

## Cytogenetics of selected *Solanum* L. species within a phylogenetic framework

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**Abstract:** We characterized 12 *Solanum* species using CMA/DAPI banding and/or genome size estimation (1C value) within a phylogenetic perspective. Here, we reported new Constitutive Heterochromatic (CH) data for *S. andreanum*, *S. peruvianum* and *S. paludosum* and new 1C value for *S. corneliomulleri* and *S. andreanum*. CH ranged from two (*S. viarum*) to 42 bands (*S. paludosum*). Potato species exhibited a higher number of CH blocks, whereas *Leptostemonum* species generally had fewer blocks, except for *S. paludosum*. However, no relationship between heterochromatin and evolutive diversification within the genus could be inferred. Mean 1C value varied from 1.0 pg in *S. melongena* (diploid) to 1.80 pg in *S. laciniatum* (octaploid), with moderate correlation ( $r = 0.69$ ) with the ploidy level of the species. Our data contribute to understanding genetic and cytogenetic diversification within *Solanum* in a phylogenetic context and are relevant to species characterization, supporting future genetic breeding programs of the genus.

**Keywords:** CMA/DAPI banding, flow cytometry, cytogenetic diversity, tomato, karyotype evolution

### INTRODUCTION

*Solanum* L. (Solanaceae Juss.), one of the largest and most diverse genera of flowering plant, comprises over 1,500 species distributed worldwide, with its center of origin and greatest genetic diversity located in South America (<https://solanaceaesource.myspecies.info/>). The genus includes 24 economically important crops, such as potato (*S. tuberosum* L.) and tomato (*S. lycopersicum* L.), which are among the most worldwide cultivated vegetables (Kawicha et al. 2023, Hilgenhof et al. 2023). Furthermore, the remarkable diversity in morphophysiological and reproductive traits, as well as ecological adaptations, makes *Solanum* an important genetic resource and an attractive model for scientific research.

These species, rich in vitamins, minerals, proteins and carbohydrates, are well known for their nutritional, medicinal and ecological importance, which

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makes them valuable resources for global food security and social stability (Balkrishna et al. 2022). Additionally, these species represent potential sources of alleles conferring tolerance to abiotic and biotic stresses. For instance, *S. pimpinellifolium* L. carries favorable alleles associated with adaptation to water and salt stress (Martínez-Cuenca et al. 2020). In *S. tuberosum* breeding programs, wild relatives have been employed, such as *S. demissum* Lindl. to improve pest resistance (Díaz-García et al. 2024), *S. acaule* Bitter to enhance tolerance to cold stress (Achakkagari et al. 2025), and *S. bulbocastanum* Dunal to improve resistance to drought stress and late blight disease (Dénes et al. 2024).

From a phylogenetic and evolutive perspective, *Solanum* is classified into three strongly supported main groups, which are denominated Thelopodium, Grade I and Clade II, with most species belonging to the Clade II (Hilgenhof et al. 2023). These groups are further divided into 12 major and 49 minor clades. The most economically important species, *S. lycopersicum* and *S. tuberosum*, are placed within Grade I, in the major Potato clade, but in two different minor clades: Tomato and Petota, respectively. Despite their high agricultural and socioeconomic relevance, several species from different groups and clades remain poorly investigated across various fields, including cytogenetics.

*Solanum* belongs to the  $x = 12$  clade, a group characterized by a putative cytological synapomorphy of having chromosome numbers based on 12 pairs. This clade was first recognized by Olmstead and Palmer (1992), and the informal name “ $x = 12$  clade” was later proposed by Olmstead and Sweere (1994). Tetraploid ( $2n = 4x = 48$ ), hexaploid ( $2n = 6x = 72$ ) and octaploid ( $2n = 8x = 96$ ) species have also been reported (Chiarini et al. 2018). Cytogenetic analyses provide karyotypic characterization that contributes to understanding the diversity, structure and organization of plant genomes, with significant implications for taxonomy, phylogeny, evolution and germplasm conservation programs (Martins et al. 2018, Almeida et al. 2022, Mesquita et al. 2025). Cytogenetic techniques, such as fluorochrome banding with Chromomycin A3 (CMA) and 4'-6-diamidino-2-phenylindole (DAPI), are approaches for karyotyping, enabling chromosome number identification, morphology characterization, asymmetry assessment, and the evaluation of constitutive heterochromatin (CH) at inter- and intraspecific levels (Deanna et al. 2022, Mesquita et al. 2025).

In addition to cytogenetic analyses, the nuclear genome size (1C value), which is estimated by flow cytometry, represents another genomic parameter relevant to understanding plant diversity and evolution. It is also used to assess inter- and intraspecific variation among populations and species (Macková et al. 2017, Braz et al. 2024). The 1C value enables the inference of ploidy levels and facilitates the detection of natural or induced polyploids (Walter et al. 2025) and hybrids (Macková et al. 2017). Furthermore, genome size data provides essential qualitative and quantitative insights into the genetic diversity, organization, and cytogenetic stability of species (Urfus et al. 2025, Zhang et al. 2025). Consequently, this information serves as a basis for developing more efficient strategies for plant breeding as well as for the preservation, management, and restoration of native flora (Urfus et al. 2025, Zhang et al. 2025).

In *Solanum*, data on the diversity of CMA banding patterns and 1C value are currently available for less than 10% of the species, mostly belonging to the Potato and Leptostemonum clades (Brasileiro-Vidal et al. 2009, Melo et al. 2011, Acosta et al. 2012, Moyetta et al. 2016, Mesquita et al. 2025, <https://cvalues.science.kew.org/>). Consequently, the full extent of the karyotype variability of *Solanum* remains largely unknown. To expand this knowledge, we performed a detailed karyotype characterization of 12 wild and domesticated species with different ploidy levels, representing different phylogenetic groups, along with 1C values for three of them, complementing our findings with literature data. Here, we discuss the heterochromatin banding pattern and 1C value among *Solanum* species in a phylogenetic framework. Our results contribute to species characterization and delimitation, providing valuable insights for breeding programs aimed at unlocking unexplored genetic diversity in *Solanum*.

