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Genetic diversity and reproductive system of *Tarenaya diffusa*

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Abstract

The Cleomaceae is a relatively small family comprising approximately 300 species distributed worldwide. *Tarenaya* is one of the main genera within this family and holds significant economic value. However, despite its beauty and remarkable diversity, it remains underrepresented in the ornamental plant market due to the scarcity of information regarding its ornamental potential. Therefore, the objective of this study was to investigate the genetic diversity among *Tarenaya diffusa* accessions through morphological characterization and to determine its reproductive system. Twenty-four accessions of *Tarenaya diffusa* were evaluated using morphological descriptors related to plant architecture, flower, and fruit traits to assess diversity. The reproductive mode

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was determined through the pollen/ovule (P/O) ratio. The experiment was conducted at the Laboratory of Biotechnology and Plant Breeding, CCA/UFPB. Data were subjected to dissimilarity analysis, from which a Euclidean distance matrix was generated, followed by clustering using Tocher's optimization method. The results revealed wide genetic diversity among *Tarenaya diffusa* accessions, and the P/O ratio was 9.45. Accessions 7, 15, and 20, belonging to groups III and V, may be recommended for breeding programs with ornamental purposes. Regarding the reproductive system, *Tarenaya diffusa* was classified as a facultative cleistogamous species.

Keywords: Cleomaceae; morphological characterization; ornamental plants; plant breeding; reproduction

Introduction

Cleomaceae is a family belonging to the order Brassicales, which encompasses significant floral diversity. It is relatively small, comprising approximately 300 species distributed worldwide, primarily in tropical and subtropical regions (Inda *et al*., 2008; Bayat *et al.*, 2018; Saunders *et al.*, 2024). In Brazil, the family is represented by nine genera and 30 species, with five genera and eleven species recorded in the state of Paraíba (Flora do Brasil, 2020; Soares Neto *et al.*, 2021).

Tarenaya Raf. is one of the main genera within this family and holds considerable economic value due to its wide range of uses, which include human food and medicine, animal feed, and, more recently, its potential as a source for the ornamental plant market, particularly the species *Tarenaya houtteana* (Schltdl.) Soares Neto & Roalson and *Tarenaya longicarpa* Soares Neto & Roalson (Iltis *et al.*, 2011; dos Santos Magalhães *et al.*, 2023; Chatara *et al.*, 2023).

Despite its beauty and remarkable diversity, *Tarenaya* remains underrepresented in the ornamental plant market, largely due to the scarcity of information regarding its ornamental potential, coupled with the lack of genetic characterization of available materials (Wu *et al*., 2018; Falquetto-Gomes *et al.*, 2026). According to Chace (2022), plants with smaller stature and larger, more abundant flowers tend to be most attractive for ornamental purposes; however, few studies have been conducted to identify this

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pattern in *Tarenaya*. One of the key strategies for developing new *Tarenaya* cultivars with ornamental potential involves studying genetic diversity, including reproductive biology, to obtain essential information about plant architecture, pollination, and floral development (Wu *et al*., 2018; Sowunmi *et al*., 2018).

The study of genetic diversity through morphological characterization is one of the fundamental tools for understanding the population under investigation and for organizing germplasm banks. This approach facilitates the efficient identification and incorporation of diverse genetic sources, including newly developed cultivars, landraces, wild relatives, and germplasm collections containing elite and/or mutant plants (Rêgo *et al*., 2015; Swarup *et al*., 2021). Recent studies on morphological characterization within the Cleomaceae family have been successfully conducted by several researchers (Masuka *et al*., 2012; Carneiro *et al*., 2018; Das *et al*., 2022), enabling both morphological and taxonomic delimitation.

In addition to studying genetic diversity, understanding the reproductive system of a population is of utmost importance for the proper development of any breeding program. Investigations into floral morphology and reproductive biology in Cleomaceae species have been carried out by Parma *et al*. (2023) in Brazil, Bhide *et al*. (2014) in Germany, and Omondi *et al*. (2017) and Zohoungbogbo *et al*. (2018) in Africa, emphasizing the study of floral structure as a source of information on mechanisms involved in the determination of reproductive traits in plants.

Therefore, the present study aimed to investigate the genetic diversity among *Tarenaya diffusa* (Banks ex DC.) Soares Neto & Roalson accessions through morphological characterization and to determine the reproductive system, to generate information that supports the development of genotypes with desirable traits.

