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CELLULAR AND MOLECULAR BIOLOGY

Comparative analysis of structural chromosome variability and heterochromatin localization in *Passiflora* L. (Passifloraceae)

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Abstract: *Passiflora* is the most diverse genus of the Passifloraceae family, and holds great economic importance. However, cytogenetic information is still limited for most species. This study aimed to analyze the karyotypes of nine *Passiflora* species, to determine the chromosome number and the distribution of heterochromatic regions by double staining with CMA/DAPI and by fluorescence *in situ* hybridization (FISH) with 35S and 5S rDNA probes. Eight species presented a karyotype with 2n=18 chromosomes, and in contrast, *P. foetida* was the only species to exhibit a karyotype with 2n=20. Double staining with CMA and DAPI revealed distinct staining patterns, with four CMA+ blocks located in the terminal regions of the chromosomes of eight species, except in *P. foetida*, where six CMA+ blocks were observed in the proximal regions. FISH identified four to six 35S rDNA sites in the terminal regions of the chromosomes in *P. alata* and *P. edmundoi*, and six sites in interstitial region in *P. foetida*. In these same species, two 5S rDNA sites were observed, and in *P. foetida* four sites in the subterminal regions. The observed chromosomal variations suggest significant genetic diversity in the genus, valuable data for studies of diversity, conservation and genetic improvement.

Key words: karyotype, CMA, DAPI, FISH, passion fruit.

INTRODUCTION

The genus *Passiflora* is considered the most representative of the Passifloraceae family, with approximately 500 species, of which approximately 160 are native to Brazil, placing the country among the main centers of genetic diversity of the genus (Bernacci et al. 2020). The Brazilian species of *Passiflora*, popularly known as "passion fruit", have great economic and social potential, being widely used in the food, medicinal and ornamental industries due to their characteristic flavor and high nutritional value (Silva et al. 2022, Nguyen et al. 2021, Monte & Santo 2021).

Cytogenetic analysis constitutes a strategic approach both for accessing biodiversity and for use in genetic improvement programs. However, cytogenetic studies in *Passiflora* are still scarce, with less than 30% of the species having published cytogenetic information. Among the species studied, most reports are limited to determining the chromosome number. In this case, of the approximately 160 species native to Brazil, the chromosome numbers are known for only 40 (Souza et al. 2008).

The species of the genus *Passiflora* present a wide variation in size and number of chromosomes, and can be classified into four groups based on their basic numbers: x=6, x=9, x=10 and x=12. Most species are diploid, with 2n=12, 2n=18 and 2n=20. However, there are also tetraploid (2n=24), hexaploid (2n=36)

and octoploid (2n=72) species (Melo & Guerra 2003, Hansen et al. 2006). In Passifloroideae (Passifloraceae), the number x=12 was probably originated by Whole Genome Duplication (WGD) after the separation of Passifloroideae and Malesherbioideae (Melo & Guerra 2021).

The analysis of the distribution of CMA/DAPI bands in the karyotype of plant species has been a useful resource in detecting genetic variability, even when confronted with the morphological uniformity of chromosomes. Despite the considerable variation in heterochromatin between species karyotypes, plant groups with very similar banding patterns in terms of number, size and location on chromosomes are occasionally observed (Cordeiro et al. 2020, Deanna et al. 2022, Pessoa et al. 2021). There are few descriptions of CMA/DAPI banding in the genus *Passiflora*, with only the report by Melo et al. (2001) standing out, who analyzed eight species of the genus, observing the presence of one to three pairs of CMA+ blocks and the absence of DAPI+ blocks. In species of economic importance, such as *P. edulis* and *P. cincinnata*, four CMA+ blocks and no DAPI+ were observed (Coelho 2009, Melo et al. 2001).

Reports investigating the number and location of 5S and 35S rDNA sites provide important information to elucidate the genome organization and chromosomal relationships of many species through fluorescence *in situ* hybridization (FISH). Zhang et al. (2016), for example, demonstrated in *Cucumis* that the number of 35S rDNA sites varied from one to five pairs in different accessions, and that most of these sites are located in the terminal regions of the chromosomes. In *Passiflora*, the analysis of 20 species revealed that the number of 5S rDNA sites was generally proportional to the ploidy level of the species, while the number of 35S rDNA sites varied from 2 to 12 among species (Melo & Guerra 2003).

In this context, the present study aimed to analyze the karyotypes of nine *Passiflora* species, comparing chromosome number and the distribution of heterochromatic regions through the double staining technique with CMA/DAPI fluorochromes and localization of rDNA sites via FISH.

MATERIALS AND METHODS

Plant material

The species *P. alata* Curtis, *P. cincinnata* Mast., *P. edmundoi Sacco*, *P. edulis* Sims, *P. foetida* L., *P. laurifolia* L., *P. ligularis* Juss., *P. luetzelburgii* Harms. and *P. setacea* DC. used in the present study were obtained from the Active Passion Fruit Germplasm Bank of Embrapa Semiarid (Petrolina-PE, Brazil, Figure 1). Plants were obtained from the germination of seeds in pots with substrate and grown in the institution's greenhouse. Samples of growing roots of each species were collected for cytogenetic analysis, following the protocol described by Guerra & Souza (2002), with some modifications.