## MATERIAL AND METHODS

### Plant materials

Twelve *Solanum* species belonging to different phylogenetic groups (Grade I and Clade II), major clades (Potato, VANAns, Leptostemonum and Geminata) and minor clades (Tomato, Petota, Acanthophora, Erythrotrichum, Eastern Hemisphere Spiny and Geminata) were analyzed. Seeds were obtained from the Germplasm Resource Information Network (GRIN Global), from the National Germplasm Resources Laboratory (United States) and from Empresa Brasileira de Pesquisa Agropecuária (Embrapa) Hortaliças (Brasília, Distrito Federal, Brazil). Seeds of *S. andreaum* and *S. macrocarpon* were multiplied at the Instituto Agronômico de Pernambuco (IPA, Pernambuco, Recife, Brazil), and *S. lycopersicum* seeds were

obtained from commercial sources. A list of the species studied, including their phylogenetic groups, voucher numbers and provenance, is provided in Table 1.

### Chromosome preparation

*Solanum* seeds were placed in Petri dishes lined with filter paper and moistened with distilled water. After over five days, root tips (~1cm in length) from germinated seeds were collected and pretreated with 0.002 M 8-hydroxyquinoline for 4.5 h at 18 °C, fixed in Carnoy's solution (methanol: acetic acid, 3:1 v/v), and stored at -20 °C until use. Root tips were macerated in an enzymatic mixture containing 2% Cellulase 'Ozonuka R-10' (Serva) and 20% Pectinase (v/v) (Sigma Aldrich) for 4.5 h at 37 °C. Slides were prepared following de Carvalho and Saraiva (1993) protocol, with minor modifications: slides were immersed in 45% acetic acid for 15 s and then air-dried at room temperature. The best slides were selected in 2 µg mL<sup>-1</sup> DAPI: glycerol (1:3, v/v), de-stained in ethanol: acetic acid (3:1, v/v) for 30 min followed by ethanol immersion for 30 min, air dried at room temperature and aged for three days.

### CMA/DAPI fluorochrome staining and microscopy

Fluorochrome staining was performed following Schweizer and Ambros (1994), with minor modifications. Slides were stained with 10 µL of 0.5 mg mL<sup>-1</sup> CMA for 1.5 h, counterstained with 10 µL of 2 mg mL<sup>-1</sup> DAPI for 1 h, mounted in glycerol/McIlvaine buffer (pH 7.0, 1:1, v/v), and stored at 4 °C for three days. The best metaphases were photographed using a DF7000GT digital camera coupled to a Leica DM4B microscope (Universidade Federal do Piauí). Images were optimized for brightness and contrast using Adobe Photoshop CS3 and Adobe Photoshop 2022.

### Chromosome measurements and idiogram construction

Three to five best metaphases from each species were used for chromosome measurements, performed using Drawid v0.26 (Kirov et al. 2017). The following morphometric parameters were determined: range of chromosome size (RCS),

**Table 1.** Chromosome number, morphometric parameters, CMA<sup>+</sup> bands, and mean 1C value (µg) in twelve *Solanum* species, distributed across two main groups, four major clades and seven minor clades, following the phylogeny of Hilgenhof et al. (2023)

Main group	Major clade	Minor clade	Scientific name	Germplasm bank/ voucher number	2n	RCS µm (n)	KF (n)	CML µm (n)	HKL (µm)	CMA <sup>+</sup> band pairs	1C (µg)	
GRADE I	VANAns	Archaeosolanum	<i>S. laciniatum</i> Ruiz & Pav.	Grin Global/ PI 337284	8x = 92	1.14 - 2.44	23M	1.67	38.46	2	1.80 <sup>#</sup>	
		Potato	Tomato	<i>S. pennellii</i> Correll	EMBRAPA/ CNPH 1759	2x = 24	2.44 - 4.73	12M	3.38	40.63	7	1.30 <sup>#</sup>
				<i>S. peruvianum</i> L.	EMBRAPA/ CNPH 1461	2x = 24	1.66 - 3.71	12M	2.61	31.35	19*	1.21*
				<i>S. lycopersicum</i> L.	Commercial	2x = 24	1.67 - 3.42	11M + 1SM	2.27	27.34	13	1.00
				<i>S. pimpinellifolium</i> L.	Grin Global/ PI 407545	2x = 24	1.41 - 2.81	11M + 1ST	2.09	25.10	4	1.15 <sup>§</sup>
				<i>S. corneliomulleri</i> J. F. Macbr.	EMBAPA/ CNPH 938	2x = 24	-	-	-	-	-	1.14*
		Petota		<i>S. andreanum</i> Baker	Grin Global/ PI 567813	4x = 48	1.77 - 4.04	12M	2.71	32.56	12*	1.29*
CLADE II	Leptostemonum	Acanthophora	<i>S. viarum</i> Dunal	EMBRAPA/ CNPH 185	2x = 24	1.97 - 3.73	11M + 1SM	2.80	33.71	1	1.32 <sup>#</sup>	
		Erythrotrichum	<i>S. paludosum</i> Moric.	EMBRAPA/ CNPH 205	2x = 24	3.82 - 6.81	11SM + 1ST	4.29	49.58	21*	-	
		Eastern Hemisphere Spiny	<i>S. melongena</i> L.	Grin Global/ PI 665010	2x = 24	1.99 - 3.89	12M	2.77	33.27	3	1.0 <sup>#</sup>	
			<i>S. macrocarpon</i> Molina	Grin Global/ PI 441914	2x = 24	1.56 - 2.78	12M	2.08	25.04	2	1.55 <sup>§</sup>	
	Geminata	Geminata	<i>S. pseudocapsicum</i> L.	Grin Global/ Grif 16422	2x = 24	1.79 - 2.81	11SM + 1ST	2.29	27.58	18	1.47 <sup>#</sup>	

