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# Karyomorphology of Caesalpinia Species (Caesalpinioideae: Fabaceae) from Caatinga and Mata Atlantica Biomes of Brazil

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**Abstract**

Out of 140 *Caesalpinia s.l.* species, only 20 species have the chromosome numbers presently known, and nine species have the karyomorphological studies available. We determine the karyotype and the chromosome morphometry in five *Caesalpinia s.l.* spp., and we describe the heterochromatin pattern in four of them. The diploid chromosome number of 24 was reported for the first time in *Caesalpinia calycina, Caesalpinia microphylla* and *Caesalpinia pluviosa var. peltophoroides*, and confirmed in *Caesalpinia ferrea* var. *leiostachya* and *Caesalpinia pulcherrima*. Different karyotype formulae were obtained for each of these five species. The chromosome asymmetry index varied from 34.94 % in *C. pluviosa* to 39.45 % in *C. ferrea*. The average chromosome length among the five species differed significantly ($P$<0.010). The heterochromatin blocks were evidenced by C-banding in both terminal and proximal regions of the *Caesalpinia* spp. chromosomes. This study represents a contribution toward an increased knowledge on cytogenetics and evolution of this genus.

**Keywords**: c-banding, cytogenetics, chromosome, evolution, karyotype

## 1. Introduction

*Caesalpinia* L. *s.l.* is comprised of approximately 140 species and belongs to Fabaceae, a plant family with the third largest number of known species (Lewis et al., 2005). Several *Caesalpinia* species suffer from the anthropogenic fragmentation of their ecosystems. For instance, six species of *Caesalpinia* from different countries are at risk of extinction as defined by the IUCN Red List of Threatened Species (IUCN, 2011). Among them, *Caesalpinia echinata* Lam. is endemic to the humid Atlantic forest along Brazil's eastern coastline and is listed as "endangered" (Varty, 1998; IUCN, 2011).

*Caesalpinia s.l.* has been analyzed phylogenetically by different approaches. The taxonomic studies have demonstrated that *Caesalpinia s.l.* is not a natural group, but rather is comprised of about 10 genera (Lewis et al., 2005; Gasson et al., 2009). Cytogenetic studies are of vital importance within systematics and elucidate phylogenetic and evolutionary relationships among groups of plants (Sumner, 2003; Biondo et al., 2005). Yet, the cytogenetics approach is still incipient in *Caesalpinia s.l.* and the chromosome numbers of about 20 species has been reported (Goldblatt, 1981; Cangiano & Bernardello, 2005). Of those, only nine species have also undergone detailed karyomorphological studies (Kumari & Bir, 1989; Cangiano & Bernardello, 2005). The haploid chromosome numbers ($n$) of 12 and diploid chromosome numbers ($2n$) of 24 are common in this group, although polyploidy has been detected in *Caesalpinia ferrea* Mart. ex Tul. var. *leiostachya* Benth. ($2n$ = 24 e 48) and *Caesalpinia bracteosa* Tul. ($2n$ = 48) (Alves & Custodio, 1989).

Cytogenetic analyses, as a description of chromosome number and size, as well as the banding pattern and position of the centromere, frequently contribute to the understanding of evolution in plants (Shan et al., 2003) and to the elucidation of factors that have been involved in the evolutionary diversification of the taxon (Pedrosa

et al., 2000; Vilatersana et al., 2000). In this work, the karyotypic constitution, the morphometry of the chromosomes and the heterochromatin distribution based on conventional coloration and C-banding were investigated in five species of *Caesalpinia s.l.* that occur in the state of Bahia, Brazil. The cytogenetic information obtained here will be useful for future studies of chromosome evolution in *Caesalpinia s.l.*

## 2. Material and Methods

Fruit of *Caesalpinia pluviosa* DC. var. *peltophoroides* (Benth.) G.P.Lewis, *C. ferrea* var. *leiostachya*, *Caesalpinia pulcherrima* Sw., *Caesalpinia microphylla* Mart. and *Caesalpinia calycina* Benth. were collected from January to April, 2007, in the state of Bahia, Brazil, respectively in the following municipalities: Itapetinga (15º15'03.15" S, 40º14'59.38" W), Itabuna (14º47'24.58" S, 39º14'54.35" W), Ilhéus (14º48'03.09" S, 39º10'29.65 W), Xique-Xique (10º50'16.20" S, 42º43'40.30" W) and Brumado (14º12'33.87' S, 41º39'59.47" W). The first three species are typically found in the Brazilian Atlantic rainforest (Mata Atlantica Brazilian biome) and the last two in the Brazilian savanna (Caatinga). These five species were selected due to the availability of flowering materials in the visited sites. These sites were designated based on records regarding the occurrence of the aforementioned plant species found at the CEPLAC Herbarium. As with most of the *Caesalpinia* species, these species have been included in different reinstated genera (Lewis et al., 2005; Gasson et al., 2009). However, in this work, we use the species nomenclature available at the IPNI (2011) for practical reasons. The botanical material was herborized and identified with the aid of analitical keys and compared with existing material in the Herbarium of Universidade Estadual de Santa Cruz (UESC), where the specimens are deposited.