Cytogenetic characterization

Slide preparation

The collected roots were pretreated with 2 mM 8-hydroxyquinoline (8-HQ) for 24 hours at approximately 4°C. They were then fixed in 8 mL of 3:1 Carnoy solution (three parts P.A. ethyl alcohol to one part glacial acetic acid) for a period of 2 to 24 hours at room temperature. They were then transferred to a new 3:1 Carnoy solution and stored at -20°C until used in mitotic preparations.

To prepare the slides, the root meristems were subjected to enzymatic digestion in 8 µL of a solution containing 2% cellulase (Onozuka) and 6% pectinase (Sigma), and then incubated in an oven at 37°C for approximately 18 hours.

Figure 1. Flowers of *Passiflora* species analyzed in the present study: *P. alata* Curtis (a), *P. cincinnata* Mast. (b), *P. edmundoi* Sacco (c), *P. edulis* Sims (d), *P. foetida* L. (e), *P. laurifolia* L. (f), *P. ligularis* Juss. (g), *P. luetzelburgii* Harms (h), and *P. setacea* DC. (i). All accessions were obtained from the Active Passion Fruit Germplasm Bank of Embrapa Semiarid (Petrolina, PE, Brazil).

Subsequently, the meristems were cut and macerated with a fine needle in a drop of acetic acid (45%) on a glass slide. They were then covered with an 18x18 mm coverslip, crushed between the slide and coverslip with filter paper, and frozen in liquid nitrogen for approximately 3 minutes. After the nitrogen was removed, the coverslips were removed with the aid of a scalpel, and the slides were left to dry at room temperature, as described by Ribeiro et al. (2017).

The freshly prepared slides were then stored in tightly sealed boxes at room temperature to prevent the material from coming into contact with dust. To select the slides containing the best metaphases, 10 µL of DAPI solution (2 µg/mL) diluted in glycerol in a 1:1 (v/v) ratio was added for 30 minutes, and then observed under a Leica DM 2000 epifluorescence microscope.

After selection, the slides containing 5 to 10 suitable metaphases were destained in 80 mL of Carnoy's 3:1 solution (three parts of ethyl alcohol P.A. to one part of glacial acetic acid) for 30 minutes, followed by immersion in 70% ethanol and absolute ethanol, both for 1 minute.

Subsequently, the slides were allowed to dry at room temperature and stored in the freezer at -20°C until double staining with CMA/DAPI was performed.

Double staining with CMA/DAPI

Staining with CMA/DAPI was performed according to the protocol described by Vaio et al. (2018). The slides stored at -20°C were thawed at room temperature, stained with 15 µL of CMA (0.5 mg/mL), covered with a 22x22 mm coverslip and kept in a dark box, protected from light, for 1 hour. After this period, the coverslips were removed with a jet of distilled water, and the slides were dried with an air pump.

When completely dry, the slides were stained a second time with 15 µL of DAPI (2 µg/mL) for 30 minutes, again kept in the dark. After this period, the coverslips were removed and the slides were dried using an air pump.

To mount the material after staining, 15 µL of mounting medium composed of Glycerol/McIlvaine Buffer (pH 7.0, 1:1, v/v) containing $MgCl_2$ (2.5 mM) was applied to the slides, covered with 22x22 mm coverslips. The slides were then left to "age" for a period of 3 days before being observed under a Leica DM 2000 epifluorescence microscope.

Obtaining 5S and 45S rDNA probes

To locate the 45S rDNA sites, the pTa71 probe from wheat (*Triticum aestivum* L.) was used. The 5S rDNA was obtained by PCR from the genomic DNA of *P. edulis*, using the primers 5'-GTGCGATCATACCAGC(AG)(CT)TAATGCACCGG-3' and 5'-GAGGTGCA ACACGAGGACTTCCCAGGAGG-3'. The 5S and 35S rDNA probes were labeled with biotin-11-dUTP and digoxigenin-11-dUTP, by bionick and nick translation, respectively.

Fluorescent *in situ* hybridization (FISH)

The slides stored at -20°C were thawed at room temperature, pretreated with absolute ethyl alcohol: glacial acetic acid (3:1, v/v), followed by a 70% and 100% alcoholic series, and dried in an oven at 60°C for later use in FISH. Denaturation of chromosomes and probes, post-hybridization baths, and the detection stage were performed according to Heslop-Harrison et al. (1991), modified by Pedrosa et al. (2002). Hybridization mixtures consisted of: 50% (v/v) formamide, 10% (w/v) dextran sulfate, 2x SSC, and 2.5-5 ng/µL of probe. The slides were denatured for 7 minutes at 75°C, placed in a humid chamber and hybridized for 18 to 42 hours at 37°C. Biotin-labeled probes were detected with mouse anti-biotin in combination with anti-mouse antibody conjugated to tetramethylrhodamine isothiocyanate (TRITC) in 1% (w/v) BSA. Digoxigenin-labeled probes were detected using sheep anti-digoxigenin conjugated to fluorescein isothiocyanate (FITC; Boehringer Mannheim) and amplified with FITC-conjugated anti-sheep antibody, also in 1% (w/v) BSA. The slides were mounted with 2 µg/mL DAPI in Vectashield H-1000 (Vector), in a 1:1 ratio. Images were captured with a Leica video camera attached to a Leica DM 2000 epifluorescence microscope, and brightness and contrast adjustments were performed in the Leica QFISH software.

