

Integrated cytogenomic analysis of *Physalis* germplasm using CMA/DAPI chromosome banding and flow cytometry

Erison Martins Souza

erisonms@hotmail.com

State University of Feira de Santana, Feira de Santana

Elyabe Monteiro Matos

Federal University of Juiz de Fora, Juiz de Fora

Lyderson Facio Viccini

Federal University of Juiz de Fora, Juiz de Fora

Natoniel Franklin Melo

Brazilian Agricultural Research Corporation

Gabriela Torres Silva

Federal Institute of Education Science and technology of Bahia

Adriana Rodrigues Passos

State University of Feira de Santana Feira de Santana

Luiz Cláudio Costa Silva

State University of Feira de Santana, Feira de Santana

Research Article

Keywords: Cytogenetics, flow cytometry, genome size, heterochromatin, ploidy variation

Posted Date: June 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9809513/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Understanding genome organization is fundamental for the characterization of plant genetic resources and for supporting conservation and breeding strategies. The genus *Physalis* (Solanaceae) comprises species of considerable biological, nutritional, and medicinal importance, including *Physalis peruviana*, *P. angulata*, and *P. ixocarpa*. Previous cytogenetic studies have reported a basic chromosome number of $x = 12$, with chromosome complements ranging from $2n = 24$ to $2n = 48$. In the present study, cytogenomic variation among *Physalis* germplasm accessions was investigated through the integration of CMA/DAPI chromosome banding and flow cytometry. Cytogenetic analyses revealed substantial variation in heterochromatin distribution and chromosome banding patterns among accessions, whereas flow cytometry detected differences in nuclear DNA content and ploidy levels. Significant intra- and interspecific variation was identified, indicating ongoing genomic differentiation within the evaluated germplasm. Distinct cytogenomic patterns were observed among the analyzed species, including diploid and tetraploid accessions, as well as accessions exhibiting multiple nuclear populations. Three major heterochromatin banding patterns were identified (DAPI⁺/CMA⁻, DAPI⁺/CMA⁺, and CMA⁺/DAPI⁻), with predominance of subterminal heterochromatic regions across most chromosomes. To our knowledge, this is the first comparative cytogenetic study integrating CMA/DAPI fluorochrome banding and flow cytometry in germplasm accessions of *P. peruviana*, *P. angulata*, and *P. ixocarpa*. The combined application of these approaches provides valuable insights into genome organization, ploidy variation, and genetic diversity within the genus *Physalis*, contributing to germplasm characterization and the conservation and utilization of plant genetic resources.

Introduction

Understanding genetic diversity within the genus *Physalis* and its germplasm collections is essential for the conservation and sustainable use of plant genetic resources, as well as for the advancement of breeding strategies. The characterization of genetic variability among accessions enables the identification of genetically divergent and reproductively compatible parental lines, thereby contributing to the expansion of the genetic base of cultivated materials and supporting hybridization programs. In this context, cytogenetic and flow cytometric approaches provide valuable insights into genome organization, ploidy variation, and nuclear DNA content, representing important tools for the characterization and management of plant genetic resources (Herrera 2007).

Most cytogenetic studies in *Physalis* have relied on conventional chromosome counting, revealing substantial variation in chromosome number among species. Diploid species with $2n = 24$ chromosomes have been described, such as *Physalis ixocarpa*, whereas polyploid species, including *P. peruviana* and *P. angulata*, commonly exhibit $2n = 48$ chromosomes (Trevisani et al. 2018). In addition, triploid cytotypes and individuals presenting multiple nuclear populations with $2n = 36$ and $2n = 32$ chromosomes, respectively, have also been reported in *P. peruviana* (Lagos et al. 2005). Polyploidy is considered a major evolutionary mechanism within Solanaceae and has been associated with genome diversification, ecological adaptation, and speciation processes (Carbajal et al. 2021).

Chromosomes of the genus *Physalis* are generally small and predominantly meta- or submetacentric (Bernadello and Anderson 1990), which limits detailed structural analyses using conventional staining techniques (Guerra 2000). Although species within Solanaceae frequently exhibit relative uniformity in chromosome number and morphology, substantial variation has been reported in the composition and distribution of repetitive DNA sequences. The characterization of these repetitive fractions contributes to understanding genome structural organization and the evolutionary dynamics underlying chromosomal diversification (Mesquita et al. 2016; Sumner 2003).

The use of base-specific fluorochromes, such as chromomycin A3 (CMA) and 4',6-diamidino-2-phenylindole (DAPI), enables the identification of heterochromatic regions and provides information regarding the repetitive component of the genome (Baguley 1982; Guerra 2000). These fluorochromes, widely employed in plant cytogenetics, discriminate GC- and AT-rich regions, allowing detailed analyses of heterochromatin composition and distribution (Schweizer 1981; Feitosa et al. 2010; Souza et al. 2012). CMA/DAPI banding has proven particularly useful for detecting variation in the number, position, and

intensity of chromosome bands, thereby contributing to karyotype characterization and the identification of structural chromosomal differences among species and populations (De melo 2004; Greilhuber 1984; Da silva et al. 2022).

Studies conducted across distinct plant groups have demonstrated that CMA/DAPI banding patterns can reveal both intra- and interspecific variation, support chromosome differentiation, and contribute to taxonomic and evolutionary interpretations. Despite the relevance of these approaches, detailed information regarding heterochromatin distribution and cytogenomic variation remains scarce for many species of the genus *Physalis* (Almeida et al. 2016; Brasileiro-Vidal et al. 2009; Rego et al. 2009).

In Brazil, several species of *Physalis* have been recorded; however, only a limited number has been investigated in detail at the cytogenetic level. Available information is largely restricted to classical karyotype analyses and, to a lesser extent, molecular cytogenetic approaches such as fluorescence in situ hybridization (FISH), which has been employed for the physical mapping of repetitive sequences, including rDNA sites. Consequently, substantial gaps remain in the cytogenomic characterization of the genus. A similar pattern has been reported in other Solanaceae taxa, in which cytogenetic information remains concentrated in a few species despite increasing evidence of genome size variation and chromosome reorganization (Liberato and Carvalho and Clarindo 2014; Deanna et al. 2022).

The characterization of heterochromatin and chromosome organization has contributed significantly to understanding karyotype evolution and genome diversification in phylogenetically related groups, emphasizing the importance of integrative cytogenomic approaches (Mesquita et al. 2016; Mesquita 2016). In this context, flow cytometry represents an important complementary tool to classical cytogenetics, enabling rapid and sensitive estimation of nuclear DNA content, genome size, and ploidy variation through the analysis of large numbers of nuclei. The integration of cytogenetic and flow cytometric data provides a broader and more robust assessment of genomic variation and genetic diversity within germplasm collections (Doležel and Bartoš 2005; Loureiro et al. 2023).

Therefore, the present study aimed to characterize cytogenomic variation among accessions from the *Physalis* Germplasm Collection of the Molecular Genetics Laboratory at the Experimental Forest Garden Unit of the State University of Feira de Santana (LAGEM/UEFS) through the integration of CMA/DAPI chromosome banding and flow cytometry, with the objective of evaluating heterochromatin distribution, ploidy variation, and genome size diversity among the analyzed accessions.

Material and methods

Plant material and sampling design

The plant material analyzed in this study comprised accessions of *Physalis angulata*, *Physalis peruviana*, and *Physalis ixocarpa* belonging to the *Physalis* Germplasm Collection maintained at the Molecular Genetics Laboratory of the State University of Feira de Santana (LAGEM/UEFS), Brazil. The accessions originated from different geographic regions and represented both cultivated and wild materials.

A total of 13 accessions of *P. angulata* were evaluated, originating from distinct Brazilian regions: Pará (PA), Piauí (PI), Candeias (CAN), Anguera (ANG and LG), Feira de Santana (G53 and FSA), Espírito Santo (ES), Serra Preta (SP1 and SP2), Pernambuco (PE), Itapetim (IT), and Lajedinho (LJ). In addition, four accessions of *P. peruviana* (Feltrin–FE, Colombia–CO, São Carlos–SC, and São Paulo–SP), two accessions of green-fruited *P. ixocarpa* (IX1 and IX2), and one accession of purple-fruited *P. ixocarpa* (IX3) were included (Table 1). Seeds from all accessions were subjected to germination in order to obtain root meristems for cytogenetic analyses and young leaves for flow cytometric analyses.

Table 1
Geographic origin and identification of the *Physalis* accessions evaluated in this study.