Grin Global (Germplasm Resource Information Network); EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária); M = metacentric; SM = submetacentric; ST = subtelocentric, according to Levan et al. (1964). <sup>#</sup>C-values from the Plant C-DNA values database, available at <https://cvalues.science.kew.org/>. <sup>§</sup>According to Arumuganathan and Earle (1991); <sup>§</sup>According to Barow and Meister (2002). \* First report on CMA bands and C-DNA values in *Solanum*.

karyotypic formula (KF), chromosome medium length (CML), and haploid karyotype length (HKL), according to Guerra (1988). Centromere position was classified according to Levan et al. 1964 (Table 1). Idiograms were constructed using Adobe Photoshop CS3 and were plotted onto the phylogenetic tree proposed by Hilgenhof et al. (2023).

### Nuclear 1C value measurement by flow cytometry

The nuclear 1C values of *S. andreaeanum*, *S. peruvianum* and *S. corneliomulleri* were determined by flow cytometry using leaf nuclei suspensions prepared according to Praça-Fontes et al. (2011) (Figure S1). *Solanum lycopersicum* 'Stupické' (2C = 2.00 pg) was used as an internal reference. This species has a well-established and stable genome size, with highly reproducible 1C values reported across independent studies, which facilitates inter-study comparability. Moreover, *S. lycopersicum* presents low interference from secondary metabolites under the extraction protocol used, resulting in well-defined G<sub>0</sub>/G<sub>1</sub> peaks and low coefficients of variation. Each species was represented by at least four plants, with three replicates per plant, and suspensions containing more than 10,000 nuclei.

The relationship between ploidy level ( $x$ ) and mean nuclear DNA content (1C value) in nine *Solanum* species (Table 1) was evaluated using simple linear regression, with the 1C value as the dependent variable and ploidy level as the independent variable. Genome size data (1C values) obtained by flow cytometry for *S. laciniatum*, *S. pennellii*, *S. pimpinellifolium*, *S. viarum*, *S. paludosum*, *S. melongena*, *S. macrocarpon* and *S. pseudocapsicum* were retrieved from the Plant C-Values Database (<https://cvalues.science.kew.org/>). The strength and direction of the association were quantified using Pearson's correlation coefficient ( $r$ ), and the proportion of explained variance was estimated by the coefficient of determination ( $R^2$ ). The statistical significance of the relationship was assessed using the associated  $p$ -value, adopting a significance level of 5%. Model assumptions were evaluated prior to interpretation. Homoscedasticity was assessed by visual inspection of residuals versus fitted values and formally tested using the Breusch-Pagan test, which indicated no significant deviation from constant variance ( $p \leq 0.05$ ). Normality of residuals was evaluated using Q-Q plots, showing no strong departures from normality. A scatterplot was generated to visualize the relationship between ploidy level and genome size, including the fitted regression line and 95% confidence intervals. All statistical analyses were performed in R version 4.2 (R Core Team 2022).

## RESULTS AND DISCUSSION

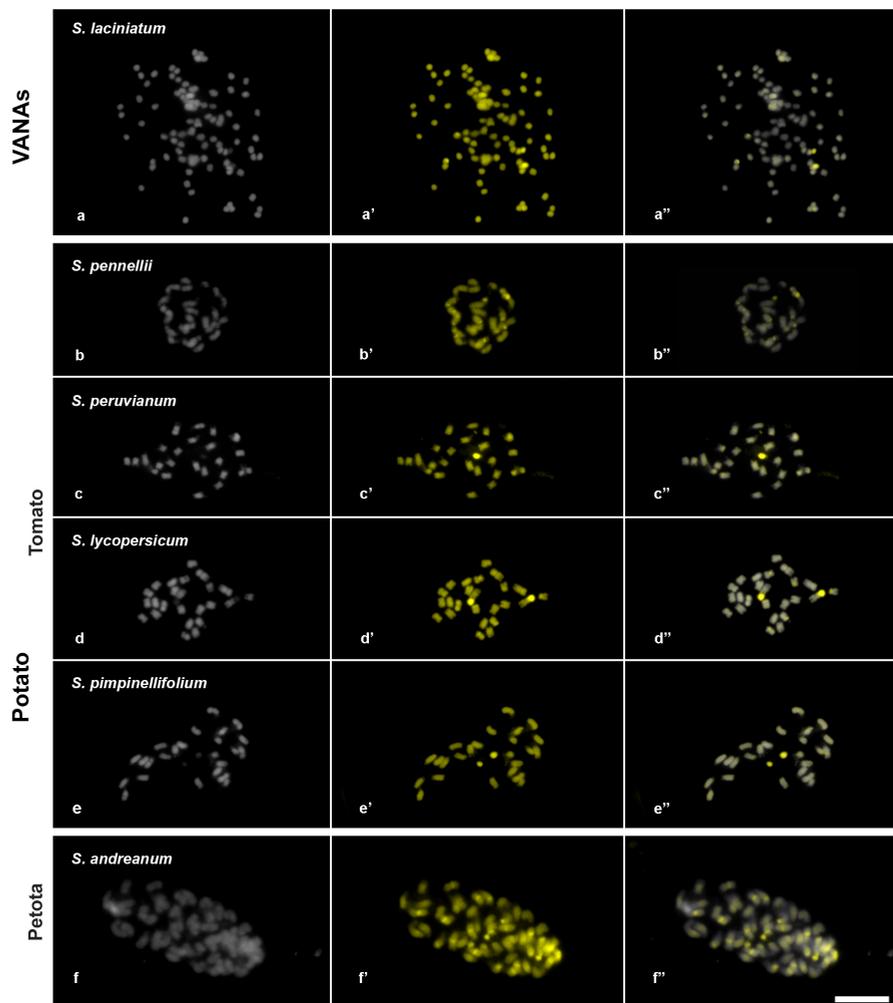
The *Solanum* species examined exhibited chromosome numbers of  $2n = 24, 48$ , and the dysploid 92 (Table 1, Figure 1a). Most species predominantly have metacentric and submetacentric chromosomes, with the most common karyotype formula being 12M (metacentric). *Solanum pseudocapsicum*, *S. paludosum* and *S. pimpinellifolium* possessed at least one pair of subtelocentric chromosomes (11SM + 1ST). *Solanum* species generally exhibited small to medium-sized chromosomes (Chiarini et al. 2018), ranging from 1.14  $\mu\text{m}$  in *S. laciniatum* (octaploid) to 6.81  $\mu\text{m}$  in *S. paludosum* (diploid), with haploid karyotype lengths varying from 25.04  $\mu\text{m}$  in *S. macrocarpon* to 49.58  $\mu\text{m}$  in *S. paludosum*. Detailed chromosome morphometric data for the analyzed species are presented in Table 1.