RESULTS

Chromosome number and double staining with CMA/DAPI in *Passiflora*

Chromosome analyses revealed that the species *P. alata, P. cincinnata, P. edmundoi, P. edulis, P. laurifolia, P. ligularis, P. luetzelburgii* and *P. setacea* presented a diploid karyotype with 2n=18 chromosomes, indicating that these eight species belong karyologically to the group with basic number x=9. In contrast, *P. foetida* was the

only species to exhibit a diploid karyotype with 2n=20 chromosomes, confirming its inclusion in the group of species with basic chromosome number x=10.

Double staining with the fluorochromes CMA and DAPI revealed distinct patterns of CMA+ blocks, with variations in size (heteromorphism), location, and staining intensity, with small blocks (dots) detached from the chromosome pairs being observed in some cases (Figures 2 and 3). *Passiflora alata*, *P. cincinnata, P. edulis, P. laurifolia, P. ligularis* and *P. luetzelburgii* presented four CMA+ blocks, all located in the terminal regions of two chromosome pairs (Figures 2b, d, f, h, j and l). In *P. edmundoi* and *P. setacea*, six CMA+ blocks were observed, distributed in the terminal regions of three chromosome pairs, with small bright CMA+ dots frequently observed, detached from the chromosomes, probably related to the nucleolar organizer region (NORs) (Figures 3b, d and f).

In *P. foetida*, the only species with a different chromosome number from the others, six CMA+ blocks were detected, all in proximal regions of the chromosomes (Figures 3f and g).

Fluorescent *in situ* hybridization in *Passiflora*

Fluorescence *in situ* hybridization (FISH) with rDNA probes allowed the localization of four 35S rDNA sites in *P. alata* (Figure 4b) and six 35S rDNA sites in *P. edmundoi* (Figure 4e), located in the terminal region of the chromosomes, and corresponding to the number of CMA+ blocks previously observed for these two species. For both species, only two 5S rDNA sites were also observed (Figures 4c and f). On the other hand, for *P. foetida*, six 35S rDNA sites were detected located in the interstitial region of six chromosomes (Figure 5b) and four 5S rDNA sites located in the subterminal region of two other chromosomes (Figures 5c and e). Figure 6 presents representative idiograms of the nine

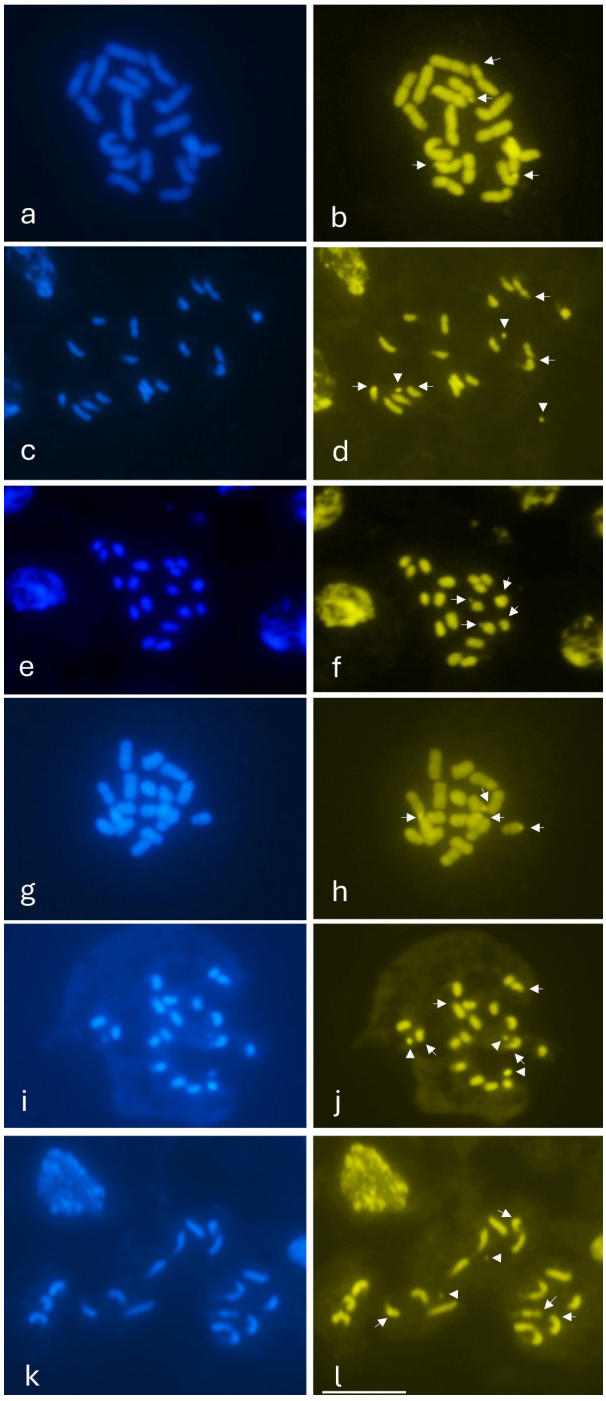

Figure 2. Differential staining of DAPI (a, c, e, g, i, k) and CMA (b, d, f, h, j, l) in *Passiflora* species with 2n=18 and 4 CMA+ blocks. a, b) *P. alata*; c, d) *P. cincinnata*; e, f) *P. edulis*; g, h) *P. laurifolia*, i, j) *P. lingularis*; k, l) *P. luetzelburguii*. Arrows indicate CMA+ blocks in the terminal region of chromosomes. Arrowheads indicate CMA+ blocks ("dots") distended or detached from the chromosomes. Bar represents 5 µm.

analyzed species, indicating the patterns of CMA+/DAPI– blocks and the location of 35S and 5S rDNA sites revealed by FISH.

