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Original Paper

Genome size in Argentinean species of *Passiflora* genus: cytological and phenotypical correlates

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Abstract

The genus *Passiflora* is the largest of the Passifloraceae family with many species and great phenotypic variability. There are nineteen species of *Passiflora* native to Argentina, distributed in four taxonomic subgenera: *Passiflora*, *Decaloba*, *Dysosmia* and *Tacsonioides*. Unlike most species of the genus, Argentinean species could tolerate colder climates. For most Argentinean species, genome size information is unavailable. The objective of this work has been to estimate the genomic size of 36 genotypes of thirteen *Passiflora* taxa by flow cytometry and to make a contrast with phenotypic ornamental characteristics. The genomic sizes of *P. tucumanensis*, *P. elegans* and *P. mooreana* are being introduced for the first time. The DNA amount per basic genome varied between 0.54 and 2.52 picograms in *P. capsularis* and *P. alata*, respectively. In the species *P. caerulea*, *P. elegans* and *P. edulis f. flavicarpa*, intraspecific variation in genomic size has been registered. The high correlation of genome size with flower diameter, and of leaf area with flower diameter can contribute to the selection of parents for the ornamental plant improvement plan. Also, the data collected in this work will be very useful in the study of inter-specific hybrids.

Key words: DNA content, flow cytometry, genome size, ornamental plant breeding, *Passiflora* native to Argentina.

Resumen

El género *Passiflora* es el más grande dentro de la familia Passifloraceae con numerosas especies y gran variabilidad fenotípica. Existen diecinueve especies de *Passiflora* nativas de Argentina distribuidas en cuatro subgéneros: *Passiflora, Decaloba, Dysosmia* y *Tacsonioides*. A diferencia de la mayoría de las especies del género, las argentinas podrían tolerar climas más fríos. Para la mayoría de las especies argentinas, la información sobre el tamaño del genoma no se encuentra disponible. El objetivo de este trabajo fue estimar el tamaño genómico de 36 genotipos de trece taxones de *Passiflora* por citometría de flujo y contrastarlos con las características fenotípicas ornamentales. Los tamaños genómicos de *P. tucumanensis, P. elegans* y *P. mooreana* se presentan por primera vez. La cantidad de ADN por genoma básico varió entre 0,54 y 2,52 picogramos en *P. capsularis* y *P. alata*, respectivamente. Se registró variación en el tamaño genómico intra-específico en las especies *P. caerulea*, *P. elegans* y *P. edulis f. flavicarpa*. La alta correlación del tamaño del genoma con el diámetro de la flor y del área foliar con el diámetro de la flor podría contribuir a la selección de progenitores para el plan de mejoramiento de plantas ornamentales. Asimismo, los datos recopilados en este trabajo serán de gran utilidad en el estudio de los híbridos interespecíficos.

Palabras clave: contenido de ADN, citometría de flujo, tamaño del genoma, mejoramiento de plantas ornamentales, *Passiflora* nativas de Argentina.

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Introduction

The *Passiflora* genus comprises 525 species distributed in tropical and subtropical areas of America, Asia and Africa (Ulmer & MacDougal 2004). Its center of origin is in South America and is the widest genus of the *Passifloraceae* family with many species and great phenotypic variability (Souza *et al.* 2004). Many *Passiflora* species are cultivated for their ornamental value, such as *P. alata* and *P. caerulea*; for their medicinal properties, such as *P. incarnata*; or for their edible fruits, such as the commercially exploited *P. edulis* (passion fruit) (Ulmer & MacDougal 2004).

There are nineteen species of *Passiflora* considered native to Argentina and they are distributed in four taxonomic subgenera. The largest one is the *Passiflora* subgenus that has 11 species, while the *Decaloba*, *Dysosmia* and *Tacsonioides* subgenus have 5, 2 and 1 species, respectively (Deginani 2001).

By their austral distribution, Argentinean *Passiflora* species could tolerate colder climates, unlike most species of the genus that prefer tropical temperatures. For this reason, they were included in breeding programs with the aim to obtain ornamental varieties tolerant to low temperatures by means of interspecific hybridization (Bugallo *et al*. 2011).