Due to recent advances in genomics over the last decades, karyotyping and genome size have become increasingly fundamental steps in biological research, particularly within the field of cytogenomics. However, few studies have addressed the distribution patterns of CH within *Solanum* (Brasileiro-Vidal et al. 2009, Rego et al. 2009, Melo et al. 2011, Acosta et al. 2012, Moyetta et al. 2016, Mesquita et al. 2025). Here, we describe the CH distribution patterns of 12 *Solanum* species, providing new data for *S. andreaeanum*, *S. peruvianum* and *S. paludosum* (Table 1, Figures 1 and 2). Each species exhibited a unique heterochromatic banding profile, enabling specific cytogenetic characterization of these wild and cultivated genetic resources. To facilitate the comparative interpretation of heterochromatin distribution across *Solanum*, idiograms were constructed for each species and plotted onto a recent phylogenetic tree of the genus (Figure 3).

In our fluorochrome-based banding analysis, we observed notable variation in the number, location, size and intensity of heterochromatic bands among analyzed species (Table 1, Figures 1 and 2). Two patterns of CH distribution were identified: i) strongly labeled CMA<sup>++</sup>/DAPI<sup>-</sup> terminal bands, related to the nucleolar organizer region (NOR); and ii) small terminal and proximal CMA<sup>+</sup>/DAPI<sup>-</sup> or CMA<sup>+</sup>/DAPI<sup>0</sup> bands not associated with the NOR. All species exhibited at least one pair of CMA<sup>++</sup>/DAPI<sup>-</sup> bands (NOR) located in the terminal region of a chromosome pair, as previously

reported by Chiarini et al. (2018). In *S. viarum* (Clade II, Leptostemonum, Acanthophora), only one CMA<sup>+</sup>/DAPI<sup>-</sup> pair was detected, which was heteromorphic in size, with one chromosome having distended NOR and their corresponding block not distended (Figure 2a-a''). Additional CMA<sup>+</sup> bands were mainly located in terminal and proximal regions of *Solanum* chromosomes. For example, the wild potato *S. andreaeanum* (Grade I, Potato, Petota) presented 12 large CH pairs preferentially distributed in proximal chromosomal regions, including six bands located in terminal/subterminal regions. One chromosome pair exhibited two CH bands, being one terminal (NOR-related) and one proximal band on the same chromosome (Figure 1f-f'', Figure 3).

*Solanum laciniatum* (Grade I, Archaesolanum), despite having the highest chromosome number among the analyzed species, exhibited one of the lowest chromosome numbers of CMA<sup>+</sup> bands (two band pairs, Figure 1 a-a''), corroborating the findings of Melo et al. (2011). In contrast, *S. peruvianum* (Grade I, Potato, Tomato) displayed the highest number of bands (19 pairs), distributed across all chromosomes, mainly in the terminal regions (Figure 1c-c'').