Seeds were treated with the fungicide Captan Fersol for 24 h and germinated at 24 ºC in Petri dishes covered with filter paper moistened with distilled water. Tips of roots measuring 1-2 cm of length were pre-treated in 8-hydroxyquinoline (0.002 mol.$L^{-1}$) for 6 h (1 h at 24 ºC and 5 h at 4 ºC). Samples were fixed in Carnoy I (ethanol/acetic acid 3:1, v:v) for 3 h at 24 ºC and later stored at -20 ºC. Roots were washed twice in distilled water (5 min each) and incubated in a solution containing cellulase (2 %, w/v) and pectinase (20 %, w/v) at 37 ºC for 1 h in a humidity chamber. Subsequently, one drop of acetic acid (45 %, w/v) was added to the roots for 5 min. The slides were prepared through maceration (Guerra and Souza 2002). The slides were immersed in liquid $N_2$ after the removal of coverslips and stored at -20 °C.

For karyotype analysis, the slides were dried at room temperature (24 ºC) and stained with Giemsa (2 %, w/v) for 10 min, then rinsed in distilled water and dried again at room temperature and mounted in Neo-Mount® (Merck, Darmstadt, Germany). For the study of the heterochromatin pattern, the previously prepared slides were treated according to Guerra and Souza (2002) protocol, with the following adaptations: the chromosomes of *C. pluviosa* var. *peltophoroides, C. ferrea* var. *leiostachya* and *C. calycina* were denaturated for 100 min and the chromosomes of *C. ferrea* var. *leiostachya* and *C. pluviosa* var. *peltophoroides* were stained for 45 min. The slides containing the metaphases and the micrometric slide were photographed using a BX51 microscope equipped with a C-7070 digital camera (Olympus, Tokyo, Japan).

Chromosome measurements were carried out in five metaphases of each species using Adobe Photoshop® CS3 version 10.0 for the following parameters: short arm length (S), long arm length (L), and absolute chromosome length (A). We used the averages of chromosome measurements to construct ideograms: to calculate the haploid karyotype length (KL = total length of all chromosomes ÷ 2), relative chromosome length (RCL % = [total length of each chromosome ÷ KL] x 100), average chromosome length (C = ∑ of the total length of all chromosomes ÷ diploid chromosome number), ratio between arms (r = long arm length ÷ short arm length), and chromosome asymmetry index (A2 = [∑ of the short arms lengths ÷ haploid karyotype length] x 100) (Huziwara, 1962). Karyotypes were classified according to the position of the centromere following Levan et al. (1964). Satellites were classified according to Battaglia's terminology (1955). The length of the satellites was not added to the length of the corresponding chromosome arm but was added to the total length of the chromosome (Cuco et al., 2003).

We used an entirely random delineation for the evaluation of intra and inter-specific karyotype variations. Chromosome length data were analyzed using ANOVA, and differences between averages were analyzed by Tukey's test ($P$<0.01) using the software GENES version 2009.7.0 (Cruz, 2006).

## 3. Results

The diploid chromosome number of for all species analyzed was 24 (Figure 1). The five studied species showed distinct features that were revealed by the morphometric analysis (Table 1). Statistical analyses showed significant differences (P<0.01) in chromosome length (Table 2), which indicates variation in genome size. The variance analysis showed significant difference (P<0.01) among chromosome pairs in each analyzed species

(Table 3). Also, there was significant difference in chromosome length among the species for each chromosome pair (Table 4). For all of the statistical analyses, the coefficients of variation (CV) were low (10.8 % on average); thus the measurements showed good replicability and the sample size was adequate for the study.