One important aspect to consider when making interspecific *Passiflora* hybrids is that related to genomic similarities between the species involved. The possibility of hybridization is an indirect measure of the degree of genomic relationship between parental species. Crossings between species with similar genomes usually produce fruits normally, while among those of different genomes, it is frequent the abortion of embryos or the sterility of hybrids, among other manifestations (He *et al*. 2022). If a hybrid is obtained, the more similar the chromosomal constitution of its parents, the more normal it will be (Poggio *et al*. 2016; Singh 2003). The inviability of the pollen of the hybrids, or the absence of fruiting, can be attributed to the poor chromosomal homology between the parents of the hybrid that results in the formation of unbalanced gametes (Singh 2003). The reduction of chromosomal correspondence between distant related species is sometimes due to structural differences between chromosomes perpetuated during speciation (Poggio *et al*. 2016; Singh 2003).

Changes in the genome size generally involving polyploidy and transposable element amplification, cause increase of nuclear DNA content; while unequal homologous recombination or illegitimate recombination could generate small deletions deriving in the shrinkage of the nuclear genome (Bennetzen *et al*. 2005). Greilhuber (1979) named the DNA content of the monoploid genome (x number) as 1Cx and used it as an abbreviation for 1C value (x-level). The basic chromosome numbers reported for the Argentinean species subgenera were x = 9 for *Passiflora* and *Tacsonioides* (2n = 18 and 2n = 36 for a cytotype of *P. mooreana*). For subgenus *Dysosmia* x = 10 (2n = 20) and x = 6 (2n = 12 and 24) for *Decaloba* (Tab. S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.23905617.v1>) (Souza *et al*. 2008; Melo *et al*. 2001; Bugallo *et al*. 2020). Polyploidy is not frequently seen in the Argentinean species of *Passiflora*, being found only in *P. suberosa* (2n = 24 and 36), *P. misera* (2n = 12 and 36), both from subgenus *Decaloba*, and in *P. mooreana* (2n = 18 and 36) from subgenus *Passiflora* (Ferreira *et al*. 2020; Bugallo *et al*. 2020). Interestingly, in recent years, many studies have been published exposing the origin of the variation in the genomic size of some *Passiflora* species. Dias *et al*. (2020) made cytogenetic maps comparing *P. alata* and *P. watsoniana* using BAC-FISH, and found numerous transposable element repeats as well as other single-copy markers in both species. However, they did not find breaks in the synteny between the two species studied, consequently, large-scale rearrangements would not have occurred. On the other hand, Ferreira *et al.* (2020) studied the karyotypes and the nuclear DNA content of 28 species of *Passiflora* and mentioned genome sizes from 2C = 0.42 in *P. organensis* to 2C = 5.36 in *P. quadrangularis*. The variation in the DNA amount exceeds 1,276% between species with divergent chromosome numbers, but they also found differences of a 925% in the 2C values within subgenera.

Diversification and evolution have triggered much interest in the variation of the DNA contents in *Passiflora* (Sader *et al*. 2019; Yotoko *et al*. 2011). The volume of the karyotype chromosomes is directly correlated with the DNA amount in the nucleus (Grif 2000). Although large variations in genomic sizes are often associated with karyotypic alterations observable under a microscope, such as polyploidy, aneuploidy, or accessory

chromosomes, in some cases these modifications are not visible (Inceer *et al*. 2018). Variation in intraspecific genomic size has been verified due to chromosomal rearrangements, gains or losses of repetitive DNA and transposable elements (Sader *et al.* 2020; Boutte *et al*. 2020; Inceer *et al*. 2018; Šmarda & Bureš 2012). Genome downsizing consists of a non-random deletion of coding and non-coding sequences, modifications in retroelements, chromosomal reorganization, loss and / or gain of individual chromosomes or entire genomes, altered patterns of gene expression and epigenetic alterations (Doyle & Coate 2019; Inceer *et al*. 2018; Leitch & Leitch 2013; Ma & Gustafson 2006; Feldman & Levy 2005).

In a previously published work, Yotoko *et al*. (2011) found a relationship between genome size and flower diameter in the genus *Passiflora*. However, the species analyzed came mostly from Brazil and comparisons with other characters of ornamental importance were not made.