Code	Accession	Origin	State	Latitude	Longitude
SP1	Serra Preta 1	Serra Preta	BA	-12.036925	-39.269081
SP2	Serra Preta 2	Serra Preta	BA	-12.036881	-39.268967
ITA	Pernambuco	Itapetim	PE	7.378150	-37.188770
W01	LG2010	Anguera	BA	-12.151111	-39.246389
W06	Lajedinho	Lajedinho	BA	-12.366667	-40.900000
W11	Candeias	Candeias	BA	-12.667778	-38.550556
W16	Piauí	Teresina	PI	-5.089167	-42.801667
W21	Genitor 53	Feira de Santana	BA	-12.273333	-38.955556
FSA	Feira de Santana	Feira de Santana	BA	-12.451944	-39.618611
AN1	Anguera	Anguera	BA	-12.160556	-39.238056
ES	Espirito Santo	Espirito Santo	ES	-18.503060	-40.678283
PA	Pará	Pará	PA	-1.366200	-48.372089
PE	Pernambuco	Pernambuco	PE	-7.377500	-37.190556
IX1 Ixocarpa verde México					
IX2 Ixocarpa verde México					
IX3 Ixocarpa roxo México					
FE Feltrin Feltrin seeds					

Cytogenetic analyses using CMA/DAPI fluorochrome staining were performed on a representative subset of accessions, including *P. angulata* (ES and PE), *P. ixocarpa* (IX1, IX2, and IX3), and *P. peruviana* (FE). To broaden genomic sampling and improve the assessment of cytogenomic variability, flow cytometric analyses were subsequently expanded to 13 accessions of *P. angulata*, four accessions of *P. peruviana*, and three accessions of *P. ixocarpa* using young leaf tissues.

Cytogenetic analysis using CMA/DAPI fluorochrome staining

Cytogenetic analyses were conducted at the Biotechnology Laboratory of Embrapa Semiárido, Petrolina, Pernambuco, Brazil. Seeds were germinated in Petri dishes containing moistened filter paper and maintained at room temperature until root emergence.

Actively growing root tips were pretreated with 8-hydroxyquinoline (8-HQ) for 24 h at 10°C to accumulate metaphases with adequate chromosome condensation. Subsequently, the roots were fixed in freshly prepared Carnoy's solution (ethanol: acetic acid, 3:1 v/v) and stored at - 20°C until use.

For slide preparation, root tips were washed twice in distilled water for 5 min and enzymatically digested in a solution containing 2% cellulase and 20% pectinase at 37°C for 2 h. Following digestion, individual root meristems were transferred onto microscope slides and macerated in a drop of 45% acetic acid. The meristematic tissue was carefully fragmented under a stereomicroscope using dissecting needles. Coverslips (18 × 18 mm) were gently applied to avoid air bubbles, followed by slight thumb pressure to improve chromosome spreading. Coverslips were subsequently removed after freezing the slides in liquid nitrogen for at least 15 min. Slides were air-dried and aged for three days at room temperature prior to fluorochrome staining.

Dual CMA/DAPI staining was performed according to Vaio et al. (2018). Slides were stained with chromomycin A3 (CMA; 0.5 mg mL⁻¹) for 1 h in a dark chamber and subsequently counterstained with 4',6-diamidino-2-phenylindole (DAPI; 2.0 µg mL⁻¹) for 30 min. Slides were mounted in glycerol/McIlvaine buffer (1:1; pH 7.0) and aged for an additional three days in the dark to allow fluorochrome stabilization (Barros and Silva et al. 2010).

Heterochromatic regions were classified according to fluorochrome affinity as CMA⁺/DAPI⁻, DAPI⁺/CMA⁻, and CMA⁺/DAPI⁺ bands. Neutral regions without preferential staining were considered CMA⁰/DAPI⁰.

Metaphase chromosomes were analyzed because this stage provided the highest degree of chromosome condensation and visualization quality. Only well-spread metaphases without chromosome overlap were selected for analysis. Images were captured using a Leica DM2000 epifluorescence microscope equipped with a digital video camera and Leica QFish software for image acquisition and chromosome counting.

Estimation of relative nuclear DNA content by flow cytometry

Seeds obtained from the *Physalis* Germplasm Collection of LAGEM/UEFS (Table 1) were surface-sterilized in 96% ethanol for 1 min, followed by immersion in 2.5% sodium hypochlorite solution (commercial bleach, QBoa®) supplemented with three drops of Tween® for 10 min. Subsequently, the seeds were rinsed three times with sterile distilled water under aseptic conditions. The disinfected seeds were inoculated into glass test tubes containing 15 mL of autoclaved culture medium composed of Murashige and Skoog (1962) basal salts and vitamins, supplemented with 30 g L⁻¹ sucrose and solidified with 6 g L⁻¹ agar. The culture medium was autoclaved at 121°C for 15 min prior to use. Tubes were sealed with polypropylene caps and maintained in a growth chamber at 35°C under a 16 h photoperiod for 30 days at the Plant Tissue Culture Laboratory of the Federal University of Bahia. After the growth period, two fully expanded leaves from each accession were collected and wrapped in moistened paper containing 0.5 mL sterile water. Samples were stored in zip-lock plastic bags and transported to the Genetics and Biotechnology Laboratory of the Institute of Biological Sciences, Federal University of Juiz de Fora, for flow cytometric analyses.

Nuclear suspensions were prepared according to Galbraith et al. (1983) by co-chopping leaf tissues with a disposable steel razor blade in WPB isolation buffer (Loureiro et al. 2007). The resulting suspension was filtered through two layers of gauze and subsequently through a 30 µm Nylon CellTrics® mesh to remove cellular debris.

For DNA staining, 25 µL propidium iodide (PI; 1 mg mL⁻¹) and 5 µL RNase (1 mg mL⁻¹) were added to each sample. After incubation for 30 min in the dark, samples were analyzed using a CytoFLEX flow cytometer (Beckman Coulter, CA, USA). Three independent replicates were performed per accession, and at least 10,000 nuclei were analyzed for each sample. *Pisum sativum* (2C = 9.09 pg) was used as the internal reference standard (Doležel et al. 1998). Histograms were generated and analyzed using CytExpert 2.0.1 software. Relative nuclear DNA content was estimated according to Doležel and Bartoš (2005) and Doležel et al. (2007). In the generated histograms, the x-axis corresponded to propidium iodide fluorescence intensity (PI-A), proportional to nuclear DNA content, whereas the y-axis represented the number of analyzed nuclei.

Differences in sampling design between cytogenetic and flow cytometric analyses were considered during data interpretation and are discussed integratively in the Results and Discussion section, taking into account the methodological specificities and complementary nature of the employed approaches.

Results and discussion

Cytogenetic analysis: heterochromatin distribution (CMA/DAPI) and ploidy

Cytogenetic and flow cytometric analyses revealed substantial cytogenomic variation among the evaluated accessions of *Physalis angulata*, *P. peruviana*, and *P. ixocarpa*. Differences in heterochromatin distribution, nuclear DNA composition, and ploidy levels indicate the occurrence of both intra- and interspecific genomic variability, highlighting the relevance of cytogenomic approaches for germplasm characterization and plant genetic resource management.

CMA/DAPI chromosome banding allowed the identification of distinct heterochromatin distribution patterns among the *Physalis* accessions. In all accessions, multiple DAPI⁺ bands were observed predominantly in subterminal chromosomal regions (Fig. 1), indicating the presence of AT-rich heterochromatin. In contrast, all accessions except ES exhibited at least one chromosome pair with strong CMA⁺ signals, likely corresponding to nucleolar organizer regions (NORs). These chromosomal regions contain ribosomal RNA genes, particularly 35S rDNA loci, which are associated with nucleolus formation and ribosome biosynthesis (Seregard 1998).

The number and distribution of CMA⁺ blocks varied markedly among accessions. Four CMA⁺ blocks were detected in PE (2n = 48), two CMA⁺ blocks in IX1, IX2 (2n = 24), and Feltrin (2n = 48), whereas approximately eight CMA⁺ blocks were identified in IX3 (Fig. 1; Table 2). Three major heterochromatin banding patterns were recognized: (i) DAPI⁺/CMA⁻ bands, (ii) DAPI⁺/CMA⁺ bands, and (iii) CMA⁺/DAPI⁻ bands. DAPI⁺/CMA⁻ bands occurred mainly in subterminal regions of ES, Feltrin, IX1, and IX2. DAPI⁺/CMA⁺ bands, frequently associated with NOR regions, were distributed in terminal, subterminal, proximal, and interstitial positions. CMA⁺/DAPI⁻ bands were observed in terminal NOR-associated regions of IX1, IX2, and Feltrin, and interstitially only in Feltrin (Table 2).

Table 2
Different chromosome banding patterns revealed by CMA/DAPI staining in *Physalis* accessions.

Accessions	CMA+ blocks (subterminal/terminal)	Interstitial heterochromatin (CMA+/DAPI-)	Proximal/subterminal heterochromatin (DAPI+/CMA+)	NOR (terminal) (CMA+/DAPI-)	Subterminal heterochromatin (DAPI+/CMA-)
ES					X
PE	4/0		X		
Feltrin	0/2	X		X	X
IX1	2/2		X	X	X
IX2	2/2		X	X	X
IX3	8/0		X		

The variation detected in CMA/DAPI banding patterns reflects differences in the composition and organization of repetitive DNA among the evaluated accessions. Such variability is commonly associated with structural changes involving satellite DNAs and other repetitive sequences, which play an important role in genome organization and chromosomal evolution in plants (Guerra 2000; Greilhuber 1998). Differences in the number, position, and intensity of fluorescent bands suggest genomic differentiation among accessions and may indicate chromosomal rearrangements or amplification of repetitive DNA associated with evolutionary divergence.