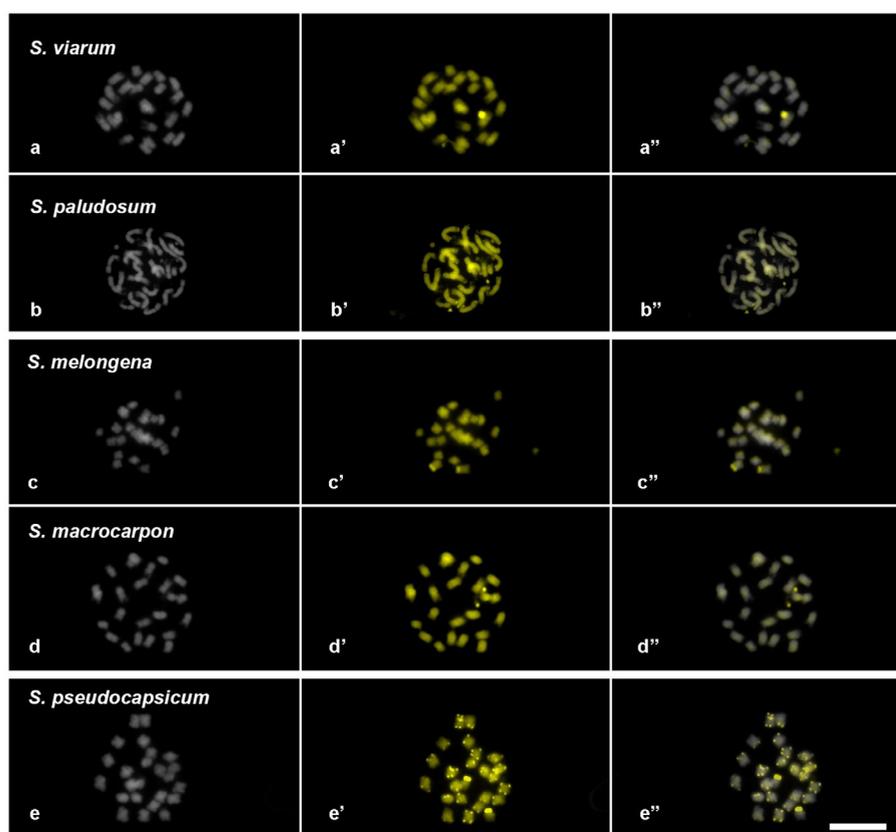


**Figure 1.** CMA/DAPI banding in metaphase chromosomes of different *Solanum* species belonging to Grade I. The chromosomes are pseudocolored in grey (DAPI, a-f), while blocks of constitutive heterochromatin are stained yellow (CMA, a'-f'). The merged images are presented in the third column (a''-f''). (a-a'') *S. laciniatum*, (b-b'') *S. pennellii*, (c-c'') *S. peruvianum*, (d-d'') *S. lycopersicum*, (e-e'') *S. pimpinellifolium*, (f-f'') *S. andreaeanum*. Bar = 10  $\mu$ m.

In general, we observed a similar number of CMA<sup>+</sup> bands among species within the same clade. For example, in the Potato (minor clade Tomato) the mean number of CH blocks was 13-14 CMA<sup>+</sup> pairs (Figures 1 and 3). Similar data were previously reported for eight of the 17 Tomato clade species, which also exhibited a high number of CMA<sup>+</sup> bands, with a mean of 19 CH pairs among the analyzed species (Brasileiro-Vidal et al. 2009). Conversely, species belonging to the *Leptostemonum* clade displayed fewer CH blocks, averaging between one and four CMA<sup>+</sup> block pairs (present work, Rego et al. 2009, Melo et al. 2011, Mesquita et al. 2025). An exception was observed in the minor clade *Erythrotrichum*, where *S. paludosum* displayed a discrepant CH pattern, with 21 terminal CMA<sup>+</sup> band pairs (Figure 2 b-b''). Cytogenetically, this species shares a more similar pattern with *S. pseudocapsicum* (Geminata, 18 pairs, Figure 2 e-e'') than its closely related species (Melo et al. 2011).

In *Solanum*, heterochromatin (a repeatome fraction) is highly dynamic, independent, and not homogeneous across the genus (Chiarini et al. 2018). Despite this heterochromatin heterogeneity, no clear evolutive trends associated with clade diversification could be inferred, probably due to the limited number of samples analyzed in this work. Broader cytogenetic sampling, including other species from additional major and minor clades, will be essential to confirm or refine these patterns.

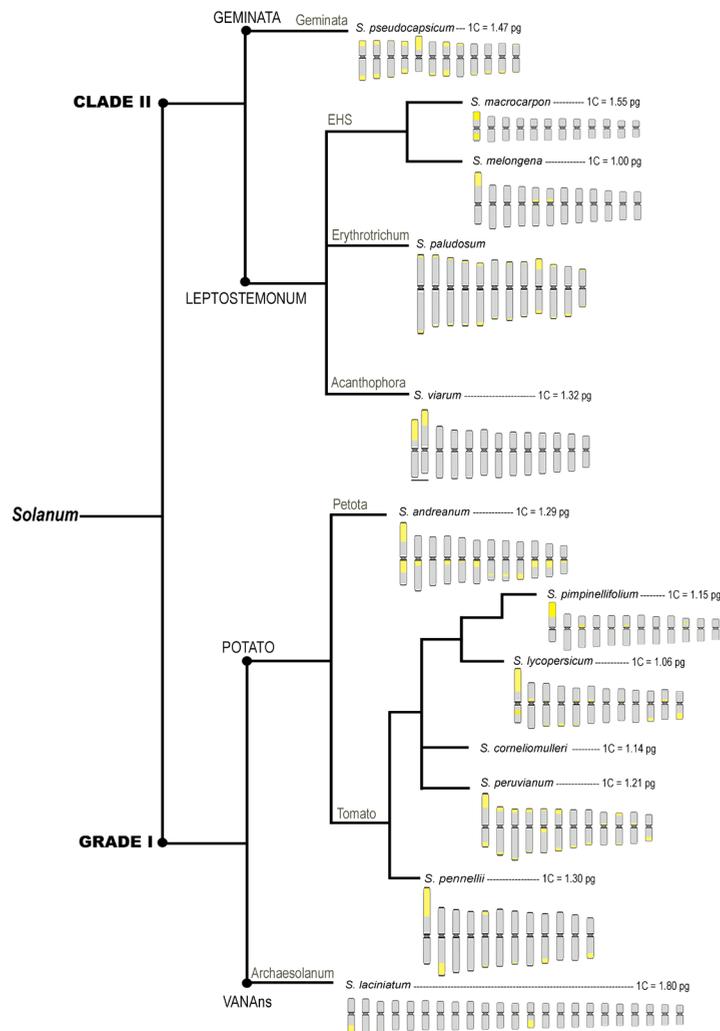
The amplification or deletion of different classes of repetitive DNA that constitute heterochromatin likely represents one of the main mechanisms underlying speciation and genomic differentiation within *Solanum*. For example, Gaiero et al. (2019) reported that the most abundant repeats in the genomes of 12 wild and cultivated species belonging to the Tomato and Petota minor clades are long terminal repeat (LRT) retrotransposons, particularly the Ty3/ Gypsy elements,



