BGC 37) karyotype with the karyotypic formula 12M. (bar = 10  $\mu$ m).

According to Moscone et al. (2007), differences in the morphology, size and number of chromosomes are common in populations of the same species or in interspecific taxa, and these differences are grouped into cytotypes or chromosomal races. These authors affirmed that such differences are frequent in the genus *Capsicum*, in which cytotypes differ mainly in karyotypic formula and chromosome size. For the *C. frutescens* accessions BGC 01 and BGC 37, secondary constrictions were observed in the homologous pairs 1 and 12 and 6 and 11, respectively (Figures 2 and 3).

Moscone et al. (1996) reported the presence of secondary constrictions in every *Capsicum* species, which ranged from 1 to 4 per karyotype.

**Table 1.** Average values, in micrometers, of CSI, CI, r, AI, and KF of four accessions of the species *C. annuum* (BGC 34, BGC 36, BGC 39, BGC 59), two accessions of the species *C. chinense* (BGC 07, BGC 49), two accessions of the species *C. frutescens* (BGC 01, BGC 37) and four accessions of the species *C. baccatum* (BGC 21, BGC 26, BGC 27, BGC 54).

Species /Accession	2n	CSI	CI	r	AI (%)	KF
<i>C. annuum</i> - BGC 34	24	6.92 – 3.64	45.34	1.22	45.61	11M+1SM
<i>C. annuum</i> - BGC 39	24	6.49 – 3.59	45.64	1.20	45.81	11M+1SM
<i>C. annuum</i> - BGC 36	24	7.05 – 3.69	45.53	1.21	45.77	11M+1SM
<i>C. annuum</i> - BGC 59	24	7.42 – 4.42	44.63	1.25	44.68	11M+1SM
<i>C. chinense</i> - BGC 07	24	6.60 – 4.56	45.80	1.19	45.97	11M+1SM
<i>C. chinense</i> - BGC 49	24	5.82 – 3.29	45.88	1.07	46.05	11M+1SM
<i>C. frutescens</i> - BGC 01	24	7.17 – 4.21	45.19	1.12	45.46	11M+1SM
<i>C. frutescens</i> - BGC 37	24	5.73 – 3.60	46.55	1.06	46.54	12M
<i>C. baccatum</i> - BGC 21	24	7.00 – 4.45	47.45	1.18	47.64	11M+1SM
<i>C. baccatum</i> - BGC 54	24	7.48 – 4.31	44.90	1.22	45.08	11M+1SM
<i>C. baccatum</i> - BGC 26	24	6.92 – 3.49	47.60	1.22	45.51	11M+1SM
<i>C. baccatum</i> - BGC 27	24	6.56 – 3.75	45.70	1.19	46.02	11M+1SM

CSI: chromosome size interval, CI: centomere index, r: chromosome arm ratio, AI: asymmetry index and KF: karyotypic formula.



The average size of the chromosomes observed in the present work varied from 3.29 (BAC 49) to 7.48  $\mu\text{m}$  (BGC 54) (Table 1). This result diverges from the data found in the literature: Souza et al. (2011) reported an average chromosome size of 2.59 to 4.12  $\mu\text{m}$  in four Brazilian *C. chinense* accessions, and Teodoro-Pardo et al. (2007) reported values of 1.6 to 8.4  $\mu\text{m}$ , 2.4 to 2.9  $\mu\text{m}$  and 2.1 to 5.2  $\mu\text{m}$  for *C. annuum* karyotypes from three Mexican states.

A high frequency of metacentric chromosomes was observed in the complements of the 12 accessions, which is indicated by the average values of the centromere indices (CI) and ratios obtained between the arms of the chromosomes (r) (Table 1). The results indicate symmetrical karyotypes in the studied accessions, especially in the *C. frutescens* accession BGC 37, which presented only metacentric chromosomes. According to Stebbins (1958), plants with higher karyotypic symmetry than others of the same genus are evolutionarily ancestral to those that possess lower symmetry.

Wadt et al. (2004) reported that although most of *Capsicum* species are  $2n = 24$  and present high similarity in chromosome morphology, the genus possesses high intra- and interspecific karyotypic variability. This observation is supported by the results of the asymmetry index (AI%) among the karyotypes of the species studied in this work (Table 1), which varied from 44.68 (BGC 59) to 47.64% (BGC 21). These values are under 50% and demonstrate that the karyotypes are asymmetrical amongst themselves according to Romero-Zarco (1986).