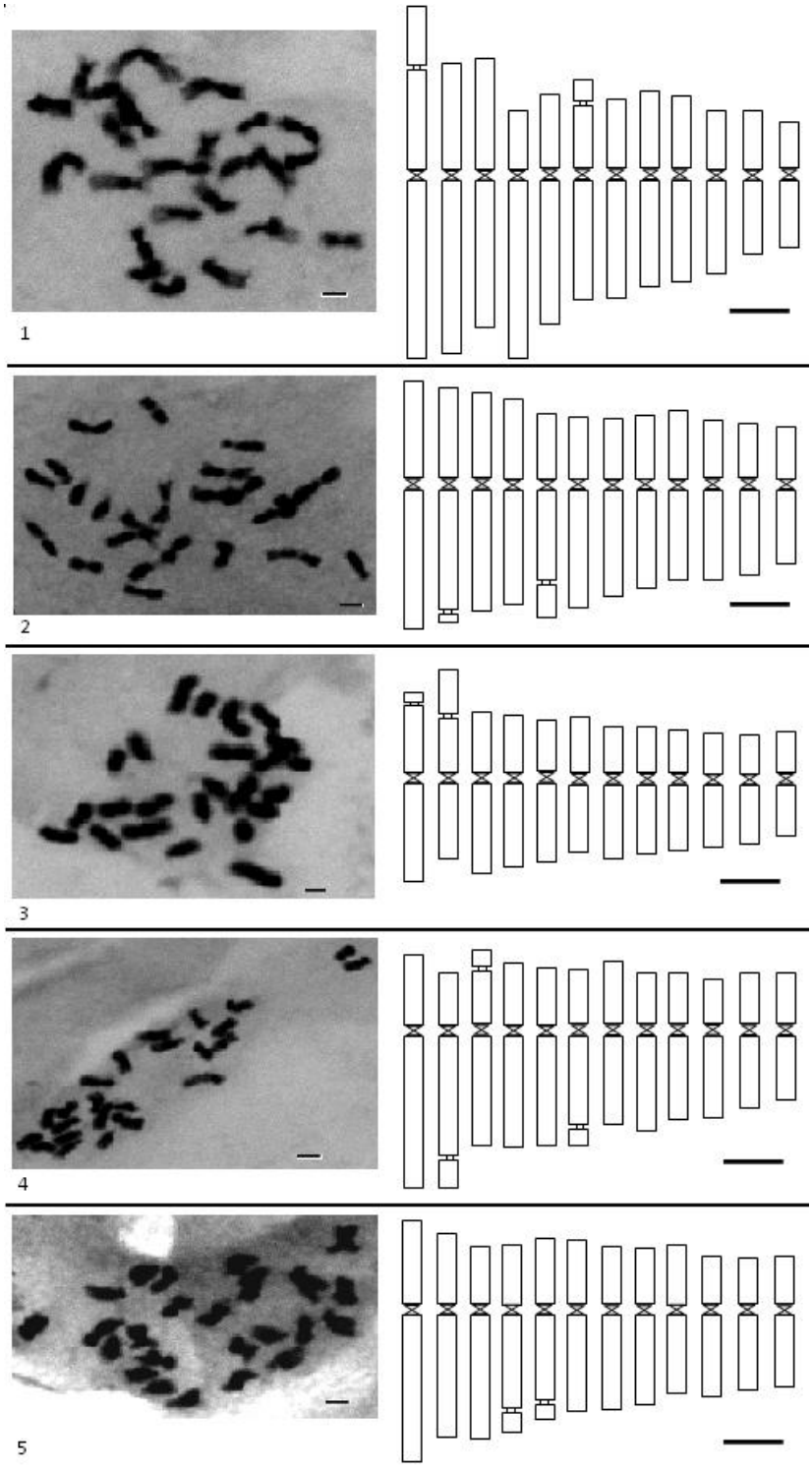


Figure 1. Mitotic metaphase chromosomes (bar = 10 µm) and ideogram (bar = 1 µm) of *C. calycina* (1); *C. ferrea* var. *leiostachya* (2); *C. microphylla* (3); *C. pluviosa* var. *peltophoroides* (4); *C. pulcherrima* (5).

Table 1. Chromosome morphometry of *Caesalpinia* species. Averages of the short arm length (S), long arm length (L), satellite size (SAT), total length of the chromosomes (C), relative chromosome length (RCL %), and ratio between arms (r). Nomenclature for chromosome (Chrom) morphology (N: M = Metacentric, $1.00 \leq r \leq 1.66$; SM = Submetacentric, $1.67 \leq r \leq 2.99$; ST = Subtelocentric, $3.00 \leq r \leq 6.99$)