DISCUSSION

In general, species of the genus *Passiflora* are cytotaxonomically distributed into three groups with different basic chromosome numbers: x=6, x=9 and x=12 (Melo et al. 2001, Hansen et al. 2006, Sader et al. 2019). These groups are associated with the four subgenera proposed by Feuillet & MacDougal (2004): *Decaloba* (x=6), *Passiflora* L. (x=9) and *Astrophea* and *Deidamioides* (x=12) (Sader et al. 2018). In this case, species that share the same basic number tend to exhibit similar karyotypic patterns (Melo et al. 2001, Melo & Guerra 2003).

The subgenus *Passiflora* L. includes species such as *Passiflora alata, P. edulis, P. cincinnata, P. edmundoi, P. luetzelburgii, P. setacea, P. laurifolia* and *P. ligularis*, all with basic number x=9 and diploid karyotype 2n= 2x= 18 (Hansen et al. 2006, Melo et al. 2015, Farias 2014, present study). The basic number x=9 may have originated through evolutionary processes involving polyploidy

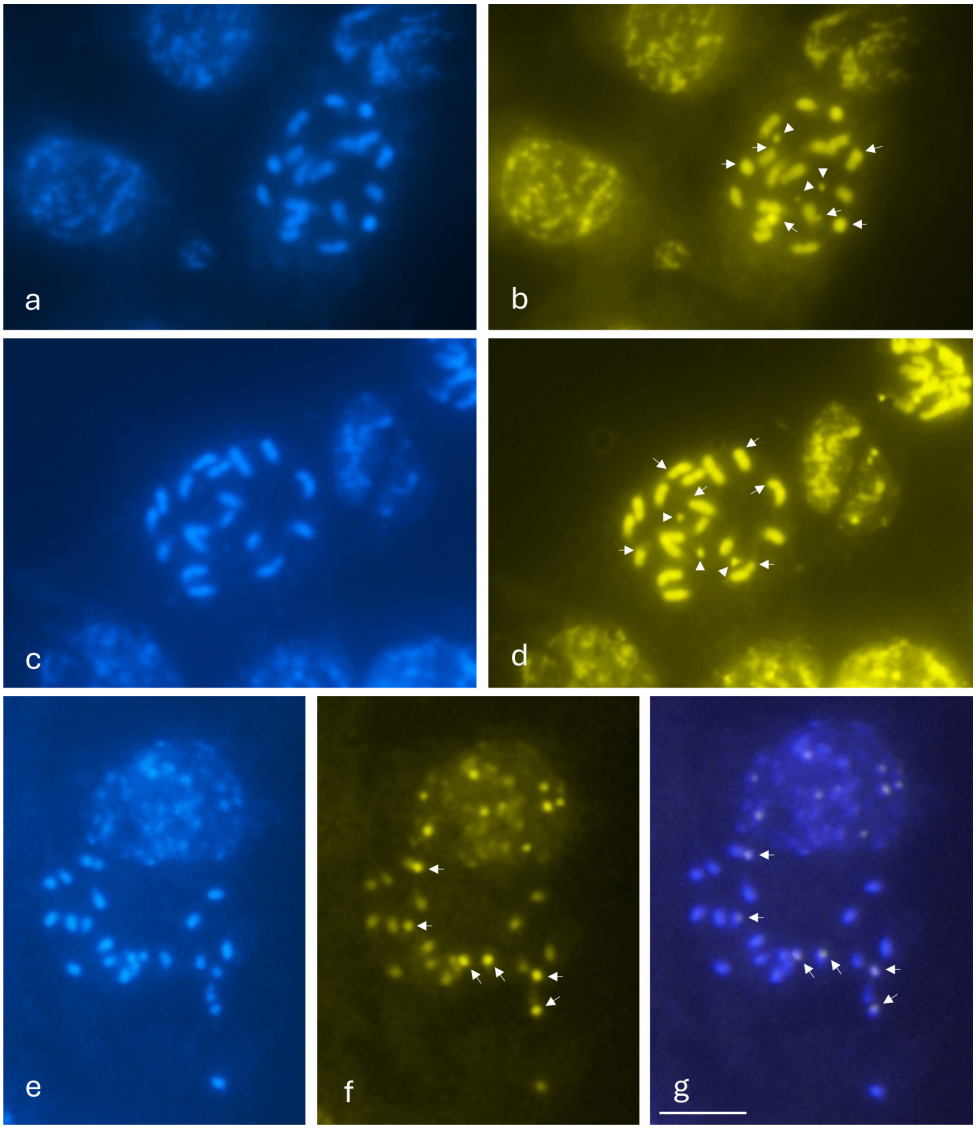

Figure 3. Differential staining of DAPI (a, c, e) and CMA (b, d, f) in *Passiflora* species with 6 CMA+ blocks. a, b) *P. edmundoi*; c, d) *P. setacea* both with 2n=18. e, f, g) *P. foetida* with 2n=20. Figure g represents the superposition of figures e, f. Arrows indicate CMA+ blocks. Arrowheads indicate CMA+ blocks ("dots") distended or detached from the chromosomes. Note CMA+ blocks in the terminal region of *P. edmundoi* and *P. setacea* ("b, d") and interstitial of *P. foetida* ("f, g"). Bar represents 5 µm.