Although species of Solanaceae frequently exhibit conserved chromosome numbers and morphology, heterochromatin composition has been recognized as an important source of genomic diversification within the family (Bernardello and Anderson 1990; Mesquita et al. 2016). The heterochromatin variability observed in *P. angulata*, *P. peruviana*, and *P. ixocarpa* reinforces the dynamic nature of repetitive DNA regions and demonstrates the usefulness of CMA/DAPI banding for detecting structural chromosome variation not revealed by conventional staining methods (Schweizer 1981).

Previous karyotypic studies in *P. peruviana* were mainly restricted to chromosome morphology and pairing analyses (Liberato et al. 2014). More recently, Garcia Paitan et al. (2023) used FISH mapping to identify 5S and 45S rDNA loci in *P. peruviana*. In *Physalis pubescens*, CMA/DAPI banding revealed AT-rich heterochromatin predominantly located in terminal chromosomal regions (Mesquita et al. 2019). However, to our knowledge, this is the first report describing heterochromatin banding patterns in *P. angulata*, *P. peruviana*, and *P. ixocarpa* using CMA/DAPI fluorochromes.

The characterization of AT- and GC-rich chromosomal regions contributes to understanding genome organization and the evolutionary dynamics of repetitive DNA, particularly under polyploidization processes. Similar heterochromatin diversity has

been described in several Solanaceae genera, including *Solanum*, *Nicotiana*, *Lycium*, and *Capsicum* (Brasileiro-Vidal et al. 2009; Rego et al. 2009; Chiarini et al. 2014; Romero-da-Cruz et al. 2015).

The fluorescent banding profiles identified here revealed substantial variation in the distribution of repetitive sequences among species and accessions. While the number of CMA⁺ sites ranged from two to eight, their chromosomal positions varied considerably, including terminal, subterminal, proximal, and interstitial regions. This pattern suggests dynamic amplification and dispersion of GC-rich repetitive sequences during genome evolution.

The occurrence of CMA⁺/DAPI⁻ terminal bands associated with NORs is considered one of the most common heterochromatin patterns in plants and has been proposed as an ancestral condition in Solanaceae (Guerra 2000). Nevertheless, not all CMA⁺ sites necessarily correspond to ribosomal DNA *loci*, since GC-rich heterochromatin may also consist of satellite DNAs independent of rDNA sequences (Jo et al., 2009). The observed variation in the number of CMA⁺ blocks, including putative 35S rDNA-associated sites, is consistent with previous reports in Solanaceae species. Similar variation has been reported in *Solanum* spp., *Nicotiana* spp., and *Capsicum* spp., where the number of 35S rDNA *loci* ranges from two to more than 30 sites depending on the species and cytotype (Matyasek et al. 2003; Kovarik et al. 2004; Moyetta et al. 2017; Romero-da-Cruz et al. 2017).

The exclusive occurrence of interstitial CMA⁺/DAPI⁻ bands in Feltrin may represent localized chromosomal rearrangements, such as inversions or transposition events, as previously suggested for other plant groups (Fonseca 2010; Shibata et al. 2005). Furthermore, the absence or weak detection of some DAPI or CMA bands may reflect reduced heterochromatin content, absence of extensive AT- or GC-rich blocks, or structural chromatin modifications affecting fluorochrome accessibility. Similar heterochromatin loss or reduction has been reported in several plant taxa (Kopecký et al. 2019).

Subterminal DAPI⁺/CMA⁻ bands rich in AT sequences were widely distributed across chromosomes of ES, IX1, IX2, and most chromosomes of Feltrin. This pattern appears to be relatively uncommon in Solanaceae and has previously been reported mainly in *Capsicum praetermissum* (Scaldeferro et al. 2013). In contrast, DAPI⁺/CMA⁺ bands revealed colocalization of AT- and GC-rich regions, especially in PE and *P. ixocarpa* accessions, where CMA⁺ blocks were distributed in subterminal and proximal positions. Similar patterns have also been described in *Physalis pubescens*, *Solanum*, and *Capsicum* species (Chiarini et al. 2014; Romero-da-Cruz et al. 2015).

Differences in fluorescence intensity among chromosomal regions likely reflect variation in the copy number of tandemly repeated sequences within heterochromatic blocks. Alterations in repeat abundance may result from amplification, deletion, or unequal crossing-over events, contributing to the observed heterochromatin polymorphism (Prado et al. 1996; Su et al. 2020).

This study represents the first comparative cytogenetic analysis using CMA/DAPI fluorochromes in *P. angulata*, *P. peruviana*, and *P. ixocarpa*. The detected variation in the number, composition, and chromosomal distribution of DAPI⁺ and CMA⁺ bands demonstrates substantial genomic differentiation among the evaluated accessions.

Chromosome counts revealed $2n = 2x = 24$ chromosomes in *P. ixocarpa* and $2n = 4x = 48$ chromosomes in *P. angulata* and *P. peruviana*. Considering the basic chromosome number $x = 12$ for the genus *Physalis* (Quiros 1984), the analyzed accessions from the LAGEM/UEFS *Physalis* Germplasm Collection were classified as diploid (*P. ixocarpa*) and tetraploid (*P. angulata* and *P. peruviana*). Detailed chromosome morphology could not be accurately established due to the small chromosome size and high condensation levels; however, chromosome number and fluorochrome banding patterns were clearly identifiable.

The chromosome numbers observed here agree with previous reports for the genus. Among the 25 *Physalis* species reviewed by Menzel (1951), most presented $2n = 24$ chromosomes, whereas *P. peruviana*, *P. minima*, and *P. angulata* frequently exhibited $2n = 48$. Tetraploid chromosome complements in *P. angulata* and *P. peruviana* have also been reported by Pedrosa (1999), Araújo et al. (2015), Azeez et al. (2019), and Carbaral et al. (2021).

Polyploidy appears to be relatively common in *P. angulata*, *P. peruviana*, and *P. minima* (Ganapathi et al. 1991; Nohra et al. 2006; Carbaral et al. 2021). Although most *Physalis* species are diploid with $x = 12$, *P. angulata* exhibits considerable cytotypic variation, including diploid and polyploid forms (Menzel 1951; Preet and Gupta 2015, 2018; Saavedra et al. 2019; Azeez and Faluyi 2019). Numerical chromosome variation reported in *P. peruviana* and *P. angulata*, including aneuploid cytotypes, has been associated with chromosomal rearrangements, inversions, and dysploidy events (Rodriguez and Bueno 2006; Guerra 2008).

The occurrence of tetraploid complements in *P. angulata* and *P. peruviana* may have practical implications for breeding programs, particularly regarding interspecific hybridization potential. Experimental crosses between these species have already demonstrated the possibility of hybrid formation and fruit production, although with differences in physiological maturation (Adeboye and Oladele 2014).

Nuclear DNA content analysis and ploidy inference by flow cytometry

Flow cytometric analyses produced predominantly low coefficients of variation (CV%) (Supplementary Material), ranging from approximately 2.3% to 5.0% for most diploid and tetraploid accessions. These values fall within the range considered optimal for reliable nuclear DNA content estimation in plants (Doležal et al. 2007). Representative histograms illustrating G0/G1 peak quality and nuclear population resolution are shown in Fig. 2. The low CV values indicate adequate G0/G1 peak resolution and efficient sample preparation, supporting the robustness of the ploidy inferences obtained.

In the histograms, the x-axis represents propidium iodide fluorescence intensity (PI-A), which is proportional to nuclear DNA content, whereas the y-axis corresponds to the number of analyzed nuclei. The G0/G1 peak, representing nuclei with non-replicated DNA content (2C), was used as the reference for genome size estimation and ploidy inference. Accessions PE_6 and ES_4 (*P. angulata*) displayed well-defined G0/G1 peaks, indicating stable nuclear DNA content. Similarly, accession IX1_1 (*P. ixocarpa*) exhibited a profile consistent with diploidy. In contrast, accession IX3_4 presented multiple G0/G1 peaks, suggesting a mixoploid condition or the coexistence of distinct nuclear populations. Mixoploidy refers to the occurrence of cells with different ploidy levels within the same individual and is characterized in flow cytometry by the presence of multiple G0/G1 peaks corresponding to nuclei with distinct DNA contents (Doležal and Bartoš 2005).

Accessions Colômbia-CO_1 and Feltrin-FE_1 (*P. peruviana*) exhibited G0/G1 peaks shifted to approximately twice the fluorescence intensity observed in diploid accessions, indicating increased nuclear DNA content.