| Species | Chrom | Average Value | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | S (µm) | L (µm) | SAT (µm) | C (µm)* | RCL (%) | r | N |
| *C. calycina* | 1 | 1.60± 0.58 | 2.87± 0.72 | 0.95± 0.22 | 5.43 ± 0.63 a | 13.6 | 1.79 | SM |
| | 2 | 1.70± 0.40 | 2.79± 0.15 | - | 4.49 ± 0.28 ab | 11.3 | 1.63 | M |
| | 3 | 1.79± 0.22 | 2.38± 0.19 | - | 4.17 ± 0.41 bc | 10.5 | 1.33 | M |
| | 4 | 0.93± 0.19 | 2.87± 0.17 | - | 3.81 ± 0.24 bcd | 9.6 | 3.07 | ST |
| | 5 | 1.18± 0.27 | 2.33± 0.27 | - | 3.51 ± 0.30 cde | 8.8 | 1.97 | SM |
| | 6 | 0.98± 0.09 | 1.91± 0.58 | 0.35± 0.10 | 3.25 ± 0.41 def | 8.2 | 1.93 | SM |
| | 7 | 1.11± 0.22 | 1.88± 0.35 | - | 3.00 ± 0.15 ef | 7.5 | 1.69 | M |
| | 8 | 1.22± 0.16 | 1.71± 0.15 | - | 2.94 ± 0.99 efg | 7.4 | 1.39 | M |
| | 9 | 1.15± 0.21 | 1.64± 0.19 | - | 2.80 ± 0.14 efg | 7.0 | 1.42 | M |
| | 10 | 0.94± 0.17 | 1.50± 0.33 | - | 2.46 ± 0.29 fgh | 6.2 | 1.59 | M |
| | 11 | 0.96± 0.06 | 1.19± 0.11 | - | 2.15 ± 0.15 gh | 5.4 | 1.24 | M |
| | 12 | 0.73± 0.06 | 1.07± 0.18 | - | 1.81 ± 0.15 h | 4.5 | 1.47 | M |
| *C. ferrea* var. *leiostachya* | 1 | 1.57± 0.17 | 2.25± 0.38 | - | 3.82 ± 0.38 a | 11.1 | 1.45 | M |
| | 2 | 1.46± 0.20 | 1.93± 0.20 | 0.14± 0.11 | 3.54 ± 0.26 ab | 10.3 | 1.34 | M |
| | 3 | 1.37± 0.38 | 1.97± 0.27 | - | 3.34 ± 0.25 abc | 9.7 | 1.62 | M |
| | 4 | 1.33± 0.19 | 1.86± 0.17 | - | 3.20 ± 0.20 abcd | 9.3 | 1.42 | M |
| | 5 | 1.05± 0.31 | 1.46± 0.31 | 0.52± 0.17 | 3.05 ± 0.28 bcd | 8.8 | 1.42 | M |
| | 6 | 1.00± 0.35 | 1.92± 0.29 | - | 3.92 ± 0.28 bcde | 8.4 | 2.16 | SM |
| | 7 | 0.99± 0.32 | 1.73± 0.34 | - | 2.73 ± 0.26 cdef | 7.9 | 2.00 | SM |
| | 8 | 1.02± 0.31 | 1.58± 0.29 | - | 2.61 ± 0.20 defg | 7.6 | 1.74 | SM |
| | 9 | 1.12± 0.13 | 1.45± 0.10 | - | 2.58 ± 0.19 defg | 7.5 | 1.30 | M |
| | 10 | 0.93± 0.16 | 1.45± 0.26 | - | 2.38 ± 0.22 efg | 6.9 | 1.62 | M |
| | 11 | 0.88± 0.25 | 1.38± 0.20 | - | 2.27 ± 0.21 fg | 6.6 | 1.75 | SM |
| | 12 | 0.84± 0.13 | 1.21± 0.14 | - | 2.05 ± 0.19 g | 5.9 | 1.46 | M |
| *C. microphylla* | 1 | 1.09± 0.12 | 1.57± 0.34 | 0.16± 0.06 | 2.83 ± 0.36 a | 11.5 | 1.44 | M |
| | 2 | 0.86± 0.23 | 1.22± 0.19 | 0.70± 0.07 | 2.79 ± 0.38 ab | 11.4 | 1.40 | M |
| | 3 | 0.98± 0.06 | 1.45± 0.16 | - | 2.43 ± 0.22 abc | 9.9 | 1.48 | M |
| | 4 | 0.91± 0.28 | 1.35± 0.14 | - | 2.27 ± 0.27 abcd | 9.2 | 1.47 | M |
| | 5 | 0.82± 0.12 | 1.27± 0.31 | - | 2.09 ± 0.31 bcde | 8.5 | 1.55 | M |
| | 6 | 0.89± 0.09 | 1.09± 0.18 | - | 1.98 ± 0.24 bcde | 8.1 | 1.23 | M |
| | 7 | 0.73± 0.14 | 1.18± 0.14 | - | 1.91 ± 0.23 cde | 7.8 | 1.62 | M |
| | 8 | 0.73± 0.14 | 1.10± 0.15 | - | 1.84 ± 0.24 cde | 7.5 | 1.51 | M |
| | 9 | 0.67± 0.16 | 1.06± 0.14 | - | 1.74 ± 0.25 de | 7.1 | 1.56 | M |
| | 10 | 0.66± 0.13 | 0.99± 0.09 | - | 1.65 ± 0.15 de | 6.7 | 1.50 | M |
| | 11 | 0.62± 0.16 | 0.94± 0.12 | - | 1.57 ± 0.20 e | 6.4 | 1.51 | M |
| | 12 | 0.65± 0.05 | 0.82± 0.14 | - | 1.47 ± 0.16 e | 5.9 | 1.26 | M |
| *C. pluviosa* var. *peltophoroides* | 1 | 1.13± 0.16 | 2.46± 0.36 | - | 3.59 ± 0.46 a | 11.6 | 2.17 | SM |
| | 2 | 0.85± 0.09 | 1.94± 0.77 | 0.46± 0.14 | 3.26 ± 0.35 ab | 10.6 | 2.33 | SM |
| | 3 | 0.86± 0.27 | 1.79± 0.35 | 0.27± 0.07 | 2.93 ± 0.31 bc | 9.5 | 2.14 | SM |
| | 4 | 1.02± 0.21 | 1.80± 0.06 | - | 2.83 ± 0.23 bcd | 9.2 | 1.81 | SM |
| | 5 | 0.94± 0.24 | 1.79± 0.10 | - | 2.73 ± 0.26 cde | 8.9 | 1.99 | SM |
| | 6 | 0.87± 0.18 | 1.45± 0.18 | 0.25± 0.03 | 2.58 ± 0.33 def | 8.4 | 1.67 | M |
| | 7 | 1.01± 0.12 | 1.43± 0.19 | - | 2.45 ± 0.29 ef | 7.9 | 1.40 | M |
| | 8 | 0.82± 0.14 | 1.53± 0.28 | - | 2.36 ± 0.30 efg | 7.6 | 1.89 | SM |
| | 9 | 0.83± 0.21 | 1.35± 0.14 | - | 2.19 ± 0.17 efg | 7.1 | 1.75 | SM |
| | 10 | 0.73± 0.10 | 1.32± 0.14 | - | 2.06 ± 0.20 fgh | 6.7 | 1.82 | SM |
| | 11 | 0.84± 0.14 | 1.18± 0.14 | - | 2.01 ± 0.21 gh | 6.5 | 1.42 | M |
| | 12 | 0.83± 0.11 | 1.04± 0.10 | - | 1.87 ± 0.19 h | 6.0 | 1.26 | M |
| *C. pulcherrima* | 1 | 1.35± 0.44 | 2.38± 0.33 | - | 3.73 ± 0.50 a | 12.0 | 1.91 | SM |
| | 2 | 1.15± 0.37 | 1.99± 0.19 | - | 3.15 ± 0.34 ab | 10.1 | 1.87 | SM |
| | 3 | 0.94± 0.23 | 2.02± 0.25 | - | 2.96 ± 0.20 abc | 9.5 | 2.30 | SM |
| | 4 | 0.97± 0.21 | 1.51± 0.22 | 0.31± 0.04 | 2.80 ± 0.28 bcd | 9.0 | 1.62 | M |
| | 5 | 1.06± 0.31 | 1.37± 0.20 | 0.23± 0.02 | 2.67 ± 0.35 bcde | 8.6 | 1.35 | M |
| | 6 | 1.03± 0.22 | 1.57± 0.14 | - | 2.61 ± 0.29 bcde | 8.4 | 1.57 | M |
| | 7 | 0.92± 0.18 | 1.55± 0.55 | - | 2.48 ± 0.26 bcde | 7.9 | 1.78 | SM |
| | 8 | 0.90± 0.10 | 1.45± 0.37 | - | 2.35 ± 0.29 bcde | 7.6 | 1.66 | M |
| | 9 | 0.97± 0.26 | 1.28± 0.16 | - | 2.25 ± 0.30 cde | 7.3 | 1.44 | M |
| | 10 | 0.77± 0.17 | 1.33± 0.41 | - | 2.10 ± 0.35 de | 6.8 | 1.84 | SM |
| | 11 | 0.75± 0.26 | 1.23± 0.25 | - | 1.99 ± 0.32 de | 6.4 | 1.90 | SM |
| | 12 | 0.77± 0.19 | 1.15± 0.13 | - | 1.93 ± 0.31 e | 6.2 | 1.54 | M |