Materials and Methods

Genetic Diversity

A total of 24 accessions of *Tarenaya diffusa* (Figure 1A) obtained from seeds collected at the Center of Agricultural Sciences (CCA) of the Federal University of Paraíba (UFPB) were evaluated. A reference specimen of *T. diffusa* is deposited in the Herbarium Lauro

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Pires Xavier (JPB), under catalog number JPB 64710 (barcode JPB0064710), collected by P.C. Gadelha Neto on 20 December 2017, at the CCA/UFPB, Areia, Paraíba, Brazil (available at: https://specieslink.net/rec/107/64710). The experiment was conducted in the Laboratory of Biotechnology and Plant Breeding at CCA/UFPB, located in the municipality of Areia, Paraíba, in the "*Brejo Paraibano*" region, at 6º 57' 48" S - 35º 41' 30" W (Antonino *et al*., 2004).

Sowing was performed in 200-cell polystyrene trays using sand as the substrate. When the seedlings had developed two to three pairs of true leaves, they were transplanted into 900 mL plastic pots containing the same substrate. The plants were kept in shaded conditions during the initial days and then transferred to a greenhouse until the end of their life cycle. After 120 days from sowing, genetic diversity among accessions was assessed based on morphological descriptors.

Fourteen morphological descriptors related to plant architecture, flower, and fruit (Figures 1B and 1C) were evaluated. For the traits related to plant architecture, the following characteristics were assessed after flowering: plant height, stem diameter, canopy diameter, number of leaflets, leaf length, and leaf width, for which fully expanded leaves were selected to ensure sample standardization during measurements. All measurements, except for the number of leaflets, were taken using a graduated ruler and a digital caliper (Leetools® digital caliper). For floral traits, after anthesis, the number of flowers per inflorescence, flower diameter, number of anthers, and number of petals were recorded. For fruit-related traits, fruit length, fruit diameter, fruit weight, and number of seeds were measured.

An average of three samples per plant was considered for the traits measured in leaves, flowers, and fruits. A standardized Euclidean distance matrix was generated to obtain the dissimilarity matrix among the accessions, and subsequently, the accessions were grouped using the Tocher optimization method. All statistical analyses were performed using the Genes software (Cruz, 2016).

Mode of Reproduction

The mode of reproduction was determined based on the pollen to ovule ratio (P/O), following the methodology and classification intervals proposed by Cruden (1977):

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Cleistogamy 2.7–5.4; Obligate autogamy 18.1–39.0; Facultative autogamy 31.9–396.0; Facultative xenogamy 244.7–2588.0; Obligate xenogamy 2108.0–195,525.0. According to this author, these values reflect the efficiency of pollination, since the more efficient the pollen transfer, the lower the P/O ratio.

A total of five flowers per plant were evaluated, comprising 10 plants. For pollen grain counting, all anthers from each flower were extracted at the pre-anthesis stage (Figure 2A) and placed in Eppendorf tubes containing 200 µL of distilled and deionized water. Subsequently, 50% of the volume was transferred to a Neubauer® chamber for counting under an Axio Vert.A1® microscope (Figure 2B). The estimation of the total number of pollen grains was performed according to the following equation:

$$NP = \frac{Vf}{Vi*Vc} * number\ of\ pollen\ grains\ counted$$

Where: NP = mean number of pollen grains; Vf = final volume, representing the difference between Vi and Vc; Vi = initial volume; Vc = volume counted across all quadrants of the chamber.

For ovule counting (Figure 2C), the ovary of each flower was excised using a scalpel blade, and the ovules were counted under a *ZEISS Stemi* 305® microscope. The P/O ratio was estimated based on the ratio between the mean values obtained for pollen and ovule numbers.

Results

Genetic Diversity

The dissimilarity matrix revealed that the diversity among pairs of accessions ranged from 0.59 to 4.73. Accessions 3 and 5 exhibited the smallest genetic distance, and accessions 1 and 20 were the most divergent (Table 1).

The Tocher clustering method allowed the division of the 24 accessions into five distinct groups (Table 2). Group I comprised the majority of the accessions (1, 2, 3, 4, 5, 6, 8, 9, 10, 16, 17, 18, 19, 21, 22, and 24). Group II (11, 12, 13, and 23) formed the second-largest cluster, followed by Group III, which included accessions 15 and 20. Groups IV and V were each composed of a single accession, 14 and 7, respectively.