**Figure 2.** CMA/DAPI banding in metaphase chromosomes of *Solanum* species belonging to Clade II. The chromosomes are pseudo-colored in grey (DAPI, a-e), while blocks of constitutive heterochromatin are stained in yellow (CMA, a'-e'). The merged images are presented in the third column (a''-e''). (a-a'') *Solanum viarum*, (b-b'') *S. paludosum*, (c-c'') *S. melongena*, (d-d'') *S. macrocarpon*, and (e-e'') *S. pseudocapsicum*. Bar = 10  $\mu$ m.

which are preferentially located in the heterochromatin, being more abundant in tomatoes. Conversely, the satellite DNA *CL14* was found to be exclusive to the Tomato species and absent in wild potatoes. Further investigation into the specific repetitive DNA families that make up heterochromatin in *Solanum* species, such as transposable elements and satellite DNAs, will be essential for elucidating the structural genomic divergence and evolutive dynamics of the genus. Such analyses will clarify how these repetitive sequences contribute to the organization and evolution of *Solanum*.

A moderate and statistically significant positive linear relationship was detected between ploidy level ( $x$ ) and mean nuclear DNA content (1C value), as evidenced by both Pearson's correlation ( $r = 0.69$ ,  $p = 0.040$ ) and simple linear regression ( $R^2 = 0.48$ ; Figure 4). These results indicate that increases in ploidy level tend to be associated with increases in genome size among *Solanum* species, although this pattern should be interpreted with caution given the limited number of species analyzed. This pattern is consistent with reports for other plant taxa, such as *Psidium guajava* L. ( $1C = 0.47 \pm 0.01$  pg;  $2n = 2x = 22$ ) and *P. cattleianum* Sabine ( $1C = 1.78 \pm 0.08$  pg;  $2n = 6x = 66$ ) (Tuler et al. 2019), as well as *Allium dentiferum* Webb & Berthel. ( $1C = 47.80 \pm 0.60$  pg;  $2n = 4x = 32$ ) and *A. dentiferum* ( $1C = 57.30 \pm 1.10$  pg;  $2n = 5x = 40$ ) (Kobrlóvá et al. 2024).



**Figure 3.** Idiograms showing the number, size and position of heterochromatic CMA<sup>+</sup> bands (yellow blocks) and/or mean 1C value (pg) of twelve *Solanum* species plotted in a phylogenetic tree based on Hilgenhof (2023). Chromosomes are traditionally ordered from the longest to the shortest. In *S. viarum*, the heteromorphic chromosome pair is underlined. EHS = Eastern Hemisphere Spiny.

Although a moderate and significant positive correlation between ploidy level and mean 1C value was detected, the relationship is not strictly proportional across *Solanum* species. Notably, *S. andreanum* (4x) exhibits 1C values comparable to or even lower than those of some diploid species, and *S. laciniatum* (8x) does not show a genome size proportional to its high ploidy level (Table 1). These patterns are consistent with genome downsizing and diploidization processes commonly observed after polyploidization, involving DNA sequence loss, particularly of repetitive elements, chromosomal rearrangements, and differential retention of duplicated genomic regions. Such processes can decouple genome size from chromosome set number, especially in older or stabilized polyploids (Wang et al. 2021, Heslop-Harrison et al. 2023). Therefore, while polyploidy contributes to an overall increase in genome size at a broader evolutionary scale, post-polyploid genomic restructuring plays a key role in shaping the observed variation in nuclear DNA content among *Solanum* species.

Despite its importance and broad applications, most cytogenetic and genetic studies remain restricted to economically important *Solanum* species and their close relatives, even when employing classical approaches, such as CMA/DAPI banding and 1C value estimation. Recent advances in next generation cytomolecular techniques, such as Fluorescent *in situ* Hybridization (FISH) using single-copy oligonucleotide probes, have been applied, but still in a limited number of species (Braz et al. 2018). Similarly, genome sequencing and synteny comparisons are restricted to a few representatives, mainly within the Potato clade (Tang et al. 2022, Benoit et al. 2025, [https://www.plabipd.de/timeline\\_view.ep](https://www.plabipd.de/timeline_view.ep)). This limitation coverage constrains our understanding of the cytogenetic and genomic diversity of the genus, hindering the identification of evolutionary patterns and the exploration of under-investigated genetic resources.

Understanding the diversity of heterochromatin and genome size is essential for elucidating genome structure and organization in plants. Here, we provide unpublished and reviewed genomic *Solanum* data, expanding the current cytogenetic and genome size knowledge of the genus, as well as a detailed characterization of CH distribution across species' karyotypes. These findings open new perspectives for future cytomolecular studies and offer new insights into the cytogenetic diversity of wild and cultivated *Solanum* within a phylogenetic context. Additional cytomolecular and genomic investigations are needed to further elucidate karyotype organization and evolutionary trends within the genus.