Although genomic size data for species of the genus *Passiflora* has been published in several papers, the information for most species from Argentina is not available. Unfortunately, studies addressing genome size diversity among closely related species and its relationship with phenotypes are also scarce (Du *et al*. 2017). The objective of this work has been to estimate the genome size of Argentinean species of the genus *Passiflora* participating in a breeding program to obtain ornamental plants. In addition, we aim at verifying the correlation between the amount of DNA with phenotypic ornamental parameters to evaluate its application in selection.

Materials and Methods

Plant material

Plants from 36 genotypes corresponding to 13 *Passiflora* taxa, with 3 replicates each, obtained by vegetative propagation, have been analyzed. The plant material used came from the *Passiflora in vivo* collection of the Instituto de Floricultura, INTA (Institute of Floriculture of the National Institute of Agricultural Technology, Argentina). The plants were grown in the same greenhouse with a controlled minimum temperature of 10 ºC, in 15-liter pots with a substrate composed of pine bark, pine leaves, river residue and soil (1:1:1:1). The samples tested involved three species of the subgenus *Decaloba*: *P. capsularis* (1 genotype), *P. morifolia* (1) and *P. suberosa* (2); one of subgenus *Dysosmia: P. foetida* (3); and nine of subgenus *Passiflora*: *P. alata* (2), *P. amethystina* (1), *P. caerulea* (10), *P. cincinnata* (2), *P. edulis f. edulis* (1), *P. edulis f. flavicarpa* (4), *P. elegans* (4), *P. mooreana* (2), *P. tucumanensis* (3). These plants were the same used to perform the crossings in the breeding ornamentals from native species program. The genotypes analyzed, their origin, chromosome numbers, haploid chromosome length (HCL) and 1Cx DNA value previously analyzed were listed in Table S1 (available on supplementary material <https://doi.org/10.6084/m9.figshare.23905617.v1>).

Flow cytometric analysis of nuclear DNA in *Passiflora*

For flow cytometry, fully expanded leaves of adult plant specimens were selected from the collection. The leaves were cleaned superficially with water and dried with a paper towel. The intact nucleus suspensions were prepared by simultaneously chopping 1 to 2 cm of *Passiflora* leaf and the same amount of leaf from the control plant in a Petri dish containing 1 ml of Otto I buffer, composed of 0.1M citric acid and 0.5% Tween 20 (Otto 1990). Control plants of *Hordeum vulgare* cv. New Golden (10.4 pg.) were used as internal standards. The chopped material was filtered by a 50 µm nylon mesh and the Otto II staining buffer, composed of 0.4M $Na_2HPO_4.12H_2O$ 14.325 g for each 100 ml, was incorporated together with propidium iodide and RNase, both in a concentration of 50 µl per ml of solution. The stained core suspension was analyzed on a Partec CyFlow Ploidy Analyzer flow cytometer (Partec, Germany) with 532 nm-30 mW green laser. The estimate of the amount of DNA in each genotype was calculated according to Dolezel & Bartos (2005), using the formula: Sample 2C nuclear DNA amount = Sample fluorescence * Control 2C nuclear DNA amount * (Fluorescence control) $^{-1}$. Estimations of 2Cx were divided by ploidy level to register 1Cx value. At least three different estimates were made for each of the genotypes analyzed, as repetitions.

Estimation of phenotypic characters

The estimated phenotypic characters were flower diameter, the area of each leaf and the length of the internodes. For the estimation, at least five completely open flowers and 10 internodal segments for each genotype were measured with

a caliber and registered in millimeters. Estimation of the mean leaf area was made by means of the measurement of this parameter in a Green Leaf Area Meter GA-5 (Tokio Photoelectrics) in at least 10 leaves for genotype.

Comparison between genome size, cytological and phenotype characters

The comparisons between the genomic size and the phenotypic characters obtained in this work, as well as with previously published cytological characters (Bugallo *et al*. 2020; Kew 2021), were made through regressions to establish correlations.

Statistical design and analysis

The hypothesis of similarity of means 1Cx value, flower diameter, leaf area and internodal length between the different genotypes and taxa of *Passiflora* was verified according to an F-test (ANOVA) in a completely randomized design and they were compared by means of a Between Sum of Squares (BSS) statistical test with significance level p 0.05. Correlations between characters were studied by means of linear regressions. All statistical analyses have been performed with the InfoStat package (version 2009, National University of Córdoba, Argentina) (Di Rienzo *et al*. 2010).