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Group I was characterized by larger canopy diameters (50.05 cm) and a higher number of seeds per fruit (22.21). Group II showed the lowest seed number, producing an average of 17 seeds per pod. Group III presented the lowest mean plant height. Group IV exhibited the highest values for leaf length (13.26 cm) and leaf width (10.73 cm), but the shortest fruit length, averaging 1.36 cm per fruit. Group V encompassed the most vigorous plants, with the greatest plant height (61.00 cm), stem diameter (5.56 mm), number of flowers per inflorescence (3.66), flower diameter (9.51 mm), and fruit length (2.46 cm).

Mode of Reproduction

An average P/O ratio value of 9.45 was found for *Tarenaya diffusa*, when comparing this result with Cruden's indices, the value obtained for this species may be classified within the category of facultative cleistogamy, which may serve as an indication of the reproductive system of these plants.

Discussion

Genetic Diversity

Diversity was observed among pairs of *Tarenaya diffusa* accessions (Table 1). In other studies on the diversity within the Cleomaceae family, Muhammad *et al.* (2018) also reported a wide range of variation among accessions based on morphological traits, particularly leaf length and width, as well as flower dimensions. The Euclidean distances between accessions further indicate the presence of substantial diversity among them (Neitzke et al., 2010).

Five distinct groups were formed using Tocher's clustering method among the 24 evaluated accessions (Table 2). The clustering observed in this study demonstrates the broad genetic diversity among *Tarenaya diffusa* accessions. Since, in general, the Cleomaceae family exhibits considerable variation, ranging from differences in photosynthetic metabolism to morphological traits, this diversity may be related to the evolutionary history of specific groups within the family (Parma *et al*., 2022).

In general, plant height ranged from 39.2 to 61 cm, with group III, composed of accessions 15 and 20, exhibiting the shortest plants compared to the other groups

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(Table 2). In studies conducted by Shilla *et al*. (2019), *T. houtteana* was characterized by an average height of approximately 150 cm; however, the same species was highlighted in the work of Parma *et al*. (2023), who, when evaluating morphological characteristics such as flower size, observed relatively larger flowers compared with those of the species analyzed in the present study. Considering the requirements of the ornamental plant market and the potential of the *Tarenaya diffusa* evaluated here in terms of plant height, along with the floral potential of *T. houtteana*, hybridization between the two species would represent a viable strategy for developing *Tarenaya* hybrids with high ornamental potential, combining shorter stature with larger flowers, since, according to Plaschil *et al.* (2021), genotypes belonging to distinct groups should be selected as parents in hybridization-based breeding programs.

Accessions 7, 15, and 20, belonging to Groups III and V, are recommended for ornamental purposes due to their higher averages for the number of flowers per inflorescence and flower diameter (Table 2), since plants with larger flowers and petals, as well as attractive colors, can be used for ornamental purposes (Souza & Lorenzi, 2012; Soares Neto *et al*., 2019; Plaschil *et al*., 2021). Onyango *et al*. (2013), Wu *et al*. (2018), and Das *et al*. (2022), in their morphological studies on Cleomaceae, also reported great diversity among species; these morphological traits, particularly leaf and flower structure, are key in distinguishing closely related species within the same genus of the Cleomaceae family.

The study of intraspecific genetic diversity through morphological descriptors is crucial for germplasm bank characterization, as it provides valuable insights for genetic improvement programs through selection and hybridization aimed at developing varieties with specific desirable traits (Wasonga *et al*., 2015).

Mode of Reproduction

The P/O ratio of *Tarenaya diffusa*, according to Cruden's indices, suggests a facultative cleistogamous reproductive system. The P/O ratio has been widely used to determine the mode of reproduction. In studies conducted by Souza & Maués (2003) on the floral biology of four species belonging to the family Meliaceae, with emphasis on floral morphology and the pollen/ovule ratio, it was observed that the results obtained for all

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species, when compared with Cruden's indices, were classified into the categories of facultative autogamy and facultative xenogamy. These studies demonstrate the great importance of the pollen/ovule ratio as a tool in plant breeding, since understanding the reproductive system through the quantification of reproductive organs helps to define crossing and selection strategies within a short period and at low cost, directly impacting the efficiency and success of breeding programs, especially in native species under initial study, such as *Tarenaya diffusa*.