Some accessions (PA_2, PI_1, G53_8 and G53_10, SP1_6, IX2_3, and IX3_6) exhibited coefficients of variation above 7% (Supplementary Material), a threshold above which flow cytometric data should be interpreted with caution due to reduced peak resolution.

Ploidy estimation by flow cytometry

Flow cytometry enabled the identification of three major cytogenomic categories among the evaluated accessions: diploid (2x), tetraploid (4x), and mixoploid individuals. Most accessions from the LAGEM/UEFS *Physalis* Germplasm Collection, originating from different regions of Brazil, exhibited relative nuclear DNA content values ranging from approximately 45,000 to 62,000 fluorescence units. This interval was highly consistent among *P. angulata* accessions (Fig. 2), regardless of geographic origin, supporting the diploid condition previously reported for the species (Preet and Gupta 2015, 2018; Saavedra et al. 2019). A similar diploid pattern was observed in green-fruited *P. ixocarpa* accessions IX1_1 (Fig. 2) and IX2_3, which are classically described as $2n = 2x = 24$ (Robledo-Torres et al. 2011).

In contrast, *P. peruviana* accessions FE, CO (Fig. 2), SC, and SP displayed nuclear DNA content values ranging from approximately 94,000 to 120,000 fluorescence units, corresponding to nearly twice the values observed in diploid accessions. The low intra-accession variation and reduced CV values associated with these samples support the interpretation of stable tetraploidy resulting from complete genome duplication.

Purple-fruited *P. ixocarpa* accessions IX3_4 (Fig. 2) and IX3_6 exhibited intermediate DNA content patterns, whereas IX3_6 also showed elevated CV values and multiple nuclear populations. Although these profiles may reflect mixoploidy or somatic chromosomal instability, the influence of tissue-specific developmental processes cannot be excluded. In plants, vegetative tissues frequently undergo endoreduplication and endocyclic variation, resulting in nuclei with distinct DNA contents without necessarily involving permanent chromosomal alterations (Galbraith et al. 1991; Jovtchev et al. 2006; Wang et al. 2021).

In this context, occasional discrepancies between cytogenetic and flow cytometric results may reflect the distinct sampling nature of these approaches, as well as the higher sensitivity of flow cytometry for detecting intraindividual variation in biologically unstable systems (Doležel and Bartoš 2005). Given the complexity of the observed patterns, particularly in accessions exhibiting multiple nuclear populations, additional analyses involving larger sample sizes and different tissue types will be necessary to clarify the origin and stability of these cytogenomic variations.

The differences in nuclear DNA content and ploidy levels detected by flow cytometry further support the existence of genome size variation among *Physalis* accessions. Genome size variation may arise from polyploidization events, amplification or loss of repetitive DNA sequences, and chromosomal rearrangements (Bennett and Leitch 2005). Polyploidy is widely recognized as a major mechanism of genome evolution in angiosperms and is particularly relevant in Solanaceae, where it contributes to genetic diversification, adaptation, and speciation (Soltis et al. 2015). The variation observed among *P. angulata* and *P. peruviana* accessions reinforces the importance of polyploidization and genome restructuring in the evolutionary history of the genus.

These findings are consistent with the cytogenetic analyses obtained through CMA/DAPI banding, which revealed variation in the distribution and intensity of heterochromatic regions among accessions. Differences in DAPI⁺ and CMA⁺ banding patterns indicate variability in AT- and GC-rich repetitive DNA fractions, suggesting that genome size variation is associated not only with changes in chromosome number, but also with amplification or reduction of repetitive DNA sequences (Schweizer 1976; Heslop-Harrison and Schwarzacher 2011). Such heterogeneity in chromatin organization may underlie the emergence of distinct nuclear populations detected by flow cytometry, reinforcing the interpretation that mixoploidy reflects ongoing processes of genome restructuring (Doležel and Bartoš 2005; Loureiro et al. 2023).

Although intraindividual variation in nuclear DNA content has been described in several Solanaceae species, including *Solanum lycopersicum* and *Capsicum annum*, often associated with endoreduplication and chromosomal variability (Galbraith et al. 1983; Moscone et al. 1995), the mechanisms underlying the patterns observed in purple-fruited *P. ixocarpa* remain poorly understood. In addition to intrinsic cytogenomic variation, the potential influence of in vitro cultivation conditions and tissue type cannot be disregarded. From an evolutionary perspective, changes in genome size and chromatin organization may reflect the remarkable plasticity of plant genomes, contributing to adaptive responses and the maintenance of genetic diversity (Van de Peer et al. 2021; Loureiro et al. 2023). Nevertheless, further studies involving larger sampling efforts, multiple tissue types, and controlled cultivation conditions will be required to clarify the origin and stability of these variations in *Physalis*.

Integrated Analysis of Flow Cytometry and DAPI/CMA Cytogenetics

Cytogenetic analysis using double CMA/DAPI staining revealed substantial variation in the number, distribution, and intensity of heterochromatic bands among the evaluated accessions. The *P. angulata* accessions from Pernambuco (PE) and Espírito Santo (ES) were interpreted as tetraploid (4x) (Table 3) based on chromosome counts and fluorescent band organization. The *P. peruviana* accession Feltrin (FE) was also consistently classified as 4x (Table 3). In contrast, the green-fruited *P. ixocarpa* accessions (IX1 and IX2) were classified as diploid (2x), with no clear cytogenetic evidence of complete chromosome duplication.

Table 3
Comparative synthesis between flow cytometry and cytogenetic analyses (DAPI/CMA) in accessions of *P. angulata*, *P. peruviana*, and *P. ixocarpa*.

Accessions	Flow cytometry	DAPI/CMA	Integrated interpretation
<i>PE</i>	2x	4x	Genomic diploidy with heterochromatin expansion
<i>ES</i>	2x	4x	Structurally complex diploidy
<i>Feltrin</i>	4x	4x	Stable tetraploidy
<i>IX3</i>	Mixoploid	2x	Nuclear heterogeneity / variation in DNA content

The integration of chromosome banding and flow cytometry proved to be a complementary strategy for assessing cytogenomic variation. Cytogenetic analysis provides structural information regarding chromosome organization and heterochromatin distribution, whereas flow cytometry enables quantitative estimation of nuclear DNA content through the analysis of thousands of nuclei, thereby increasing sensitivity to intraspecific variability (Doležal et al. 2007). The combined use of these approaches allows a broader assessment of genome organization and improves the reliability of germplasm characterization.

The results obtained suggest the predominance of nuclear DNA content compatible with diploidy in the Brazilian accessions of *P. angulata* analyzed here, although discrepancies between the approaches indicate greater cytogenomic complexity. The purple-fruited *P. ixocarpa* accessions exhibited multiple nuclear populations, whereas *P. peruviana* showed stable tetraploidy. By directly quantifying nuclear DNA content, flow cytometry proved particularly efficient in discriminating complete genome duplications from structural genomic variation.

The discrepancies observed in the *P. angulata* accessions PE and ES, classified as tetraploid by DAPI/CMA cytogenetics but diploid by flow cytometry, represent one of the central findings of this study. Critically, these results suggest that interpretations based exclusively on chromosome morphology may be influenced by differential heterochromatin expansion, particularly GC-rich regions evidenced by CMA⁺ bands. Amplification of these regions, including possible expansion of nucleolar organizer regions (NORs), may substantially alter chromosomal appearance without implying complete genome duplication (Guerra 2000; Hirai 2020; Mcstay 2016).

From an evolutionary perspective, the occurrence of multiple nuclear populations and discrepancies between chromosome structure and nuclear DNA content may reflect elevated genomic plasticity in *P. angulata* and purple-fruited *P. ixocarpa*. Such plasticity may be associated with adaptation to distinct environments, recent colonization history in Brazil, and the maintenance of genetic diversity even in populations derived from relatively restricted gene pools (Adams and Wendel 2005; Heslop-Harrison et al. 2023; Hollister 2015; Van de Peer et al. 2021).

These patterns can be visualized integratively in Fig. 3, in which differences in nuclear DNA content detected by flow cytometry are associated with the respective heterochromatic banding patterns revealed by DAPI/CMA staining.

The complete agreement between flow cytometry and DAPI/CMA analyses observed in the Feltrin, CO, SC, and SP accessions reinforces the interpretation of stable tetraploidy, possibly resulting from ancient polyploidization events that became fixed over evolutionary time. This pattern contrasts with that observed in IX3, in which cytogenetic analyses revealed only diploid metaphases, whereas flow cytometry detected multiple nuclear populations. This discrepancy is interpreted as a consequence of the distinct sampling nature of the techniques: cytogenetics evaluates a limited number of metaphases, while flow cytometry integrates information from thousands of nuclei and is therefore more sensitive to cellular variation (Steinarsdóttir et al. 1997; Robinson et al. 2023).

Additionally, the influence of factors associated with in vitro cultivation and the type of tissue analyzed cannot be excluded, as these may contribute to variation in nuclear DNA content in vegetative tissues.