(from x=6 to x=12), followed by aneuploidy with loss of chromosome pairs in ancestral species, which would lead the basic number x=12 to reduce to x=9, resulting in diploid species with 2n=18 chromosomes (Melo et al. 2001). The presence of species with intermediate chromosome numbers, such as 2n = 20 and 2n = 22, supports this hypothesis (Soares-Scott et al. 2005), as is the case of *P. foetida*.

Unlike the other species studied, *P. foetida* presented a karyotype with a distinct basic number. This result is consistent with previous studies by Melo & Guerra (2003), and other reports of variation in chromosome number for this species: 2n = 2x = 18 (Janaki-Ammal & Darlington 1945), 2n = 2x = 20 (Storey 1950) and 2n = 2x = 22 (Bowden 1945, Harvey 1966). According to Melo et al. (2001), these cytotypes may present proximal secondary constrictions, which distend in one of the smaller chromosome pairs during prophase or prometaphase. This phenomenon can lead to a misinterpretation of the chromosome number, suggesting the existence of distinct chromosome sets and resulting in an incorrect count of 2n=2x=22 instead of 2n=2x=20. Another hypothesis to explain the numerical chromosome variation in *P. foetida* is suggested by Soares-Scott et al. (2005) and Sader et al. (2019), who pointed out the possibility that this species originated from ancestors with 2n=24 chromosomes and suffered chromosome losses throughout evolution. This process would help to explain the observed variations in chromosome number. It is worth noting that the evolution of chromosome number in a species can occur either through ascending dysploidy, through chromosome fission, in which a chromosome divides at the centromere, forming two acrocentric chromosomes, or through descending or reductive dysploidy, which occurs through Robertsonian fusion, where two acrocentric chromosomes lose their short arms and fuse at the centromere. These mechanisms contribute to the diversification of the genome

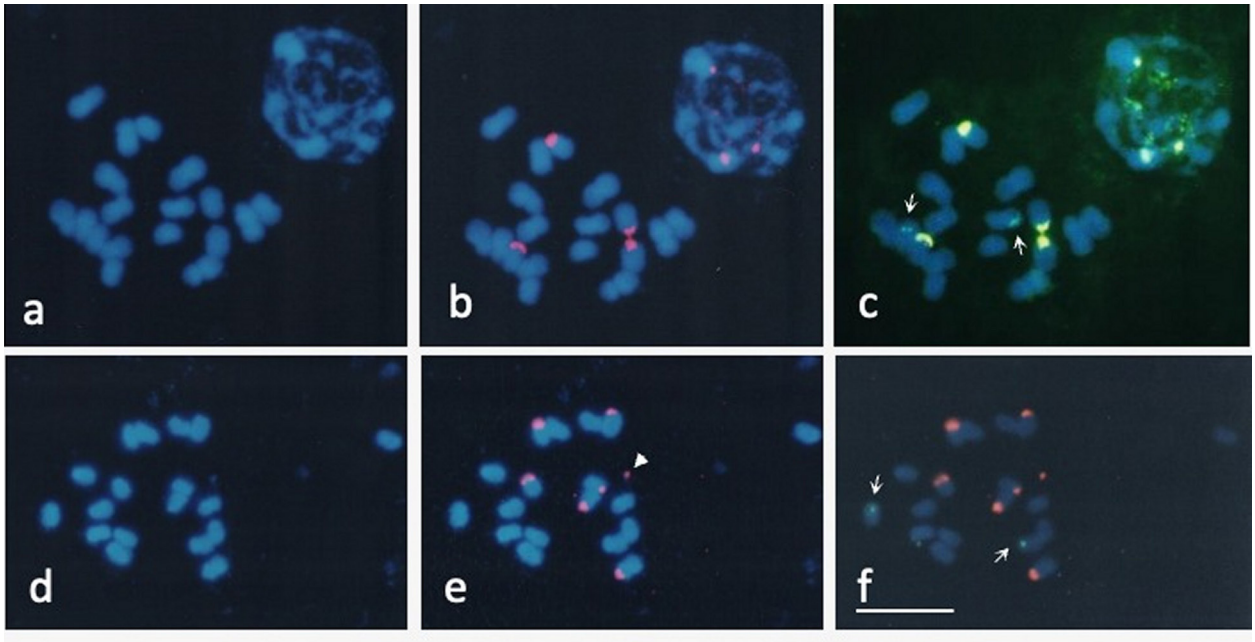

Figure 4. Fluorescent *in situ* hybridization with 35S (red or yellow) and 5S (green) rDNA probes in *Passiflora* species with 2n=18. a, b, c) *P. alata*. Note chromosome morphology in a, four 35S rDNA sites in b, and two 5S rDNA sites in c (arrows). d, e, f) *P. edmundoi*. Note six 35S rDNA sites and highlighted 35S rDNA block (arrowhead) in e, and two 5S rDNA sites in f (arrows). Bar represents 5 µm.

of species over time (Melo & Guerra 2021, Udall et al. 2019).