On the other hand, Harder & Johnson (2023), studying species from the families Convolvulaceae, Polemoniaceae, and Plantaginaceae, concluded that the P/O ratio provides ambiguous information about the relative production of female and male gametophytes and their evolution, thus recommending its discontinuation. However, in the present study, the P/O ratio proved to be an efficient parameter for determining the reproductive system in *Tarenaya diffusa*, as it allowed its classification without environmental interference, an advantage in breeding programs. Although there is coherence in the arguments presented by Harder & Johnson (2023), abandoning the P/O methodology would not be an appropriate solution. Therefore, it is recommended that the P/O ratio continue to be used as one of the techniques to obtain a comprehensive and accurate understanding of reproductive capacities and breeding potential in other plant species.

In studies on floral biology characterization based on manual crosses and the number of seeds produced in 10 species of Cleomaceae, Parma *et al*. (2023) described *Tarenaya diffusa* as presenting a reproductive system mainly characterized by autogamy, corroborating the findings of the present study and demonstrating that classification based on the pollen/ovule ratio allows a more precise characterization of the reproductive system, with low interference from environmental factors.

One of the main limitations associated with facultative cleistogamy in this species is the production of interspecific and intraspecific hybrids, as in the previously mentioned cross between potential genotypes of *T. diffusa* and *T. houtteana* aimed at increasing ornamental potential, due to difficulties in pollination as well as incompatibility (Cheptou, 2026). However, traditional approaches such as the manual opening of floral buds before self-pollination and emasculation may constitute an alternative for

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obtaining hybrids with desirable traits, as has been reported for cleistogamous species such as tomato (Du *et al*., 2025).

Thus, it was concluded that there is genetic diversity among the accessions of *Tarenaya diffusa*. Accessions 7, 15, and 20, belonging to Groups III and V, are recommended for breeding programs with ornamental purposes. Regarding its reproductive system, *Tarenaya diffusa* is classified as facultatively cleistogamous.

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Authors' Contributions

António Fanuel Boa – Research conception, data collection, analysis and interpretation, manuscript preparation, and literature review. Mailson M. do Rêgo – Research conception, data analysis, and supervision of the study. Elizanilda R. do Rêgo – Research conception, data analysis, and supervision of the study. Aline C. Dantas – Data collection and organization. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available in the: https://doi.org/10.48331/SCIELODATA.V0WSW5

AI Usage Statement

During the preparation of this work the author(s) used ChatGPT (OpenAI) in order to assist in the English language translation. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication

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Table and Figure Captions

Figures:

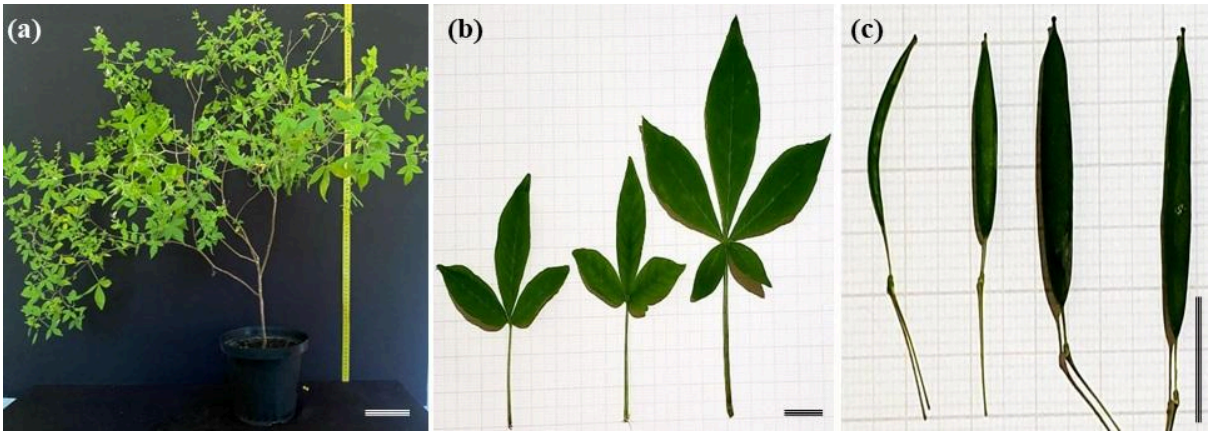


Figure 1. Morphology of *Tarenaya diffusa* (a) Adult plant of *Tarenaya diffusa*. Scale bar = 10 cm; (b) Leaf morphology of *Tarenaya diffusa*; (c) Fruit structure of *Tarenaya diffusa*. Scale bars = 1.0 cm in (b) and (c).