Results

As a result of the flow cytometric analysis, histograms of the relative content of nuclear DNA of Argentinean genotypes of *Passiflora* and *Hordeum vulgare* cv. New Golden were obtained. The amount of 1Cx DNA estimated for *Passiflora* taxa from Argentina ranged between 0.54 and 2.52 picograms in *P. capsularis* and *P. alata* species, respectively (Tab. S2, available on supplementary material <https://doi.org/10.6084/m9.figshare.23905617.v1>).

The BSS statistical comparison carried out between the analyzed taxa showed the existence of similarity between the sizes of the genomes in the *Decaloba* and *Dysosmia* subgenus species, differing with the *Passiflora* subgenus species, which presented higher amounts of DNA. Comparison among the amounts of DNA in the monoploid complements (1Cx) of the taxa allowed the recognition of five groups: a) *P. capsularis*, *P. suberosa*, *P. morifolia* and *P. foetida* with 0.54 to 0.72 picograms of DNA per genome; b) *P. tucumanensis* with 0.91 pg.; c) *P. elegans* and *P. cincinnata* with 1.29 and 1.35 pg.; d) *P. edulis f. edulis*, *P. edulis f. flavicarpa*, *P. caerulea*, *P. mooreana* (diploid and tetraploid) and *P. amethystina* with 1.52 to 1.76 pg., e) *P. alata* with 2.52 pg. This analysis differentiated the *Passiflora* subgenus, which presented a greater amount of DNA in its genome (0.91 to 2.52 pg.), from the *Dysosmia* and *Decaloba* subgenera (0.54 to 0.72 pg.).

Within the subgenus *Passiflora*, there was variation between the C values of the different taxa. In addition, intraspecific differences in the size of the genomes were recorded in the species *P. caerulea*, *P. elegans* and *P. edulis f. flavicarpa*. The species with the highest number of genotypes in this work was *P. caerulea*, of which 10 accessions were studied. There were found 3 different statistical groups (ANOVA-BSS) and a variation from 1.33 to 1.80 pg. of DNA per haploid genome.

In the studied species of *Passiflora*, variation in the phenotypic characters, flower diameter, length of internodes and leaf area were found (Tab. S2, available on supplementary material <https://doi.org/10.6084/m9.figshare.23905617.v1>). In linear regression studies (Fig. 1), genome size (Cx value) and flower diameter correlated significantly ($R^2 = 0.75$), as well as leaf area and flower diameter ($R^2 = 0.58$). Correlations of less than 0.5 were found between genome size and leaf area ($R^2 = 0.40$), and between the length of the internodes and the rest of the studied characters: with the leaf area ($R^2 = 0.31$), with genome size ($R^2 = 0.15$) and flower diameter ($R^2 = 0.13$). The three phenotypic characters studied in this work, flower diameter, leaf area and length of internodes, showed intra and inter-specific variation.

A linear regression was performed between the total length of the genome, estimated by measuring the chromosomes in the genotypes of the collection (Bugallo *et al*. 2020), and the amount of DNA per haploid genome estimated in this work. The results showed that the two variables were closely correlated, with a coefficient of determination $R^2 = 0.95$ (Fig. 1). When the total length of the genome was compared with the estimated genomic sizes for other genotypes of these species, known prior to this work, the same correlation was found ($R^2 = 0.95$).

Discussion

In this work, the nuclear DNA content of 30 genotypes corresponding to 13 taxa of