Similar discrepancies have been reported in plant species exhibiting genomic instability or tissue-specific variation in ploidy levels (Doležal et al. 2007). In the *Festuca yvesii* complex (Poaceae), for example, the combined use of flow cytometry and chromosome counting across different populations within the same species revealed cytotype diversity and variation in nuclear DNA content that were neither linear nor uniform, thus requiring the integration of both methods for robust cytogenomic interpretation (Martínez-Sagarra et al. 2021).

Together, these findings reinforce the complementary nature of the approaches employed, since flow cytometry demonstrated greater sensitivity for detecting variation in nuclear DNA content, whereas classical cytogenetics enabled detailed characterization of chromosome organization and heterochromatin distribution.

The cytogenomic variation observed among the accessions highlights the genetic diversity preserved within *Physalis* germplasm collections. Differences in heterochromatin organization and ploidy levels suggest ongoing genomic differentiation, which may influence adaptive potential and reproductive compatibility. The identification of genetically divergent accessions therefore represents valuable information for the selection of parental lines and for the conservation of genetic diversity in germplasm collections.

Finally, the difference in the number of accessions analyzed between the two approaches reflects biological limitations inherent to plant material establishment and the specific technical requirements of each method, rather than methodological constraints of the analyses themselves. Accordingly, cytogenetics was applied with a qualitative and structural focus, aiming at the detailed chromosomal characterization of representative accessions, whereas flow cytometry was used as a quantitative tool for estimating nuclear DNA content and determining ploidy levels in a broader sampling framework. The integration of these approaches enabled a robust and complementary assessment of cytogenomic variation among the analyzed accessions.

Conclusion

The results obtained indicate a predominance of nuclear DNA content compatible with diploidy in *P. angulata* and green-fruited *P. ixocarpa*, whereas *P. peruviana* exhibited a pattern consistent with stable tetraploidy. In contrast, purple-fruited *P. ixocarpa* accessions displayed multiple nuclear populations, suggesting heterogeneity in nuclear DNA content. Double CMA/DAPI staining revealed three major heterochromatin patterns: DAPI⁺/CMA⁻, DAPI⁺/CMA⁺, and CMA⁺/DAPI⁻ bands, with a predominance of subterminal DAPI⁺ bands across nearly all analyzed chromosomes.

The integration of flow cytometry and DAPI/CMA cytogenetics proved to be an effective approach for the cytogenomic characterization of the evaluated accessions, allowing the discrimination of variation in nuclear DNA content and structural differences associated with heterochromatin organization. The discrepancies observed between the two approaches highlight the complexity of genome organization within the analyzed species and reinforce the importance of combining complementary methodologies in studies of genetic diversity, genome evolution, and conservation of plant genetic resources. Furthermore, the cytogenomic variability detected represents a valuable source of genetic resources for conservation and breeding programs within the genus *Physalis*.

Declarations

Ethics Statement

Conflicts of Interest

The authors declare no competing interests.

Ethics approval

Not applicable

Informed consent:

Not applicable

Consent for publication:

Yes

Funding

We declare that the manuscript received financial support from the Bahia Research Foundation (FAPESB), the National Council for Scientific and Technological Development (CAPES), and the National Council for Scientific and Technological Development (CNPq 409827/2023-1).

Author Contribution

EMS: methodology, investigation, formal analysis, writing of the original version. LCCS: concept, formal analysis, supervision, review, and editing. NFM: GTS: formal analysis, review, writing, and editing. ARP: formal analysis and supervision. EMM: LFV: formal analysis and supervision.

Acknowledgments

The authors would like to thank the Bahia Research Foundation (FAPESB), the National Council for Scientific and Technological Development (CAPES), and the National Council for Scientific and Technological Development (CNPq 409827/2023-1) for their financial support. We also thank the State University of Feira de Santana, the Brazilian Agricultural Research Corporation (Embrapa Semiárido), and the Federal University of Juiz de Fora for providing the plant material and for their experimental and technical support.

Data Availability

Data availability The datasets generated and/or analyzed during the current study are included in this published article and its supplementary information files.

References

1. Acosta MC, Guerra M, Moscone EA (2012) Karyological relationships among some South American species of *Solanum* (Solanaceae) based on fluorochrome banding and nuclear DNA amount. *Plant systematics and evolution* v. 298, p. 1547–1556
2. Adams KL, Wendel JF (2005) Polyploidy and genome evolution in plants. *Current Opinion in Plant Biology*, London, v. 8, n. 2, p. 135–141
3. Adeboye AO, Oladele FA (2014) Hybridization in four Nigerian *Physalis* species. *Notulae Scientia Biologicae* 6(1), 45–52
4. Almeida EM, Wanderley AM, Nollet F, Costa FR, Souza LGR, Felix LPA (2016) New Species of *Ameroglossum* (Scrophulariaceae) Growing on Inselbergs in Northeastern Brazil. *Systematic Botany* 41(2): 423–429
5. Andras SC, Hartman TPV, Marshall JA, Marchant R, Power JB, Cocking EC, Davey MR (1999) A drop-spreading technique to produce cytoplasm-free mitotic preparations from plants with small chromosomes. *Chromosomes Research* v. 7, p. 641–647

6. Araújo FL, Queiroz SROD, Passos AR, Osuna JTA (2015) Caracterização cromossômica em *Physalis angulata* L. e *P. peruviana* L. Magistral, Cruz das Almas-BA, v.27, n.1, p 82–89
7. Azeez SO, Faluyi JO (2019) Karyotypic studies of four *Physalis* species from Nigeria. Acta Botanica Hungarica v. 61, n. 1–2
8. Azeez SO, Faluyi JO, Oziegbe M (2019) Cytological, foliar epidermal and pollen grain studies in relation to ploidy levels in four species of *Physalis* L. (*Solanaceae*) from Nigeria. International Journal of Biological and Chemical Sciences 13(4): 1960–1968
9. Báez M, Souza G, Guerra M (2020) Does the chromosomal position of 35S rDNA sites influence their transcription? A survey on *Nothoscordum* species (*Amariyllidaceae*). Genetics and molecular biology, 43(1): e20180194
10. Baguley, BC (1982) Nonintercalative DNA-binding antitumor compounds. Molecular and Cellular Biochemistry, 43: 167–181
11. Barros-Silva AE, Marques A, Santos KGB, Guerra M (2010) The evolution of CMA bands in *Citrus* and related genera. Chromosome Research, 18:503–514
12. Bennett MD, Leitch IJ (2005) Genome size evolution in plants. In: Gregory, T. R. (ed.). The evolution of the genome. San Diego: Elsevier, p. 89–162
13. bernardello LM, anderson GJ (1990) Karyotypic Studies in *Solanum* section *Basarthurum* (*Solanaceae*). American Journal of Botany 77:420–431
14. Brasileiro-Vidal AC. et al (2009) Different chromatin fractions of tomato (*Solanum lycopersicum* L.) and related species. Micron v. 40, n. 8, p. 851–859
15. Carbajal Y, Bonilla H, Siles-Vallejos M, López A (2021) Citogenética comparativa de *Physalis peruviana* em três populações cultivadas em Cajamarca, Peru. Revista Peruana de Biología, 28(2): e20462
16. Chiarini FE. et al (2014) Genome size and karyotype diversity in *Solanum* sect. *Acanthophora* (*Solanaceae*). Plant Systematics and Evolution v. 300, p. 113–125
17. Da silva RT, Nollet F, Santos DC, Felix LP, Buril MT, Bruno RLA (2022) Heterochromatin and polyploidy in *Opuntia* Mill. (*Cactaceae: Opuntioideae*): implications for plant breeding. New Zealand Journal of Crop and Horticultural Science, 51(4), 527–546
18. Deanna R, Chiarini F, Acosta MC. et al (2022) Chromosome evolution in the family *Solanaceae*. Frontiers in Plant Science, Lausanne, v. 13
19. De melo NF (1998) Citogenética vegetal aplicada à taxonomia e ao melhoramento. XXVII Reunião Nordestina de Botânica. Petrolina, 3–5, 2004. Dolezel J, Greilhuber J, Lucretti S, Meister A, Lysak MA, Nardi L, Obermayer R. Plant genome size estimation by flow cytometry: Inter-laboratory comparison. Annals Botany 82 (Suppl. A): 17–26
20. Dolezel J, Bartos J (2005) Plant DNA Flow Cytometry and Estimation of Nuclear Genome Size. Annals of Botany, 95: 99–110
21. Doležel J, Greilhuber J, Suda J (2007) Estimation of nuclear DNA content in plants using flow cytometry. Nature Protocols v. 2, p. 2233–2244
22. Faria LA (1976) The chromosome field. I. Prediction of the location of ribosomal cistrons. Hereditas 83(1): 1–22
23. Feitoza LL, Martins MIG, Castro AAJF, Felix LP, Carvalho R (2010) Cytogenetics of *Alismataceae* and *Limncharitaceae*: CMA/DAPI banding and 45S rDNA sites. Plant Systematics and Evolution 286: 199–208
24. Fonsêca A, Ferreira J, Dos santos TRB, Mosiolek M, Bellucci E, Kami J, Gepts P, Geffroy V, Schweizer D, dos santos KGB, Pedrosa-Harand A (2010) Cytogenetic map of common bean (*Phaseolus vulgaris* L. L.). Chromosome Research, v. 18, p. 487–502
25. Galbraith DW, Harkins KR, Maddox JM, Ayres NM, Sharma DP, Firoozabady E (1983) Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science 220:1049–1051
26. Galbraith DW, Harkins KR, Knapp S (1991) Systemic endopolyploidy in *Arabidopsis thaliana*. Plant Physiology, Rockville, v. 96, n. 3, p. 985–989