Averages with similar letter dont differ statistically according to the Tukey's test ($P<0.01$).

Table 2. Parameters of the analysis of variance (ANOVA) for chromosomes length among five *Caesalpinia*: *C. calycina*, *C. microphylla*, *C. ferrea* var. *leiostachya*, *C. pluviosa* var. *peltophoroides* and *C. pulcherrima* (2*n* = 24)

| Source of variation | Degrees of freedom | Mean Square |
|---|---|---|
| Taxa | 4 | 2.5915** |
| Error | 55 | 0.4223** |
| CV (%) | | 24.21 |

** Significative (*P*<0.01, F test). CV, Coeficient of Variation.

Table 3. Parameters of the analysis of variance (ANOVA) for lengths of 12 chromosome pairs within each *Caesalpinia* species: *C. calycina* (CC), *C. microphylla* (CM), *C. ferrea* var. *leiostachya* (CFL), *C. pluviosa* var. *peltophoroides* (CPP) and *C. pulcherrima* (CP) (2*n* = 24; *n* = 12)

| Source of variation | Df | Mean Square | | | | |
|---|---|---|---|---|---|---|
| | | CC | CM | CFL | CPP | CP |
| Pairs of chromosomes | 11 | 4.8874** | 1.4410** | 0.9091** | 1.3743** | 1.3720** |
| Error | 48 | 0.0980 | 0.0647 | 0.0702 | 0.0845 | 0.1055 |
| CV (%) | | 9.53 | 8.84 | 13.01 | 11.28 | 12.54 |

** Significative (P<0.01, F test); Df, degrees of freedom.

Table 4. Summary of the analysis of variance (ANOVA) for chromosomes lengths pairs among *Caesalpinia* species: *C. calycina*, *C. microphylla*, *C. ferrea* var. *leiostachya*, *C. pluviosa* var. *peltophoroides* and *C. pulcherrima* (2*n* = 24; *n* = 12).

| Sv | Df | Mean square | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| *Taxa* | 4 | 3.6294** | 2.3249** | 2.1083** | 1.6208** | 1.3606** | 0.0895** | 0.8103** | 0.0811** | 0.8197** | 0.5020** | 0.3492** | 0.2341** |
| Error | 20 | 0.2325 | 0.1094 | 0.0853 | 0.0632 | 0.0887 | 0.1020 | 0.0618 | 0.0586 | 0.0489 | 0.0658 | 0.0532 | 0.0457 |
| CV(%) | | 12.60 | 9.66 | 9.21 | 8.42 | 10.58 | 12.10 | 9.88 | 9.99 | 9.55 | 12.01 | 11.53 | 11.68 |