According to Moscone et al. (2007), a higher asymmetry index among karyotypes of species in the same genus is correlated to higher genetic variability among them. In the species studied here, the various fruit shapes and sizes can be cited as examples of variability: the fruit of *C. annuum* is a small berry with a smooth texture (MOSCONE et al., 2007); the fruits of *C. chinense* are elongated, which measure approximately 1.0 to 1.5 cm in diameter and possess a smooth texture (NUEZ-VIÑALS et al., 2003); the fruit of *C. frutescens* is a smooth fusiform berry (BENTO et al., 2007); and the fruits of *C. baccatum* are smooth and round but variable in size and shape and average 0.6 cm in diameter and 10 cm in length (CARVALHO et al., 2009).

According to Carvalho and Bianchetti (2008), the flowers of *Capsicum* can also be used to observe the genetic variability of the genus because there is significant diversity in the number of flowers per node, flower and peduncle position, corolla and

anther color, presence or absence of spots on the petal lobes and cup edge.

The studied accessions were grouped using Tukey's test at an error rate of 5% (Table 2), and the results showed that significant differences do not exist among the accessions BGC 07, BGC 21, BGC 54, BGC 36, BGC 34 and BGC 27 in terms of the TCL and TLHB parameters.

**Table 2.** Average TCL and TLHB values, expressed in micrometers ( $\mu\text{m}$ ), for the accessions of the species *C. annuum* (BGC 34, BGC 39, BGC 36, BGC 59), *C. chinense* (BGC 07, BGC 49), *C. frutescens* (BGC 01, BGC 37) and *C. baccatum* (BGC 21, BGC 54, BGC 26, BGC 27).

Accession	Species	TCL	Group	TLHB	Group
BGC 01	<i>C. frutescens</i>	6.02	a	72.22	a
BGC 59	<i>C. annuum</i>	5.82	ab	69.89	ab
BGC 07	<i>C. chinense</i>	5.78	abc	69.35	abc
BGC 21	<i>C. baccatum</i>	5.69	abc	68.26	abc
BGC 54	<i>C. baccatum</i>	5.63	abc	67.51	abc
BGC 36	<i>C. annuum</i>	5.42	abc	65.01	abc
BGC 34	<i>C. annuum</i>	5.34	abc	64.11	abc
BGC 26	<i>C. baccatum</i>	5.28	c	63.38	c
BGC 27	<i>C. baccatum</i>	5.24	abc	62.84	abc
BGC 39	<i>C. annuum</i>	4.89	bc	58.65	bc
BGC 49	<i>C. chinense</i>	4.65	ab	55.86	ab
BGC 37	<i>C. frutescens</i>	4.41	ab	52.96	ab

TCL: Total chromosome length; TLHB: Total Length of Haploid Batch. Accessions followed by the same letter were grouped by Tukey test at 5%.

The accession BGC 01 differs significantly from the accessions BGC 39 and BGC 26, which were all collected in Piauí, the accession BGC 26, which was from Piauí, and differs significantly from the accessions BGC 37 and BGC 49, which were collected in Piauí and São Paulo, respectively, in relation to these parameters (Table 2). Davide et al. (2007) claim that the presence of karyotypic asymmetry that is associated with significant differences of TLHB and TCL among individuals of the same or closely related species may be the result of chromosomal alterations, such as Robertsonian translocations, inversions, unequal translocations, deletions and duplications. The same authors explain that these alterations can occur because of the environmental conditions, including climate, soil, temperature and moisture, that the individuals are subjected. According to Livingstone et al. (1999) chromosomal alterations, such as translocations, duplications and deletions, have been observed in a large number of plants in the species *C. annuum* and *C. chinense*.

The results obtained in this work demonstrate variation among the studied species, which is in agreement with the scientific literature; however, the species presented symmetrical karyotypes and identical chromosomal numbers. Thus, wider sampling and more detailed characterization of the

chromosomes, through heterochromatin distribution and identification of sequences by *in situ* hybridization, will be necessary to determine if the differences among the species characterize intra- and/or interspecific differentiation. Such approaches will be able to discriminate among species with the same karyotypic formula.

## Conclusion

The results of the present study, when combined with future cytogenetic studies using the BGC - UFPI accessions, will be of significant value to genetic enhancement programs for *Capsicum* in the state of Piauí. This research will aid in the development of higher quality and more productive varieties that are more resistant to pests and diseases, which will encourage farmers to produce more of these vegetables. Increased production will generate economic benefits by employing more labor during planting and meet the market demands of the state.

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