27. Ganapathi A.; et al (1991) The diploid taxon in Indian natural populations of *Physalis peruviana* L. and its taxonomic significance. *Cytologia* v.56, n.2, p.283–288
28. Garcia S, Kovařík A, Leitch AR, Garnatje T (2017) Características citogenéticas de genes de rRNA em plantas terrestres: análise do banco de dados de rDNA de plantas. *The Plant Journal: para biologia celular e molecular*. 89(5): 1020–1030
29. Garcia Paitan M, Postillos-Flores M, Rojas Vasquez L, Siles Vallejos M, López Sotomayor A (2023) Nuclear DNA content and comparative FISH mapping of the 5s and 45s rDNA in wild and cultivated populations of *Physalis peruviana* L. *Caryologia*, 75(3), 123–134
30. Grabiele M, Debat HJ, Scaldaferrero MA, Aguilera PM, Moscone EA, Seijo JG, Ducasse DA (2018) Highly GC-rich heterochromatin in chili peppers (*Capsicum*-Solanaceae): A cytogenetic and molecular characterization. *Scientia Horticulturae*, 238: 391–399
31. Greilhuber J (1984) Chromosomal evidence in taxonomy. In: HEYWOOD, V. H.; MOORE, D M., eds. *Current Concepts in Plant Taxonomy*. Academic Press, London, p. 157–180
32. Greilhuber J (1998) Intraspecific variation in genome size: a critical reassessment. *Annals of Botany*, Oxford, v. 82, suppl. A, p. 27–35
33. Guerra M (2000) Patterns of heterochromatin distribution in plant chromosomes. *Genetics and Molecular Biology*, 23, 4: 1029–1041
34. Guerra M (2008) Chromosome numbers in plant cytotaxonomy: concepts and implications. *Cytogenetic and Genome Research* v. 120, p. 339–350
35. Herrera JC (2007) A citogenética molecular e sua aplicação no estudo dos genomas vegetais. *Agronomia Colombiana* 25(1): 26–35
36. Heslop-Harrison JSP, Schwarzacher T (2011) Organisation of the plant genome in chromosomes. *The Plant Journal*, Oxford, v. 66, n. 1, p. 18–33
37. Heslop-Harrison JSP, Schwarzacher T, Liu Q (2023) Polyploidy: its consequences and enabling role in plant diversification and evolution. *Annals of Botany*. Feb 7;131(1):1–10
38. Hirai H (2020) Chromosome dynamics regulating genomic dispersion and alteration of nucleolus organizer regions (NORs). *Cells* 9: 971
39. Hollister JD (2015) Polyploidy: adaptation to the genomic environment. *New Phytologist*, v. 205, n. 3, p. 1034–1039
40. Jo SH, Koo DH, Kim J. et al (2009) Evolution of ribosomal DNA-derived satellite repeat in tomato genome. *BMC Plant Biology*, 9: 42
41. Jovtchëv G, Schubert I (2006) Differential regulation of DNA replication and endoreduplication in plants. *Plant Molecular Biology*, Dordrecht, v. 60, p. 107–118
42. Kopecký D, Horáková L, Duchoslav M, Doležel J (2019) Selective elimination of parental chromatin from introgression cultivars of x *Festulolium* (*Festuca* x *Lolium*). *Sustainability*, 11: (11), 3153
43. Kovarik A, Matyasek R, Lim KY, Skalická K, Koukalová B, Knapp S, Chase M, Leitch, AR (2004) Concerted evolution of 18-5.8-26S rDNA repeats in *Nicotiana* allotetraploids. *Biological journal of the Linnean Society*. Linnean Society, 82(4): 615–625
44. Lagos TC, Vallejo FA, Caetano CM, Muñoz JE, Criollo EH (2005) Comportamiento meiótico de algunos genótipos de *Physalis peruviana* L. *Fitotecnia Colombiana*. 5:1–12
45. Liberato SA. et al (2014) Citogenética de genótipos de uchuva, *Physalis peruviana* L., y *Physalis floridana* Rydb., con respuesta diferencial a *Fusarium oxysporum* Corpoica. *Ciencia y Tecnología Agropecuaria* v.15, n.1, p.51–61
46. Liberato MAR, Carvalho CR, Clarindo WR (2014) Karyotype characterization and genome size variation in species of *Physalis* (Solanaceae). *Plant Systematics and Evolution*, Vienna, v. 300, n. 6, p. 1231–1242
47. Lopez FB, Fort A, Tadini L, Probst AV, Mchale M, Friel J, Ryder P, Pontvianne FDR, Pesaresi P, Sulpice R. et al (2021) Gene dosage compensation of rRNA transcript levels in *Arabidopsis thaliana* lines with reduced ribosomal gene copy number. *The plant cell*. 33(4): 1135–1150

48. Loureiro J, Rodriguez E, Doležel J, Santos C (2007) Two New Nuclear Isolation Buffers for Plant DNA Flow Cytometry: A Test with 37 Species. *Annals of Botany* 100(4), 875–888
49. Loureiro J, Čertner M, Lučanová M, Šliwińska E, Kolář F, Doležel J, Garcia S, Castro S, Galbraith DW (2023) The use of flow cytometry for estimating genome sizes and DNA ploidy levels in plants. *Methods in Molecular Biology* v. 2672, p. 25–64
50. Martínez-Sagarra G, Castro S, Mota L, Loureiro J, Devesa JA (2021) Genome size, chromosome number and morphological data reveal unexpected infraspecific variability in *Festuca* (Poaceae). *Genes* 12(6), 906
51. Matyasek R, Lim KY, Kovarik A, Leitch AR (2003) Ribosomal DNA evolution and gene conversion in *Nicotiana rustica*. *Heredity* 91(3): 268–275
52. Mcstay B (2016) Nucleolar organizer regions: genomic ‘dark matter’ requiring illumination. *Genes e Development* 30: 1598–1610
53. Mesquita AT (2016) Estudos cromossômicos em 16 espécies pertencentes à família Solanaceae Juss. 96f. Dissertação (Mestrado em Biologia Vegetal) – Instituto de Biologia, Universidade Estadual de Campinas. Campinas, SP
54. Mesquita AT, Cruz MVR, Forni Martins ER (2016) Distribution of Repetitive DNA Sequences in Seven Species of the Plant Family Solanaceae Juss. *Cytogenetic and Genome Research* 148 (2–3): 83–155
55. Mesquita A, Teixeira et al (2019) Chromosome number and genome size diversity in five Solanaceae genera. *Caryologia*, v. 72, n. 3, p. 105–115
56. Moscone EA, Loidl J, Ehrlich SD, Schweizer D (1995) Analysis of active nucleolus organizing regions in *Capsicum*. *Chromosome Research*, Dordrecht, v. 3, p. 123–130
57. Moyetta NR, Urdampilleta JD, Chiarini FE, Bernardello GL (2017) Heterochromatin and rDNA patterns in *Solanum* species of the Morelloid and Dulcamaroid clades (Solanaceae). *Plant biosystems*. 151(3): 539–547
58. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant*, 15:473–497
59. Nohra C, Rodriguez C (2006) Study of the cytogenetic diversity of *Physalis peruviana* L. (Solanaceae). *Acta Biológica Colombiana*, 11(2), 75–85
60. Pedrosa A, Gitai J, Silva AEB, Felix LP, Guerra M (1999) Citogenética de angiospermas coletadas em Pernambuco V. *Acta Botanica Brasilica*, Feira de Santana, v. 13, n. 1, p. 49–60
61. Prado EA, Faivre-Rampant P, Schneider C, Darmency M (1996) Detection of a variable number of ribosomal DNA loci by fluorescent in situ hybridization in *Populus* species. *Genome*. 39(5): 1020–1026
62. Preet R, Gupta RC (2015) Cytomorphological diversity of *Physalis angulata* L. (Ciplukan herb) from different geographical regions of Rajasthan. *Chromosome Botany*, v. 10, n. 4, p. 125–127
63. Preet R, Gupta RC (2018) Quantification of withaferin-A and withanolide-A in diploid (n = 12) and tetraploid (n = 24) cytotypes of “rassbhary”, *Physalis angulata* L. *Natural Product Research*, 33: 3157–3160
64. Quiros C (1984) Overview of the genetics and breeding of husk tomato. *Hortscience*, v. 19, n. 6, p. 872–874, Dec.
65. Rego LNAA, Da Silva CRM, Torezan JMD, Gaeta ML, Laforga Vanzela AL (2009) Cytotaxonomical study in Brazilian species of *Solanum*, *Lycianthes* and *Vassobia* (Solanaceae). *Plant Systematics and Evolution*, v. 279, p. 93–102
66. Robinson JP, Ostafe R, Iyengar SN, Rajwa B, Fischer R (2023) Flow Cytometry: The Next Revolution. *Cells* 12(14):1875
67. Robledo-Torres V. et al. (2011) Development of tomatillo (*Physalis ixocarpa* Brot.) autotetraploids and their chromosome and phenotypic characterization. *Breeding Science* v.61(3). p.288–293
68. Rodríguez NC, Bueno ML (2006) Estudio de la diversidad citogenética de *Physalis peruviana* L. (Solanaceae). *Acta Biológica Colombiana*, Facultad de Ciencias Universidad Nacional, Bogotá, v.11, n.2, p.33–43
69. Romero-da-Cruz MV. et al (2015) Cytogenetic markers for the characterization of *Capsicum annum* L. cultivars, *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology*, v. 151, n. 1, p. 84–91
70. Rosato M, Kovařík A, Garilleti R, Rosselló JA (2016) Conserved Organisation of 45S rDNA Sites and rDNA Gene Copy Number among Major Clades of Early Land Plants. *PLoS ONE*, 11(9): e0162544