** Significative (*P*<0.01, F test). Sv, Source of variation; Df, Degrees of freedom; CV, coefficient of variation.

Table 5. Haploid karyotype length (KL), average chromosome length (C), asymmetry index (A2) and karyotipic formula (KF) for five Caesalpinia species

| Species | KL (µm) | C(µm) | A2 (%) | KF |
|---|---|---|---|---|
| *C. calycina* | 39.86 | 3.32 | 36.00 | 8M + 3SM + 1ST |
| *C. ferrea* var. *leiostachya* | 34.54 | 2.87 | 39.45 | 8M + 4SM |
| *C. microphylla* | 24.63 | 2.05 | 39.17 | 12M |
| *C. pluviosa* var. *peltophoroides* | 30.90 | 2.57 | 34.94 | 4M + 8SM |
| *C. pulcherrima* | 31.07 | 2.58 | 37.44 | 6M + 6SM |

The most variation in chromosome length was observed in *C. calycina* and the least variation within *C. microphylla* (P<0.01). Chromosome pairs 8 and 9 were not significantly different either in *C. calycina* or in *C. ferrea* var. *leiostachya*. In *C. pulcherrima* the average chromosome length of chromosome pairs 5, 6, 7, and 8 did not vary significantly. Except for *C. calycina*, the other four species showed at least one chromosome pair which was morphologically similar (Table 1).

The average chromosome length varied from 3.32 µm in *C. calycina* to 2.05 µm in *C. microphylla*, and the haploid karyotype length varied from 39.86 µm in *C. calycina* to 24.63 µm in *C. microphylla* (Table 5). The karyotype formulae were characteristic for each of the species analyzed, ranging from karyotype with all chromosomes metacentric (*C. microphylla*, KF=12M) to three different tipes of chromosomes (*C. calycina* KF=8M+3SM+1ST). The chromosome asymmetry index in the current study varied from A2 = 34.94 % in *C. pluviosa* var. peltophoroides (KF=4M+8SM) to A2 = 39.45 % in *C. ferrea* var. *leiostachya* (KF=8M+4SM).

The relative chromosome length of each species is showed (Table 1). The RCL of chromosome pair 1 (13.63 %) of *C. calycina* differed considerably when compared to pair 12 (4.54 %); whereas in *C. ferrea* var. *leiostachya, C. microphylla, C. pluviosa* var. *peltophoroides,* and *C. pulcherrima* a smaller variation was observed, with RCL ranging from 11 to 12 % for pair 1 and of about 6 % for pair 12.

The satellites differed among these species regarding number, size, and chromosome location (Figure 2). In *C. calycina,* satellites were found in the short arm of pairs 1 and 6, in *C. microphylla* in the short arm of chromosome pairs 1 and 2, in *C. ferrea* var. *leiostachya* in the long arm of chromosome pairs 2 and 5, in *C. pulcherrima* in the long arm of chromosome pairs 4 and 5, and in *C. pluviosa* var. *peltophoroides* in the long arm of chromosome pairs 2 and 6 as well as in the short arm of chromosome 3. The largest satellite was found in *C. calycina* (0.95 µm) and the smallest in *C. ferrea* var. *leiostachya* (0.14 µm). The satellites found in *C. pluviosa* var. *peltophoroides* were of the microsatellite type in both pair 2 and in pair 6 and were linear in pair 3. In *C. pulcherrima*, linear satellites were found in pair 4 and microsatellites were found in pair 5. These were different from the satellites found in *C. ferrea* var. *leiostachya* and *C. microphylla,* which had only satellites of the linear type, and from those in *C. calycina*, which were only of the microsatellite type. There was a prevalence of linear satellites compared to microsatellites in four of the five species. The exception to this pattern was *C. calycina.*

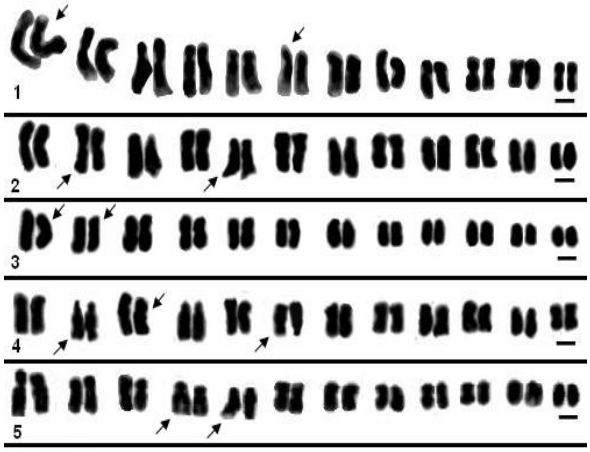


Figure 2. Karyograms of *C. calycina* (1); *C. ferrea* var. *leiostachya* (2); *C. microphylla* (3); *C. pluviosa* var. *peltophoroides* (4); *C. pulcherrima* (5). Arrows indicate satellites pairs. Bar = 10 µm