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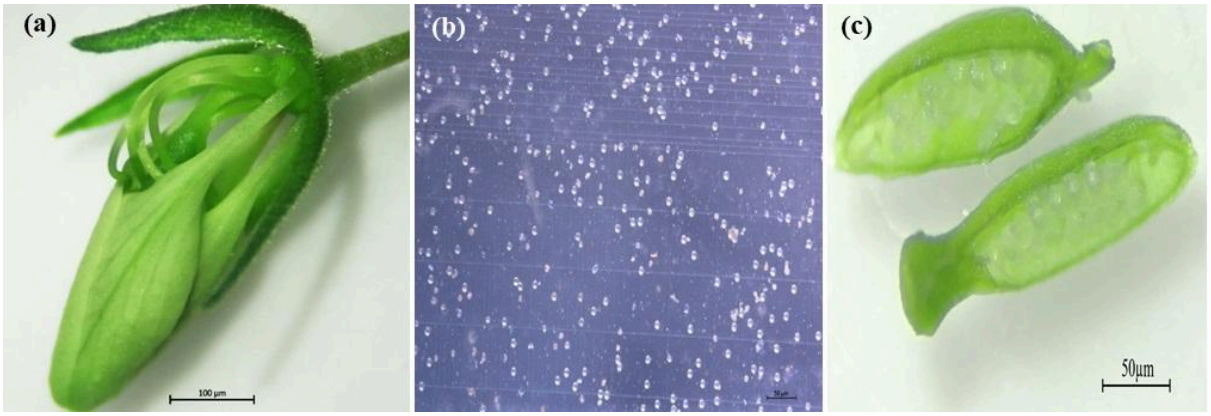


Figure 2. Determination of the reproductive mode of *Tarenaya diffusa* (a) *Tarenaya diffusa* flower at pre-anthesis stage; (b) Pollen grain counting using a Neubauer® chamber under an Axio Vert.A1® microscope with 0.4X objective; (c) Ovary structure of *Tarenaya diffusa*.

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Tables

Table 1. Dissimilarity matrix of 24 *Tarenaya diffusa* accessions based on Euclidean distance. Federal University of Paraíba, Center of Agricultural Sciences, 2025.

Accessions*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	-	0.95	1.30	2.04	1.62	1.26	2.23	3.18	1.03	2.21	1.40	2.33	2.32	2.19	3.19	2.34	1.49	2.19	1.46	4.73	2.35	1.86	2.90	1.77
2		-	1.385	2.17	1.46	0.68	1.43	2.61	1.44	1.99	1.40	1.87	2.44	2.44	2.91	1.35	0.75	1.93	1.52	3.38	2.29	1.81	2.38	1.92
3			-	1.586	0.59	1.39	1.79	2.17	1.54	1.23	1.60	0.92	1.78	3.22	2.75	1.76	0.98	1.39	1.35	2.77	1.96	1.74	1.30	1.51
4				-	1.30	1.88	2.52	1.18	1.21	1.35	2.25	2.17	1.98	3.42	3.00	1.90	1.28	1.38	1.37	2.81	1.66	0.86	2.10	1.46
5					-	1.36	1.27	0.93	1.59	0.63	1.20	1.37	1.41	2.87	2.72	1.61	0.97	0.74	0.72	2.49	1.16	1.18	0.92	0.97
6						-	0.95	2.21	0.73	1.12	1.75	1.09	1.93	1.91	1.94	0.94	0.86	2.35	1.58	2.42	1.78	1.489	2.32	2.09
7							-	1.927	1.53	1.21	2.24	1.67	3.03	3.23	2.74	1.87	1.08	2.38	2.03	3.68	2.96	1.98	3.16	2.67
8								-	2.48	1.25	2.89	2.64	2.24	3.64	2.75	2.05	1.66	0.99	0.95	2.59	1.79	1.06	2.29	1.19
9									-	1.19	2.51	1.99	2.46	3.02	3.39	1.99	1.12	2.51	1.78	3.74	2.09	1.28	3.06	2.10
10										-	1.91	1.03	2.20	3.68	2.99	1.81	1.12	1.70	1.61	2.44	1.45	1.37	1.49	1.65
11											-	1.82	1.96	2.42	2.70	1.75	1.82	1.93	1.66	3.32	1.98	2.40	1.22	2.05
12												-	1.94	2.75	2.35	1.99	1.60	2.88	2.53	2.45	2.18	2.33	1.47	2.45
13													-	1.86	2.10	2.03	2.49	2.02	1.62	2.53	1.07	1.47	1.28	1.56
14														-	2.34	2.88	2.95	3.60	2.86	3.89	1.84	2.33	3.29	2.83
15															-	1.01	2.62	2.40	2.01	1.71	2.80	2.01	3.09	2.35
16																-	0.98	1.36	1.28	0.90	1.85	1.30	1.83	1.75
17																	-	1.06	1.56	2.19	1.52	0.89	1.92	1.62
18																		-	0.69	2.11	1.37	0.77	1.55	0.66
19																			-	2.57	1.80	1.04	1.85	0.47
20																				-	1.78	1.88	1.61	2.26
21																					-	0.80	1.00	1.25
22																						-	1.94	0.62
23																							-	1.48
24																								-