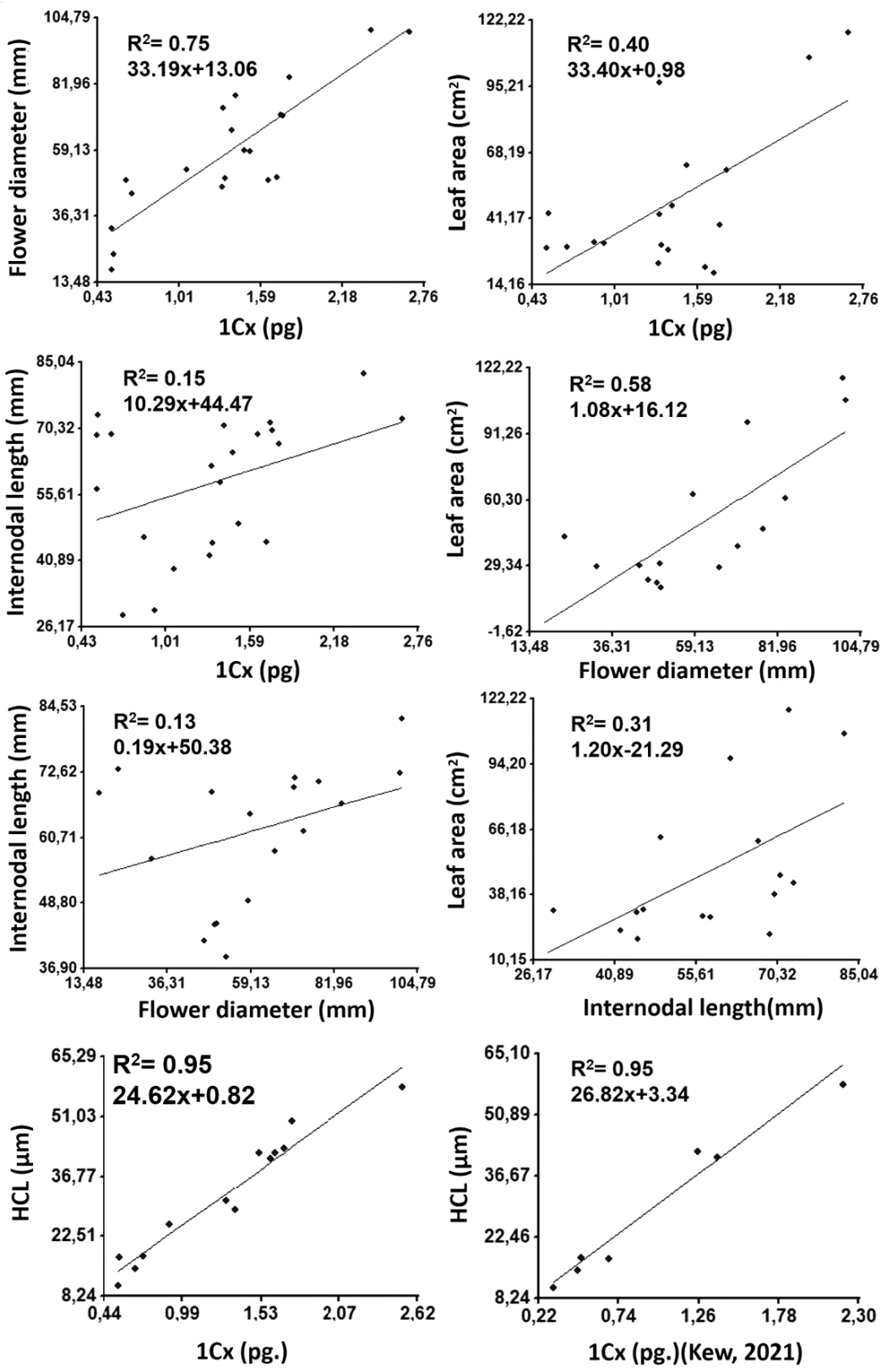

Figure 1 – Linear regressions between genome size and phenotypic characteristics of Passiflora.

Argentinean *Passiflora* species was estimated. The genomic sizes of *P. tucumanensis*, *P. elegans* and *P. mooreana* are introduced for the first time.

The DNA amount per Cx genome in the studied species varied between 0.54 and 2.52 picograms in *P. capsularis* and *P. alata* species, respectively. The results for *P. suberosa* and *P. edulis f. edulis* were similar to those published by Souza *et al*. (2004) and our results for *P. edulis f. flavicarpa* were nearer to the Mexican than to the Brazilian genotype studied by these authors. The results published by Kew (2021) for *P. caerulea* and *P. alata* are also similar to the values obtained in this work. This would indicate that the Argentinean *Passiflora* species, like those studied by Souza *et al.* (2004), have an average genome size within the plant kingdom.