Despite presenting stability in terms of chromosome number, it is important to consider the possibility of karyotypic variations among representatives of *Passiflora*, especially with regard to the number and location of CMA+ heterochromatic bands (Melo et al. 2001).

Staining with CMA and DAPI fluorochromes in *Passiflora* species revealed consistent patterns of CMA+ blocks and the absence of DAPI+ blocks. However, the observed CMA+ blocks showed variations in size (heteromorphism) and staining intensity, which may indicate structural differences in chromosomes between species. These variations may be due to structural changes that occurred throughout the evolutionary process of the different species (Soares-Scott et al. 2005).

CMA+ bands highlight regions of the genome rich in GC base pairs (Guanine and Cytosine), playing an important role in the characterization of species and populations (Almeida et al. 2016). This technique allows visualizing these bands as more intensely stained areas in the chromosomes, which significantly improves karyotypic characterization (Faleiro et al. 2005, Lira et al. 2024).

The four CMA+/DAPI- bands observed in *Passiflora alata, P. cincinnata, P. edulis, P. laurifolia, P. ligularis* and *P. luetzelburgii* were located in terminal regions of two chromosome pairs, corroborating the report by Melo et al. (2001). The same pattern observed in *P. cincinnata* was also reported by Bezerra (2020), who associated some of these terminal CMA+ bands with nucleolar organizer regions (NORs) commonly surrounded by constitutive heterochromatin

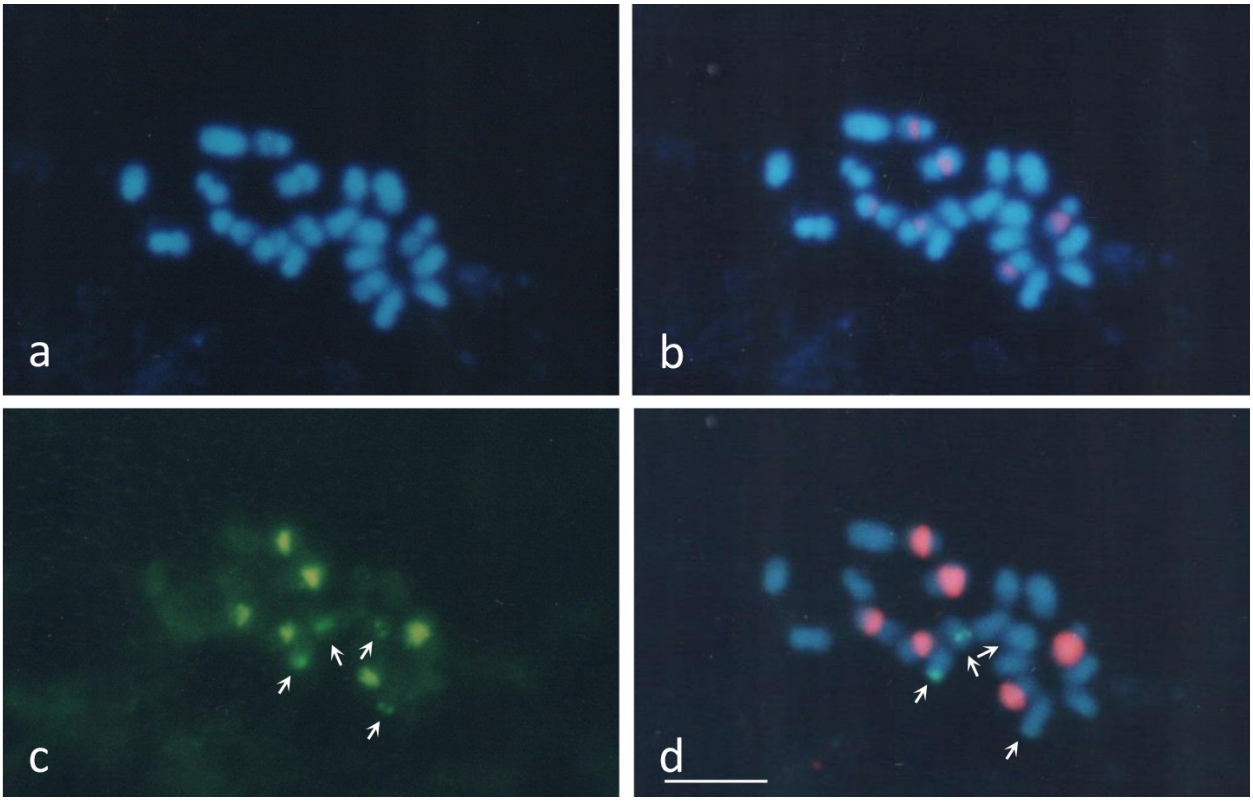

Figure 5. Fluorescent *in situ* hybridization with 35S (red or yellow) and 5S (green) rDNA probes in *Passiflora foetida* with 2n = 20. In (a), observe chromosome morphology; in (b), six 35S rDNA sites located at proximal secondary constrictions; and in (c) and (d), four 5S rDNA sites (arrows). Bar represents 5 µm.

rich in GC base pairs. According to Guerra (2000) and Cordeiro et al. (2017), in plant cytogenetics, the most common pattern of heterochromatic bands includes two terminal CMA+ bands corresponding to nucleolar organizer regions (NORs). These regions, known as satellites or NORs, function as useful chromosomal markers for confirming crosses in hybrids (Melo et al. 2001, Emiliano et al. 2022).