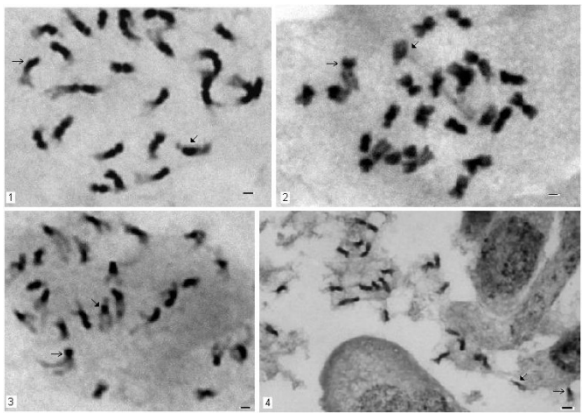

Figure 3. C-banding in *C. calycina* (1), *C. ferrea* var. *leiostachya* (2), *C. pluviosa* var. *peltophoroides* (3), *C. pulcherrima* (4), iferred as heterochromatin regions. Horizontal arrows indicate terminal C-bands and angle arrows proximal C-bands. Bar = 10 µm

Terminal bands, evidenced by C-banding, were more frequent than proximal bands in all the species analyzed (Figure 3). Interstitial bands were not visualized in any of the species studied. In *C. pluviosa* var. *peltophoroides* and *C. pulcherrima*, small blocks of heterochromatin were observed; whereas in *C. ferrea* var. *leiostachya* and *C. calycina*, large blocks of heterochromatin were found. In the four of the five species studied, there was at least one pair of almost entirely heterochromatic chromosomes. C-band was not detected for *C. microphylla.*

## 4. Discussion

Taxonomic characterization in the genus *Caesalpinia* L. remains unresolved, mainly due to the difficulty in identifying the numerous species within the genus using morphological characteristics, which leads to frequent revisions in phylogenetic studies (Lewis, 1998; Gasson et al., 2009). Cytogenetic data can be useful in systematic studies. However, there is a paucity of such data for this genus. The five analyzed species had 2n = 24. This chromosome number was reported for the first time in *C. pluviosa* var. *peltophoroides, C. calycina* and *C. microphylla* in the present work. Additionaly, we confirmed 2n = 24 in *C. pulcherrima* (Atchison, 1951). We find only 2n = 24 in *C. ferrea* var. *leiostachya*, while 2n = 24 e 2n = 48 are related in *C. ferrea* (Beltrão and Guerra 1990).

The first chromosome studies carried out on *Caesalpinia* species revealed 2*n* = 24 and n = 12, which were considered common chromosome numbers in this group (Goldblatt, 1981). However, 2n = 24 and 2n = 48 were found in *C. ferrea* (Beltrão & Guerra, 1990), and 2n = 48 was found in *C. bracteosa* (Alves & Custódio, 1989). These different chromosome numbers indicate the occurrence of polyploidy within the genus. Other exceptions were found for *C. pulcherrima, Caesalpinia japonica* Siebold and Zucc.*, Caesalpinia cucullata* Roxb.*,* and *Caesalpinia kavaiensis* H.Mann., which were initially described as having 2n = 22. This difference in the numerical pattern was attributed to mistakes in chromosome counting, both in *C. pulcherrima* and in *C. japonica.* However, n = 11 was confirmed in *C. cucullata* and *C. kavaiensis*. These latter two species were placed in the *Mesoneuron* genus (Lewis, 1998, Lewis et al., 2005).

Other *Caesalpinia* species with 2n = 24 were described*:* i) the n = 12 was confirmed in *Caesalpinia melanadenia* Standl.*, Caesalpinia nelsonii* (Britton and Rose) J.L.Contr.*, Caesalpinia exostemma* DC.*,* and *Caesalpinia hughesii* G.P.Lewis (Goldblatt, 1981); ii) the 2n = 24 was confirmed in *C. exostemma, Caesalpinia cacalaco* Humb. and Bonpl., and in *Caesalpinia bonduc* (L.) Roxb.*, Caesalpinia velutina (Britton and Rose) Standl., Caesalpinia vesicaria* L.*, Caesalpinia gilliessi* (Wall. ex Hook.) Benth.*, Caesalpinia yucatanensis Greenm,* and *Caesalpinia decapetala* (Roth) Alston - (Lewis, 1998); iii) the 2n = 24 was confirmed in *C. gilliessi*; the 2n = 24 and n = 12 were reported in *Caesalpinia paraguariensis* (D.Parodi) Burkart and *Caesalpinia mimosifolia* Griseb (Cangiano & Bernardello, 2005); iv) the 2n = 24 also was reported in *Caesalpinia crista* L. (Jena et al., 2004) and *Caesalpinia violaceae* (Mill.) Standl. (Jarolímová, 1994).