*Accessions of *Tarenaya diffusa* from TUFPB001 to TUFPB024.

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Table 2. Tocher clustering of 24 *Tarenaya diffusa* accessions based on genetic dissimilarity among 14 evaluated morphological descriptors. Means, minimum and maximum values. Federal University of Paraíba, Center of Agricultural Sciences, 2025.

Groups	Accessions		PH	SD	CD	NL	LL	LW	NFI	FD	NA	NPe	FrL	FrD	FrW	NS
I	1, 2, 3, 4, 5, 6, 8, 9, 10,16, 17, 18,19, 21, 22 e 24.	Mean	56.96	4.50	50.05	3.83	9.93	7.49	2.60	7.37	5.54	3.85	2.21	3.21	0.14	22.21
		Minimum	44.50	3.80	39.50	3.00	9.13	5.40	1.66	5.47	4.67	3.67	1.53	1.84	0.10	19.33
		Maximum	63.20	5.70	72.40	5.00	10.90	9.40	3.70	9.87	6.00	4.00	2.67	4.87	0.18	25.67
II	11, 12, 13 e 23	Mean	51.85	4.40	48.25	4.33	10.07	8.78	2.08	8.19	5.41	3.91	2.00	3.58	0.14	16.50
		Minimum	48.50	3.91	38.30	4.00	8.80	7.93	1.33	5.67	5.00	3.66	1.73	3.38	0.08	15.67
		Maximum	56.70	5.05	54.90	5.00	10.90	9.50	3.00	10.07	6.00	4.00	2.27	3.93	0.18	18.00
III	15 e 20	Mean	43.05	3.26	36.75	4.83	10.71	8.95	2.00	8.25	5.50	3.50	2.30	2.38	0.15	23.00
		Minimum	39.20	3.01	23.50	4.67	8.93	8.33	1.67	7.82	5.33	3.33	2.10	2.33	0.13	22.67
		Maximum	46.90	3.51	50.00	5.00	12.50	9.56	2.33	8.67	5.66	3.66	2.50	2.44	0.16	23.33
IV	14	Mean	54.90	4.11	44.20	4.33	13.26	10.73	2.33	4.97	5.00	4.00	1.36	1.92	0.11	20.66
V	7	Mean	61.00	5.56	49.0	4.33	11.56	8.33	3.66	9.51	6.00	4.00	2.46	1.93	0.15	22.00

PH – Plant height (cm); SD – Stem diameter (mm); CD – Canopy diameter (cm); NL – Number of leaflets; LL – Leaf length (cm); LW – Leaf width (cm); NFI – Number of flowers per inflorescence; FD – Flower diameter (mm); NA – Number of anthers; NPe – Number of petals; FrL – fruit length (cm); DFr – Fruit diameter (mm); FrW – fruit weight (g); NS – number of seeds.

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