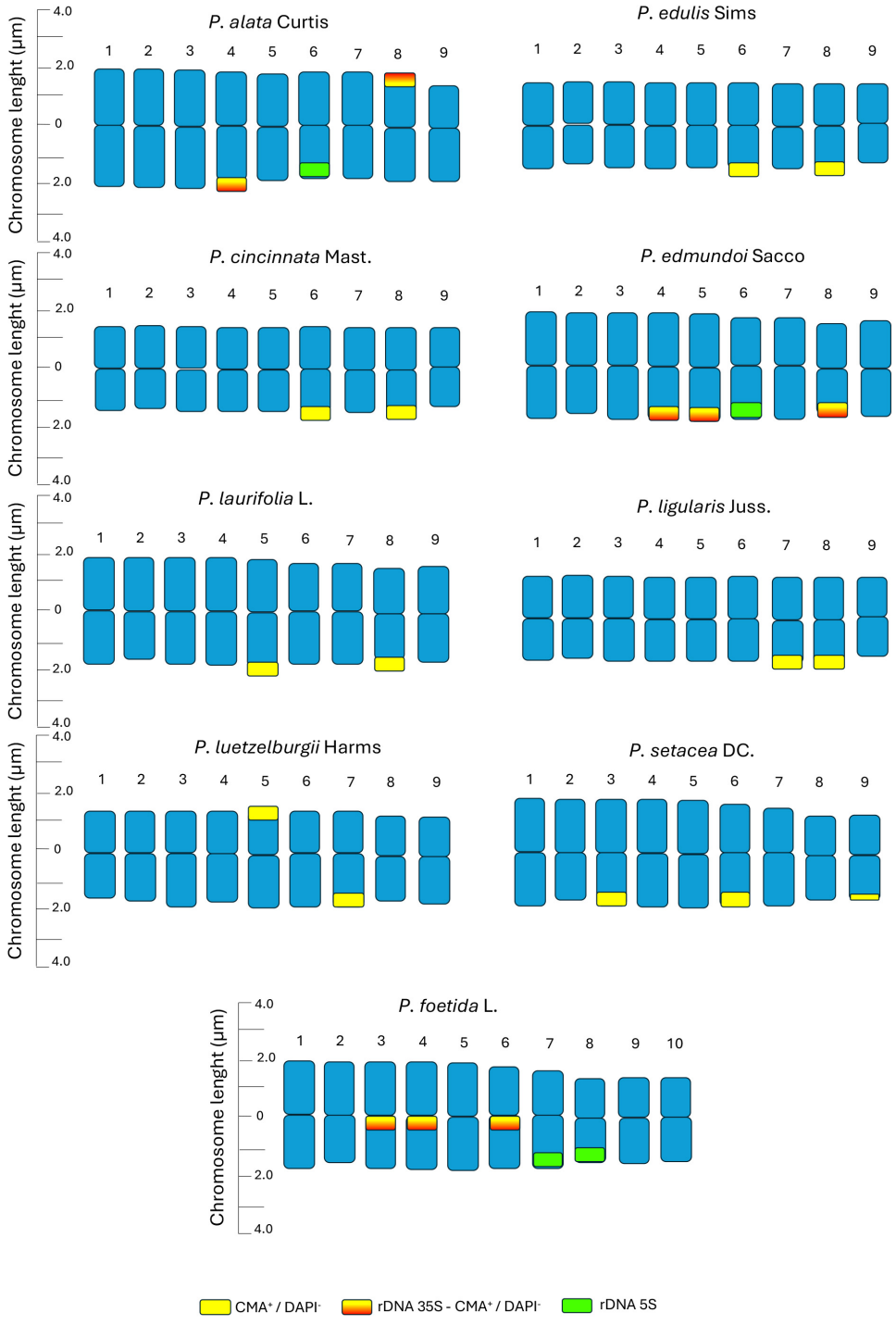

Figure 6. Idiograms of *P. alata*, *P. cincinnata*, *P. edmundoi*, *P. edulis*, *P. foetida*, *P. laurifolia*, *P. ligularis*, *P. luetzelburgii*, and *P. setacea* showing chromosome morphology and the distribution of CMA+/DAPI– heterochromatin (yellow), 35S rDNA loci co-localized with CMA+ blocks (red/yellow), and 5S rDNA loci (green). Chromosomes were arranged in decreasing order based on short arm length and identified by number.

Passiflora foetida presented six CMA+/DAPI- bands in the proximal region of three pairs of chromosomes. These results are also in agreement with Melo et al. (2001), who observed proximal CMA+ bands in *P. foetida*.