In the current study, the average chromosome length varied from C = 3.33 µm in *C. calycina* to C = 2.05 µm in *C. microphylla.* Cangiano and Bernardello (2005) reported C values of 1.90 µm in *C. gilliessi, C. paraguariensis,* and *C. mimosifolia*. Within the Caesalpinioideae, the C values reported by Auler and Battistin (1999) and Biondo et al. (2005) were equal to or inferior to 2.0 µm. The haploid karyotype length obtained in our study varied from 39.86 µm in *C. calycina* to 24.63 µm in *C. microphylla.* In other species of *Caesalpinia l.s.,* this trait ranges from 20.67 µm in *C. mimosifolia* to 24.74 µm in *C. gilliesii* (Cangiano & Bernardello, 2005). This shows a continuum between Brazilian and Argentinean species, while the larger haploid karyotype lengths were found in Brazilian species.

The chromosome asymmetry index in the current study indicates that *C. ferrea* var. *leiostachya* has the most ancestral condition of the karyotype (A2 = 39.45 %) while *C. pluviosa* var. *peltophoroides* has the most derived condition (A2 = 34.94 %) among the five species studied. In spite of *C. ferrea* var. *leiostachya* having the largest A2 value, this index did not vary considerably between *C. pulcherrima* (A2 = 37.44 %) and *C. calycina* (A2 = 36 %). The Argentinean species of *Caesalpinia* l.s. have A2 values ranging from 17 to 24 % (Cangiano and Bernardello 2005), thus showing more symmetrical karyotypes in Argentinean species then in the Brazilian ones. The variation in A2 values among species from the same geographical areas was generally smaller them between the variation in A2 among species from the two geographical areas.

The karyotypic formulae showed relatively symmetrical karyotypes and a prevalence of metacentric and submetacentric chromosomes in the species of *Caesalpinia s.l.* studied. This same tendency was observed in Caesalpinioideae by Stebbins (1971), Souza and Benko-Iseppon (2004), Kumari and Bir (1989), and Auler and Battistin (1999). Although the presence of subtelocentric chromosomes in the subfamily is rare (Kumari & Bir 1989), in the present work one submetacentric pair was observed in *C. calycina.* According to Stebbins (1971), karyotypic asymmetry is involved in the speciation process, and symmetrical karyotypes are the more ancestral

condition. Indeed, the A2 describes the variation in chromosome length in a complement, not only the centromere position (Paszko, 2006). Therefore, despite the 12 pairs of metacentric chromosomes in *C. microphylla*, this species had the second largest A2 among the studied species.

Dissimilarities regarding chromosome morphometry were observed in the karyotypes of the species in this study, mainly in the chromosome length and in the presence and location of satellites. Since chromosomes are not rigidly stable structures, chromosome variations play an important role in evolution of practically all species (Souzad & Benko-Iseppon, 2004).

All five *Caesalpinia* from Brazil studied herein had satellites visible in the mitotic chromosomes. In *C. calycina, C. microphylla, C. ferrea* var. *leiostachya* and *C. pulcherrima*, we find two pairs of satellites. However, satellites varied in size, position along the arms, as well as in the pairs of chromosomes where they were found. Three pairs of satellites were detected in *C. pluviosa* var. *peltophoroides.* The second chromosome pair showed satellites in *C. ferrea* var. *leiostachya, C. microphylla* and *C. pluviosa* var. *peltophoroides.* The presence of satellites in pair 3 and in pair 4 was verified only in *C. pluviosa* var. *peltophoroides* and *C. pulcherrima*, respectively. Three different *Caesalpinia s.l.* from Argentina also had satellites observed in their chromosomes (Cangiano & Bernardello, 2005). Although there is a tendency of maintenance of a similar karyotype pattern among related species, the variations regarding number, shape, position, and length of satellites are frequently observed in plants and have been used as markers (Moscone, 1993). However, these markers can be incorporated or suppressed over the evolutionary process.

C-banded heterochromatin was distributed preferentially in the proximal and telomeric regions in *C. ferrea* var. *leiostachya, C. pluviosa* var. *peltophoroides*, *C. pulcherrima* and *C. calycina,* however, no interstitial bands were found. Our findings corroborate those of Guerra (2000) who reported that plant species with small chromosomes do not usually show interstitial heterochromatin. *C. pluviosa* var. *peltophoroides* and *C. pulcherrima* showed small blocks of heterochromatin, whereas *C. ferrea* var. *leiostachya* and *C. calycina* showed large blocks of heterochromatin. No C-banding data were obtained for *C. microphylla*, indicating the need to improve the application of this technique for the study of this species. It is noteworthy that this is the first study applying C-banding to species within this genus.

Our results revealed inter-specific differences regarding C-banding patterns and karyomorphology of chromosomes that contribute to our understanding of the karyotypic pathern within *Caesalpinia s.l.* This study represents a contribution toward an increased knowledge on karyomorphology of *Caesalpinia* s.l. species. The most *Caesalpinia s.l.* species have been assigned to reinstated nine segregated genera based on wood anatomy (Gasson et al., 2009). However, our data together with those of Cangiano and Bernadello (2005) are still insufficient for a representative support to these genera. Therefore, we suggest expanding this analysis to other species within *Caesalpinia s.l.*, including at least two species from each new assigned genus. So, this data can be usefull for future evolutionary studies in Caesalpinia.

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