The species of the *Decaloba* and *Dysosmia* subgenera did not differ in DNA amount of the basic complement. This similarity in genome size between both subgenera is in coincidence with an evolutionary closeness, supported by karyotype analysis and in the haploid chromosome length (HCL) and karyotypical symmetry indices. Nonetheless, results of GISH analysis showed that there is greater homology between the genomes of the *Passiflora* and *Dysosmia* subgenera (Bugallo *et al*. 2020), agreeing with the molecular phylogenies of Muschner *et al.* (2003) and Sader *et al.* (2019). However, the hypothesis made by Sader *et al*. (2019), asserting that the species of the subgenus *Dysosmia* derives from the subgenus *Passiflora* also assumes that there would be an intermediary between the subgenera *Decaloba* and *Passiflora* with a basic number x = 8, an unknown number within species of the genus.

The species of the subgenus *Passiflora* showed the largest monoploid genomes (Tab. S2, available on supplementary material <https://doi.org/10.6084/m9.figshare.23905617.v1>). Nevertheless, variations were found among the species. The largest monoploid genome was presented by *P. alata* (2.52 pg.), with intermediate values in *P. amethystina*, *P. mooreana*, *P. edulis* and *P. caerulea*, and smaller amounts in *P. elegans*, *P. cincinnata* and *P. tucumanensis.* The last one was the species with the smallest genome in the subgenus *Passiflora* (0,90 pg.).

Sader *et al*. (2020) identified LTR (long terminal repeat) retrotransposons as the most abundant elements in the genomes of three *Passiflora* species (*P. cincinnata*, which was also studied in this work, *P. quadrangularis* and *P. organensis*), while Pamponét *et al.* (2019) came to the same conclusion for *P. edulis*. According to the authors, the larger genomes of the genus evolved by accumulation of retrotransposons, while the smaller genomes evolved by diversification of different types of repeats, especially satDNAs. Among the species analyzed, two polyploids were studied: *P. suberosa* (2n = 4x = 24) and *P. mooreana* (2n = 4x = 36) (Bugallo *et al*. 2013). Interestingly, there were analyzed two natural cytotypes of *P. mooreana*, one diploid 2n = 18 and the other tetraploid 2n = 36. Although downsizing is usually reported in most polyploids, in this work the size of the genome Cx was similar between the specimens of the two ploidy levels.

In the species *P. caerulea*, *P. elegans* and *P. edulis f. flavicarpa*, intraspecific variation in genomic size was registered. In *P. caerulea*, for which the DNA amount was estimated in eight genotypes, a variation was found from 1.33 to 1.80 pg. DNA per haploid genome, indicating a variation of 26%. Considering the conversion formula, 1 pg. = 978 Mbp, (Dolezel *et al*. 2003), the intraspecific variation found in this work for *P. caerulea* would be equivalent to 460 Mbp per genome.

Balant *et al*. (2022) made a summary of the cases of intraspecific genome size variation at the same ploidy level and the found amount of variation. Some examples are *Festuca pallens* (16.6% variation within diploid and 15% in tetraploid individuals), *Picris hieracioides* (37.6%), *Senecio carniolicus* (13.1% in 2x, 10.2% in 4x, 5.4% in 5x, and 10.5% in 6x populations), *Ranunculus parnassifolius* (8.58% in 2x and 1.29% 4x) and *Euphrasia arctica* (27.4%). The authors also state that, in the absence of chromosome number variation, another possible explanation for intraspecific genome size variation could be the differences in repetitive DNA sequence content. In *Zea mays*, Silva *et al*. (2020) found intraspecific DNA content variation (2C = 2.00pg to 2C = 6.10pg) and the differences were attributed to repetitive sequences, structural chromosome alterations, heterozygous terminal deletion and conspicuous insertion of LTR-retrotransposons in the karyotype.

In a previous study analyzing the amount of DNA in *Passiflora* (Souza *et al.* 2004), it is argued that the intraspecific variation in genome size would be associated with environmental parameters such as latitude, altitude and annual rainfall. In addition,

these authors refer to the fact that stress can cause destabilization and chromosomal rearrangements, producing variability subject to selection due to adaptation to environmental changes.