## DATA AVAILABILITY

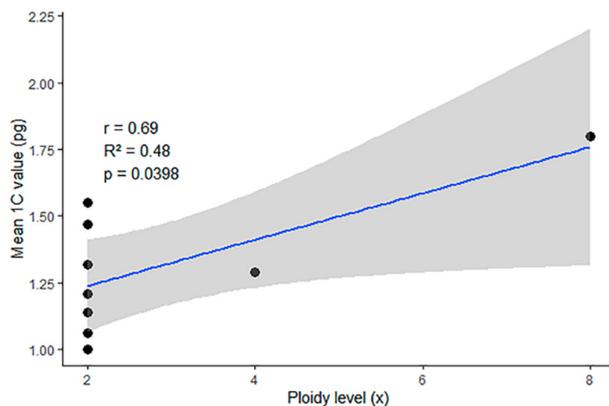
The data generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

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## REFERENCES

Achakkagari S, Camargo-Tavares JC, Praslickova D, Martini C, Bizimungu B, Anglin NL, Manrique-Carpintero N, Lindqvist-Kreuzer H, Tai HH and



**Figure 4.** Relationship between ploidy level (x) and mean nuclear DNA content (1C value) in *Solanum* species. Points represent species mean values. The solid line indicates the fitted linear regression, and the shaded area corresponds to the 95% confidence interval.

Strömvik MV (2025) Better together: Subgenomes for allotetraploid potato wild relative *Solanum acaule* Bitt. reveal origins in Petota Clade 3 and 4. **The Plant Genome** 18: e70095.

Acosta MC, Guerra M and Moscone EA (2012) Karyological relationships

## Cytogenetics of selected *Solanum* L. species within a phylogenetic framework

- among some South American species of *Solanum* (Solanaceae) based on fluorochrome banding and nuclear DNA amount. **Plant Systematics and Evolution** **298**: 1547-1556.
- Almeida BM, Martins LV, Lopes ACA, Gomes RLF, Valente SES, Peron AP, Silva VB and Feitoza LL (2022) Karyotype polymorphism of GC-rich constitutive heterochromatin in *Capsicum* L. pepper accessions. **Crop Breeding and Applied Biotechnology** **22**: e38642113.
- Arumuganathan K and Earle ED (1991) Nuclear DNA content of some important plant species. **Plant Molecular Biology Reporter** **9**: 208-218.
- Balkrishna A, Shankar R, Rashmi AJ, Prajapati UB, Srivastava A, Vidyarthi P, Lathwar R and Arya V (2022) A comparative nutraceutical analysis of edible *Solanum* L. species reported in India. **Food Science & Nutrition Research** **5**: 1-6.
- Barow M and Meister A (2002) Lack of correlation between AT frequency and genome size in higher plants and the effect of nonrandomness of base sequences on dye binding. **Cytometry** **47**: 1-7.
- Benoit M, Jenike KM, Satterlee JW, Ramakrishnan S, Gentile I, Hendelman A, Passalacqua MJ, Suresh H, Shohat H, Robitaille GM, Fitzgerald B, Alonge M, Wang X, Santos R, He J, Ou S, Golan H, Green Y, Swartwood K, Karavolias NG, Sierra GP, Orejuela A, Roda F, Goodwin S and Lippman ZB (2025) *Solanum* pan-genetics reveals paralogues as contingencies in crop engineering. **Nature** **640**: 135-145.
- Brasileiro-Vidal AC, Melo-Oliveira MB, Carvalheira GMG and Guerra M (2009) Different chromatin fractions of tomato (*Solanum lycopersicum* L.) and related species. **Micron** **40**: 851-9.
- Braz GT, He L, Zhao H, Zhang T, Semrau K, Rouillard JM, Torres GA and Jiang J (2018) Comparative Oligo-FISH mapping: an efficient and powerful methodology to reveal karyotypic and chromosomal evolution. **Genetics** **208**: 513-523.
- Braz GT, Van-Lume B, Resende KFM, Cardoso FP, Oliveira L, Andrade MJG, Souza G and Torres GA (2024) Cytomolecular trends in *Chamaecrista* Moench (Caesalpinioideae, Leguminosae) diversification. **Genetica** **152**: 51-61.
- Carvalho M and Saraiva L (1993) A technique for air-drying chromosomes from plants. **Revista Brasileira de Genética** **16**: 395-399.
- Chiarini F, Sazatornil F and Bernardello G (2018) Data reassessment in a phylogenetic context gives insight into chromosome evolution in the giant genus *Solanum* (Solanaceae). **Systematics and Biodiversity** **16**: 397-416.
- Deanna R, Acosta MC, Scaldaferrero M and Chiarini F (2022) Chromosome evolution in the family Solanaceae. **Frontiers in Plant Science** **22**: 787590.
- Dénes TE, Molnár I, Vass IZ, Vass I and Rákossy-Tican E (2024) Selection and phenotyping for drought tolerance in somatic hybrids between *Solanum tuberosum* and *Solanum bulbocastanum* that show resistance to late blight, by using a semi-automated plant phenotyping platform. **Agriculture** **14**: 48.
- Díaz-García G, Enciso-Maldonado GA, Díaz-García LA, Legaria-Solano JP, Bamberg J and Lozoya-Saldaña H (2024) Field screening of *Solanum demissum* confirms its late blight resistance in the Toluca Valley, Mexico. **American Journal of Potato Research** **101**: 122-131.
- Gaiero P, Vaio M, Peters SA, Schranz ME, de Jong H and Speranza PR (2019) Comparative analysis of repetitive sequences among species of the potato and tomato clades. **Annals of Botany** **123**: 521-532.
- Guerra MS (1988) **Introdução à citogenética geral**. Guanabara Koogan, Rio de Janeiro, 140p.
- Heslop-Harrison JS, Schwarzacher T and Liu Q (2023) Polyploidy: its consequences and enabling role in plant diversification and evolution. **Annals of Botany** **131**: 1-10.
- Hilgenhof R, Gagnon E, Knapp S, Aubriot X, Tepe J, Bohs L, Giacomini LL, Gouvêa YF, Christopher TM, Orejuela A, Clara IO, Peralta IE and Särkinen T (2023) Morphological trait evolution in *Solanum* (Solanaceae): Evolutionary lability of key taxonomic characters. **Taxon** **72**: 811-847.
- Kawicha P, Tongyoo P, Wongpakdee S, Rattanapolsan L, Duangjit J, Chunwongse J, Suwor P, Sangdee A and Thanyasiriwat T (2023) Genome-wide association study revealed genetic loci for resistance to fusarium wilt in tomato germplasm. **Crop Breeding and Applied Biotechnology** **23**: e43532311.
- Kirov I, Khrestaleva L, Van Laere K, Soloviev A, Meeus S, Romanov D and Fesenko I (2017) Drawid: user-friendly Java software for chromosome measurements and idiogram drawing. **Comparative Cytogenetics** **11**: 747-757.
- Kobrová L, Jandová M, Vojtěchová K, Šafářová L and Duchoslav M (2024) New estimates and synthesis of chromosome numbers, ploidy levels and genome size variation in *Allium* sect. *Codonoprasum*: advancing our understanding of the unresolved diversification and evolution of this section. **Botanical Studies** **65**: 40.
- Leitch IJ, Johnston E, Pellicer J, Hidalgo O and Bennett MD (2019) Plant DNA c-values database (Release 7.1). Available at <<https://cvalues.science.kew.org/>>. Accessed on September 15, 2025.
- Levan A, Fredga L and Sandberg A (1964) Nomenclature for centromeric position on chromosomes. **Hereditas** **52**: 201-220.
- Macková L, Vít P, Ďurišová L, Eliáš Junior P and Urfus T (2017) Hybridization success is largely limited to homoploid *Prunus* hybrids: a multidisciplinary approach. **Plant Systematics and Evolution** **303**: 481-495.
- Martínez-Cuenca MR, Pereira-Dias L, Soler S, López-Serrano L, Alonso D, Calatayud A and Díez MJ (2020) Adaptation to water and salt stresses of *Solanum pimpinellifolium* and *Solanum lycopersicum* var. *cerasiforme*. **Agronomy** **10**: 1169.
- Martins LV, Peron AP, Lopes ACA, Gomes RFL, Carvalho R and Feitoza LL (2018) Heterochromatin distribution and histone modification patterns of H4K5 acetylation and phosphorylation in *Capsicum* L. **Crop Breeding and Applied Biotechnology** **18**: 161-168.