71. Saavedra JCM, Rodríguez Zaragoza FA, Cabrera Toledo D, Sánchez Hernández CV, Vargas-Ponce O (2019) Agromorphological characterization of wild and weedy populations of *Physalis angulata* in Mexico. *Scientia Horticulturae*, v. 246, p. 86–94
72. Scaldaferrro MA. et al (2013) Heterochromatin type, amount and distribution in wild species of chili peppers (*Capsicum*, Solanaceae). *Genetic Resources and Crop Evolution*, v 60, p. 693–709
73. Schweizer D (1976) Reverse fluorescent chromosome banding with chromomycin and DAPI. *Chromosoma*, Berlin, v. 58, p. 307–324
74. Schweizer D (1981) Counterstain-enhanced chromosome banding. *The American Journal of Human Genetics* v. 57, p. 1–4
75. Soltis PS, Soltis DE, Van de Peer Y (2015) Polyploidy and genome evolution in plants. *Current Opinion in Genetics & Development*, London, v. 35, p. 119–125
76. Stefan Seregard MD (1998) Conjunctival melanoma. *Survey of ophthalmology*, v. 42, p. 321–350
77. Shibata F, Matsusaki Y, Hizume M (2005) AT-rich sequences containing Arabidopsis -type telomere sequence and their chromosomal distribution in *Pinus densiflora*. *Theoretical and Applied Genetics*, 110:1253–1258
78. Souza LGR. et al (2012) Cytogenetic and molecular evidence suggest multiple origins and geographical parthenogenesis in: *Nothoscordum gracile* (Alliaceae). *Annals of Botany*, v. 109, n. 5, p. 987–999
79. Steinarssdóttir M, Jonasson JG, Ogren M, Baagøe H (1997) A comparison of cytogenetic studies and flow cytometry in breast carcinomas. *Cancer Genetics and Cytogenetics*, New York, v. 95, n. 2, p. 91–98
80. Su D, Chen L, Sun J, Zhang L, Gao R, Li Q.; Han Y, Li Z (2020) Comparative chromosomal localization of 45S and 5S rDNA sites in 76 purple-fleshed sweet potato cultivars. *Plants*, 9(7): 865
81. Sumner AT (2003) *Chromosomes: Organization and function*. Blackwell Publishing
82. Trevisani NM, Melo RC, Oliveira PM, Colli MP, Meirelles JL, Frederico A (2018) Ploidia e conteúdo de DNA de populações de groselha-do-cabo cultivadas no Sul do Brasil. *Caryologia*. 71(4): 414–419
83. Vaio M. et al (2018) Multiple karyotype changes distinguish two closely related species of *Oxalis* (*O. psoraleoides* and *O. rhomboides-ovata*) and suggest an artificial grouping of section *Polymorphae* (Oxalidaceae). *Botanical Journal of the Linnean Society* v. 188, n. 3, p. 269–280
84. Van de Peer Y, Ashman TL, Soltis PS, Soltis DE (2021) Polyploidy: an evolutionary and ecological force in stressful times. *The Plant Cell*, Oxford, v. 33, n. 1, p. 11–26
85. Wang Y, Prigent C, Bardin A (2021) Polyploid mitosis, endoreplication and depolyploidization promote genomic instability and polyan euploidy. *Cells*, Basel, v. 10, n. 7, p. 1–23

Figures

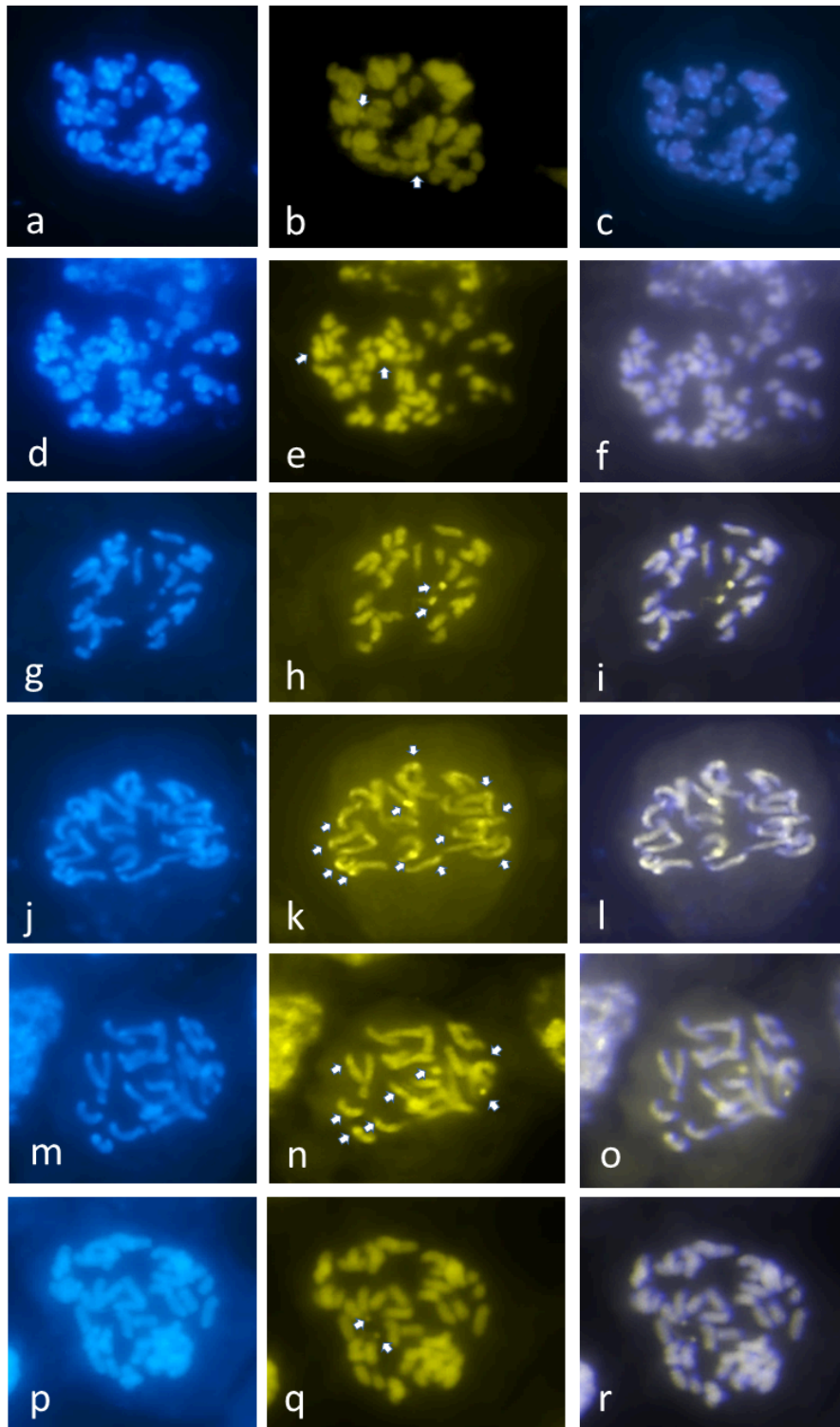


Figure 1

CMA/DAPI staining in *Physalis* accessions showing subterminal DAPI⁺ bands (blue) in all chromosomes and CMA⁺ blocks (yellow) with different distribution patterns. a, b, c – accession ES; d, e, f – accession PE; g, h, i – accession IX1. In h, note two terminal CMA⁺ blocks detached from the chromosomes with their corresponding heterochromatin threads; j, k, l – accession IX2. In k, note several subterminal and proximal CMA⁺ blocks in most chromosomes; m, n, o – accession IX3. In n, note approximately eight subterminal and proximal CMA⁺ blocks in approximately eight chromosomes; p, q, r – accession Feltrin. In q, note two terminal CMA⁺ blocks detached from the chromosomes with their corresponding heterochromatin threads.