The location and distribution pattern of heterochromatic regions present great variability in plants, and polymorphism may occur. Melo et al. (2001) observed double staining with CMA/DAPI in eight species of passion fruit (*P. amethystina, P. caerulea, P. capsularis, P. edulis f. edulis, P. foetida, P. racemosa, P. rubra* and *P. tricuspis*). In these species, four to six CMA+ blocks were observed, without the presence of DAPI+ heterochromatin. In addition, some structural alterations were observed, such as in *P. racemosa*, which presented five CMA+ blocks, one of which was larger and the other four smaller. In the study by Passos (2007), differences were reported in the number of CMA/DAPI blocks in *P. mucronata*, which presented five CMA+ blocks together with DAPI bands, and in *P. galbana*, with four CMA+ blocks. Comparing the results of these studies with those of the present study, structural similarities can be observed between the species analyzed, such as in the number of CMA+ blocks.

Double staining with CMA and DAPI revealed the presence of four to six CMA+ bands distributed predominantly in terminal regions in the *Passiflora* species analyzed, with blocks in pericentromeric regions only being observed in *P. foetida*. These results corroborate the hypothesis of Melo et al. (2001) and Melo & Guerra (2003), who proposed that species that share the same basic number tend to exhibit similar karyotypic patterns.

On the other hand, the number and position of 35S rDNA sites detected by FISH appear to coincide with the CMA+ bands and secondary constrictions observed in the karyotypes of this genus. In species with 2n=18, it was observed that there is variation in the number of 35S rDNA sites (four or six), while the number of 5S rDNA sites did not vary, with only two sites observed. However, four 5S rDNA sites were observed in *P. foetida*, making it more similar to tetraploids than to the recognized diploid karyotypes reported for the genus (Melo & Guerra 2003). In this case, *P. foetida* could be derived from a tetraploid karyotype with n=12 (x=6), passing to n=10 by descending dysploidy.

The application of chromosome banding with CMA and DAPI and FISH have proven to be informative tools, providing valuable data on variability and heterozygosity, essential for the characterization of species and the origin of hybrids (Guerra 1993, Carvalho et al. 2005). When combined with other techniques, such as BAC-FISH and Oligo-barcoding, this methodology allows a more in-depth analysis of the repetitive fraction of the DNA of the species, enabling more detailed analyses of karyotypic evolution (Mata-Sucre et al. 2024).

The genus *Passiflora* presents a high genetic diversity, offering significant opportunities for agronomic research and development. Cytological analyses, such as those performed in this study, are essential not only for the conservation of genetic resources, but also for targeted genetic improvement. Understanding the chromosomal and genetic variability of *Passiflora* species is essential to explore their agronomic and adaptive potential, promoting the development of new varieties with desirable characteristics (Faleiro et al. 2017). Understanding the chromosome number of a species is also essential for carrying out interspecific crosses aimed at obtaining artificial hybrids, since success in hybridization depends directly on the genetic compatibility between species (Silva et al. 2014). In other words, species with similar karyotypes have a greater probability of success in hybridization.

CONCLUSIONS

Based on the results presented, we can conclude that:

a) Chromosome analyses showed that most of the *Passiflora* species analyzed, including *P. alata, P. cincinnata, P. edmundoi, P. edulis, P. ligularis, P. laurifolia, P. luetzelburgii* and *P. setacea*, have a diploid chromosome number of 2n=18, indicating that they all belong to the group with basic chromosome number x=9. In contrast, *P. foetida* presented a distinct diploid number of 2n=20, confirming its classification in a group with basic chromosome number x=10. This difference in chromosome number suggests a possible evolutionary and genetic differentiation between *P. foetida* and the other species analyzed.

b) Analysis with CMA and DAPI fluorochromes revealed significant variations in the distribution and intensity of CMA+ blocks among the species. Most species presented four CMA+ blocks, located in the terminal regions of two pairs of chromosomes. However, *P. edmundoi* and *P. setacea* showed a higher number of CMA+ blocks (six), with small bright spots (dots) detached from the chromosomes, probably related to the nucleolar organizer regions (NORs). These findings indicate a variation in heterochromatin patterns among species and suggest that CMA+ blocks may be associated with nucleolar organization, playing important functional roles.

c) FISH experiments confirmed the patterns observed with CMA+, showing that 35S rDNA sites are located in the terminal regions of the chromosomes in *P. alata* and *P. edmundoi*, with the number of sites corresponding to the number of CMA+ blocks observed. In *P. foetida*, the 35S rDNA sites were found in interstitial regions of the chromosomes, indicating both chromosomal differentiation and a unique structural organization of the genome when compared to the other species analyzed.

d) The results obtained reinforce the hypothesis that the variation in chromosome number and rDNA organization in the different *Passiflora* species may reflect specific evolutionary processes and genetic adaptations. The presence of differentiated characteristics, such as the number of CMA+ blocks and the location of 35S and 5S rDNA sites, suggests a dynamic and diverse chromosomal evolution within the genus *Passiflora*.

These findings provide valuable insights into the chromosomal structure and genomic organization of *Passiflora* species, highlighting the complexity and diversity within this genus.

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