The phylogeny of the genus *Passiflora* has been addressed on several occasions. The variation in the DNA content obtained in this work agrees with the results of the phylogeny of Muschner *et al*. (2003), Krosnick *et al*. (2013) and Ramaiya *et al*. (2014), separating the *Decaloba* and *Passiflora* subgenera. However, the similarity in genome size between *P. foetida* and the species of the *Decaloba* subgenus contrasts with the molecular affinity between *P. foetida* and the *Passiflora* subgenus (Bugallo *et al*. 2020).

The genomic size analyzed in conjunction with cytogenetic data avoids misinterpretations, especially in species with several cytotypes (Kolár *et al*. 2017; Pellicer & Leitch 2019). It was found that regression tests between the length of the haploid chromosomal length (HCL) of these same genotypes (Bugallo *et al*. 2020), and the amount of DNA per haploid genome estimated in this work showed a close correlation between the two variables ($R^2 = 0.95$) (Fig. 1). The correlation between the sizes of haploid genomes, estimated by measurement in photomicrographs of the chromosomes of each species, and the amount of DNA estimated by flow cytometry strengthens both results.

It is interesting to compare if the Cx value is consistent at individual level or if it is at species or population level (between genotypes). In the comparison of the total length of the genome (HCL) (Bugallo *et al*. 2020) and data of the genomic sizes for each species, obtained from the Plant DNA C-values Database (Kew 2021), the same correlation was found ($R^2 = 0.95$) (Fig. 1). This information reinforces the validity of the relationship, in diploid species, between HCL and 1Cx, and vice versa. The cytogenetic analysis should always be present as a control that cannot be absent in the estimates made by flow cytometry.

The variation in genome sizes among genotypes of *P. caerulea* was not related to its origin since, for example, the Cae5 and Cae9 genotypes have the same site of origin but showed differences in the amount of DNA in their genome (Tabs. S1; S2, available on supplementary material <https://doi.org/10.6084/m9.figshare.23905617.v1>). Also, genotypes of *P. elegans* from the same area, presented a different genome size. As Argentinean species represent the extreme latitudinal distribution of the genus, it would be interesting to compare the genomic sizes in this work with those from tropical zones, in future research.

In breeding ornamental *Passiflora* plants, some phenotypical characters are important. Although several phenotypic parameters have been correlated with the amount of nuclear DNA in other species (weight of leaves and seeds, size of pollen grains, size of stomatal cells, among others) (Inceer *et al*. 2018), in this work, the leaf surface and the length of the internodes in the genus *Passiflora* have been examined by first time. In addition to the size of the flowers, the leaf area and the length of the internodes are relevant because of its application to green walls and living fences, where the surface covered by leaves determines the fulfillment of its objective (Busilacchi *et al*. 2008). It was found correlation between leaf area and flower diameter ($R^2 = 0.58$). This relationship could be very useful to estimate flower size and make an early selection when plants have not yet reached the reproductive stage.

The correlation found between genome size and flower diameter ($R^2 = 0.75$) was higher than that found by Yotoko *et al*. (2011) ($R^2 = 0.54$).

The genome size did not correlate so strongly either with the leaf area ($R^2 = 0.40$) or with the length of the internodes. The diameter of the flowers, the leaf area, and the length of internodes showed intra and inter-specific variation. This, in addition to the existence of genetic variability, suggests the influence of the environment on these variables (nutritional level, water availability, growth rate, etc.) (Pellicer *et al*. 2018).

Based on the results obtained in this work, we conclude that there are differences for DNA amount between the genomes of the species of the *Decaloba* subgenus compared to *Dysosmia* and *Passiflora subgenera*. In addition to the interspecific differences within the subgenus *Passiflora*, we also found intraspecific variation in *P. caerulea*, *P. edulis f. flavicarpa* and *P. elegans*. The correlation of DNA content with phenotypic characteristics such as flower diameter, leaf area and intermodal length, and with karyotype data, which allows differentiating groups of species, showed that the size of the genome is useful in the characterization of inter and intraspecific variability in the genus. These results contribute to the selection of parents for the ornamental plant-breeding plan. The data collected in this work will also result especially useful in the study of inter-specific hybrids.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that all data is available within the manuscript.

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