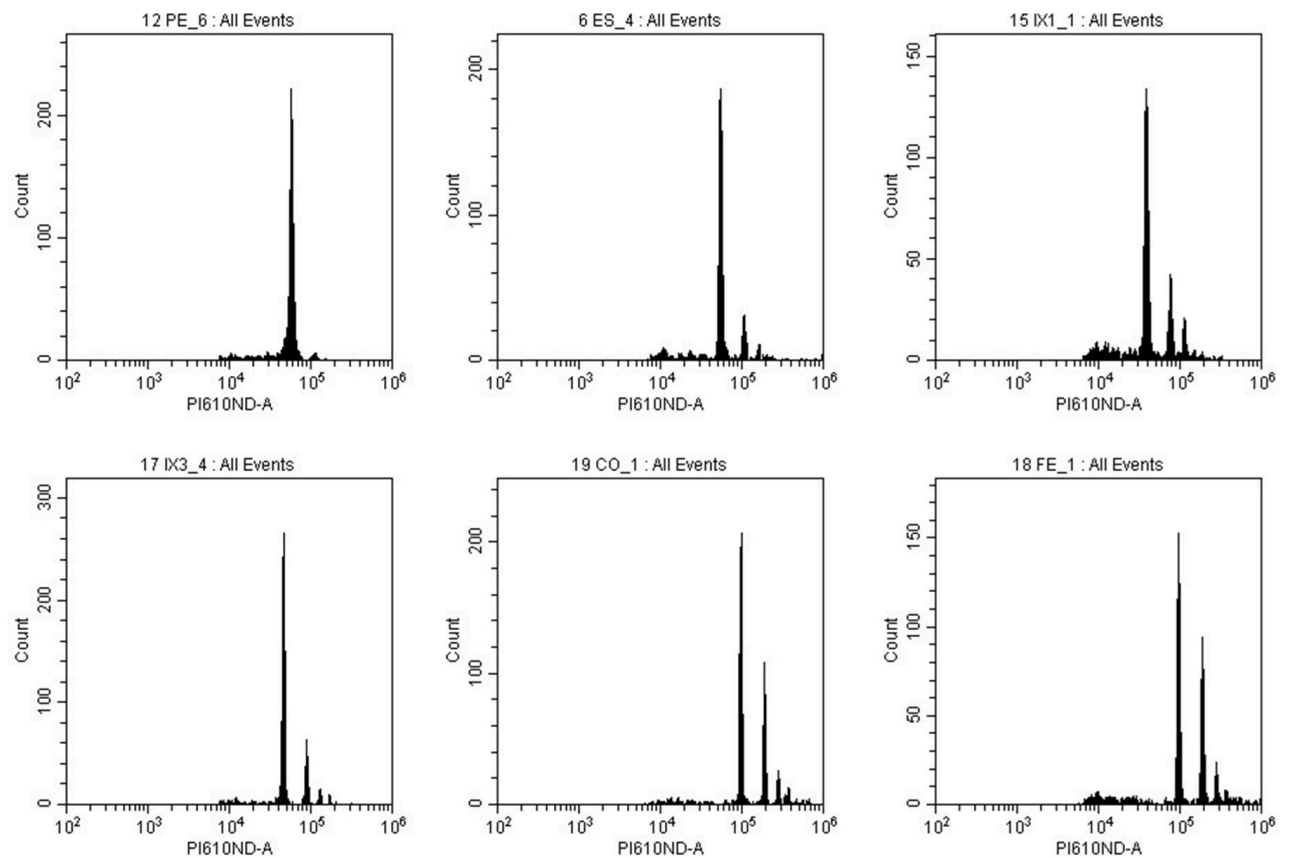


Figure 2

Flow cytometry histograms of *Physalis* accessions showing variation in nuclear DNA content and ploidy levels. Accessions PE_6 and ES_4 exhibited well-defined G0/G1 peaks and a diploid pattern, similar to accession IX1_1. The mixoploid accession IX3_4 displayed multiple nuclear populations, whereas accessions Colômbia-CO and Feltrin-FE showed a G0/G1 peak shifted to approximately twice the DNA content observed in diploid accessions.

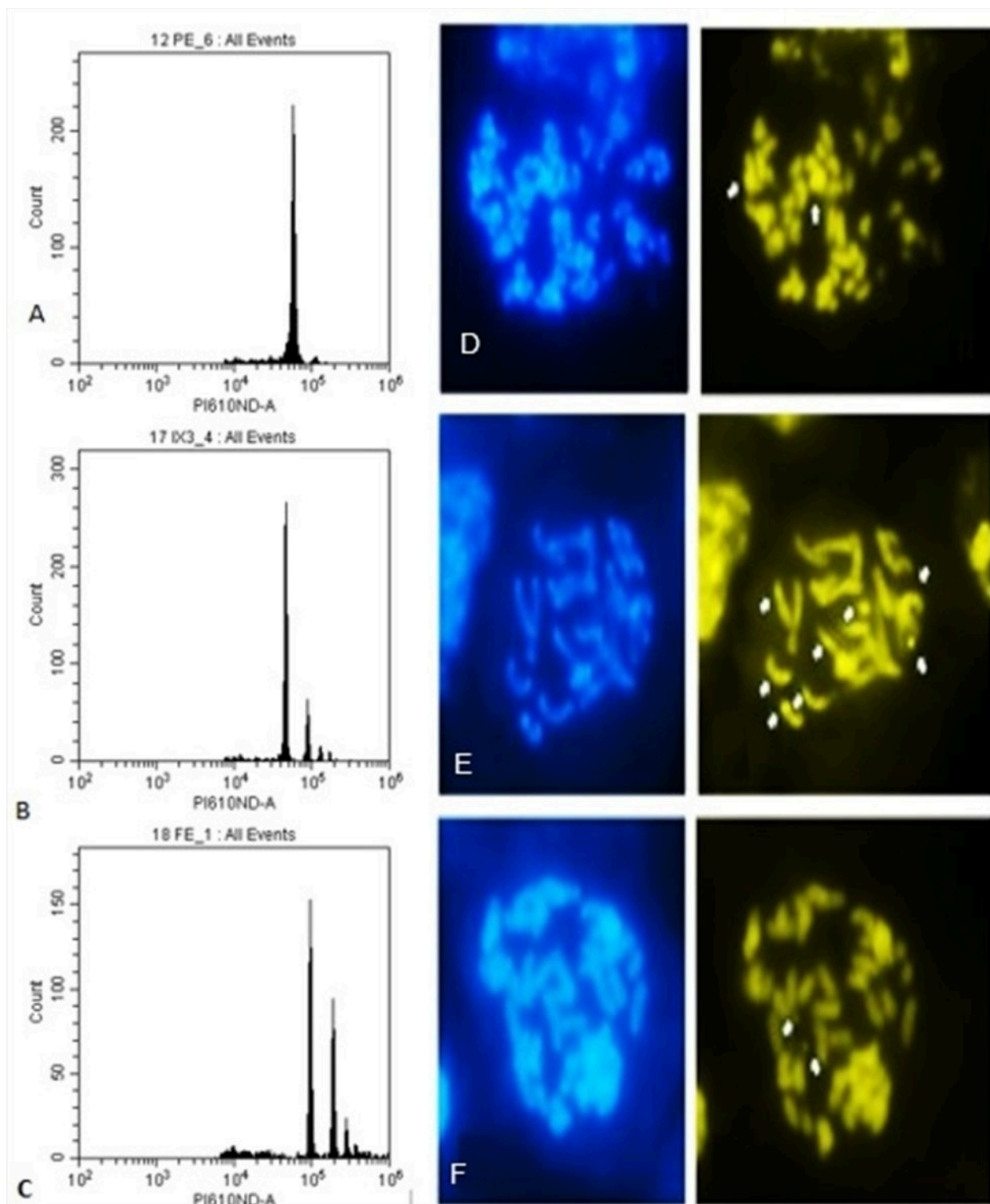


Figure 3

Integration of flow cytometric and cytogenetic analyses in *Physalis* accessions. Flow cytometry histograms (A–C) showing variation in nuclear DNA content associated with their respective DAPI/CMA-stained metaphases (D–F). The PE accession (*P. angulata*) exhibited a stable cytometric profile and apparently conserved heterochromatin distribution; the IX3_4 accession (purple-fruited *P. ixocarpa*) displayed multiple nuclear populations and variation in CMA⁺ band distribution; whereas the Feltrin accession (*P. peruviana*) showed increased nuclear DNA content relative to the remaining accessions.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial.docx](#)