- Melo, CAF, Martins MIG, Oliveira MBM, Benko-Iseppon AM and Carvalho R (2011) Karyotype analysis for diploid and polyploid species of the *Solanum* L. **Plant Systematics and Evolution** **293**: 227-235.
- Mesquita AT, Braz GT, Shimizu GH, Machado RM, Cruz MVR and Forni-Martins ER (2025) Karyotype diversity and genome size in the Cyphomandra clade of *Solanum* L. (Solanaceae). **Botanical Journal of the Linnean Society** **207**: 240-252.
- Moyetta NR, Urdampilleta JD, Chiarini FE and Bernardello G (2016) Heterochromatin and rDNA patterns in *Solanum* species of the Morelloid and Dulcarnaroid clades (Solanaceae). **Plant Biosystems** **151**: 539–547.
- Olmstead RG and Palmer JD (1992) A chloroplast DNA phylogeny of the Solanaceae: subfamilial relationships and character evolution. **Annals of the Missouri Botanical Garden** **79**: 346-360.
- Olmstead RG and Sweere JA (1994) Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. **Systematic Biology** **43**: 467-481.
- Praça-Fontes MM, Carvalho CR, Clarindo WR and Cruz CD (2011) Revising the DNA C-values of maize (*Zea mays* L.). **Genetics and Molecular Research** **10**: 3738-3745.
- R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at <<https://www.R-project.org/>>. Accessed on November 6, 2025.
- Rego LNAA, Silva CRM, Torezan JMD, Gaeta ML and Vanzela ALL (2009) Cytotaxonomical study in Brazilian species of *Solanum*, *Lycianthes* and *Vassobia* (Solanaceae). **Plant Systematics and Evolution** **279**: 93-102.
- Schweizer D and Ambros PF (1994) Chromosome banding: stain combinations for specific regions. In Gosden JR (ed) **Chromosome analysis protocols**. Humana Press, Totowa, p. 97-112.
- Tang D, Jia Y, Zhang J, Li H, Cheng L, Wang P, Bao Z, Liu Z, Feng S, Zhu X, Li D, Zhu G, Wang HR, Zhou Y, Zhou Y, Bryan GJ, Buell CR, Zhang C and Huan S (2022) Genome evolution and diversity of wild and cultivated potatoes. **Nature** **606**: 535-541.
- Tuler AC, Carrijo TT, Peixoto AL, Garbin ML, Ferreira MFS, Carvalho CR, Spadeto MS and Clarindo WR (2019) Diversification and geographical distribution of *Psidium* (Myrtaceae) species with distinct ploidy levels. **Trees** **33**: 1101-1110.
- Urfus T, Chrték J, Kaplan Z, Prančl J, Ponert J and Trávníček P and Slovák M (2025) Flow cytometry in conservation: detecting hybridization risks in threatened plant species. **Biodiversity and Conservation** **34**: 2337-2358.
- Walter E, Lyrene PM and Chu Y (2025) Polyploidy induction of wild diploid blueberry *V. fuscatum*. **Horticulturae** **11**: 921.
- Wang X, Morton, JA, Pellicer J, Leitch IJ and Leitch AR (2021) Genome downsizing after polyploidy: mechanisms, rates and selection pressures. **The Plant Journal** **107**: 4.
- Zhang W, Mei Y and Wang J (2025) Verification of the chromosome number using cytogenetics and estimation of genome size via flow cytometry and k-mer analyses for representative *Anoectochilus roxburghii* accessions. **PLOS ONE** **20**: e0322457.