

Biometrics and seed germination of *Eremanthus incanus* (Asteraceae): strategies for valorization, conservation, and cultivation

Biometría y germinación de semillas de *Eremanthus incanus* (Asteraceae): estrategias para la valorización, conservación y cultivo

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SUMMARY

Eremanthus incanus is a native tree of Brazil with economic, ecological, and social importance, but it faces risks due to predatory exploitation and a lack of research. The objective of this study is to evaluate the biometrics and seed germination of different mother trees in order to contribute to the valorization, conservation, and cultivation of the species. Phenotypically, 10 mother trees were selected from a Candeia population located in the Diamantina region of Minas Gerais, from which seeds resulting from open pollination were collected. For the biometric analysis, 100 seeds from each mother tree were randomly sampled, distributed on millimeter paper in four repetitions of 25 units each, and evaluated for weight, length, and width. The germination study began with disinfection and selection of seeds using the densimetric method. Subsequently, the material was placed in germination boxes containing germitest paper. The experiment was set up in BOD (Biochemical Oxygen Demand) germination chambers following a randomized block design, with ten mother trees and four repetitions of 25 seeds per plot. Germination was evaluated on alternate days, until a constant point was reached. Significant effects of *E. incanus* mother trees were observed on biometric variables as well as on the germination rate and germination speed index of the seeds. Understanding these effects advances knowledge about the species and its potentialities, contributing to the development of more efficient propagation and conservation strategies.

Keywords: forest seed, native species, candeia.

RESUMEN

Eremanthus incanus es un árbol nativo de Brasil con importancia económica, ecológica y social, pero enfrenta riesgos debido a la explotación predatoria y a la falta de investigaciones. El objetivo de este estudio es evaluar la biometría y la germinación de semillas de diferentes árboles madre, con el fin de contribuir a la valorización, conservación y cultivo de la especie. Fenotípicamente, se seleccionaron 10 árboles madres de una población de candeia ubicada en la región de Diamantina, Minas Gerais, de los cuales se recolectaron semillas resultantes de polinización abierta. Para el análisis biométrico, se muestrearon aleatoriamente 100 semillas de cada árbol madre, distribuidas sobre papel milimetrado en cuatro repeticiones de 25 unidades, y se evaluaron en cuanto a peso, longitud y ancho. Para el estudio de germinación, se inició con la desinfección y selección de semillas mediante el método densimétrico. Posteriormente, el material se colocó en cajas de germinación con papel germitest. El experimento se instaló en cámaras de germinación DBO (Demanda Bioquímica de Oxígeno) siguiendo un diseño de bloques al azar, con diez árboles madre y cuatro repeticiones de 25 semillas por parcela. La germinación se evaluó en días alternos hasta alcanzar un punto constante. Se observaron efectos significativos de las madres de *E. incanus* en las variables biométricas, así como en la tasa y el índice de velocidad de germinación de las semillas. La comprensión de estos efectos avanza el conocimiento sobre la especie y sus potencialidades, contribuyendo al desarrollo de estrategias de propagación y conservación más eficientes.

Palabras clave: semilla forestal, especie nativa, candeia.

INTRODUCTION

Biodiversity conservation is a pressing global concern, particularly in regions such as the Brazilian Cerrado. Recognized for its exuberant biological richness, this ecosystem faces growing threats that demand effective action to ensure its protection (Rocha *et al.* 2020, Rodrigues *et al.* 2022).

Among the treasures inhabiting the Cerrado, *Eremanthus incanus* (Less.) Less stands out. Popularly known as candeia, this forest species is part of the Asteraceae family and is widely distributed in the campos rupestres of the Espinhaço Range in the states of Minas Gerais and Bahia (Rocha *et al.* 2020). Its economic value is widely recognized because of its natural durability, which is widely used in the manufacture of fence posts (Arriel *et al.* 2023). In addition, it is also a source of essential oil, the active ingredient of which is alphasabolol, with applications in the cosmetic and pharmaceutical industries (Gimenes *et al.* 2018). Because of its ability to develop in shallow and stony soils, *E. incanus* plays a crucial ecological role in restoring degraded ecosystems (Gomes *et al.* 2015). Its adaptation to adverse conditions contributes significantly to the regeneration of areas affected by environmental degradation, helping protect and conserve local biodiversity.

However, the challenges faced by this species are numerous, and in many cases, obtaining candeia products is the result of the predatory exploitation of native candeias that, at best, is supported by management plans. According to information from the Botanic Gardens Conservation International (BGCI) and the International Union for Conservation of Nature (IUCN), the species is on the Red List of Threatened Species, classified as Least Concern (LC) (Global Tree Specialist Group 2019). In addition, the species was classified as endangered by the flora of Minas Gerais. Although there is still insufficient information to definitively categorize it as threatened, there are indications that may eventually endorse this condition (Arriel *et al.* 2023), especially when we consider the rapid pace of degradation and fragmentation of the Cerrado.

Among the main aspects that underlie the sustainable management of native forest species, biometrics and seed germination stand out. Obtaining these data, in addition to optimizing restoration and conservation efforts, significantly contributes to promoting the domestication and long-term sustainability of forest genetic resources (Nunes *et al.* 2021).

Seeds are the main propagation vehicles of tree species and mark the beginning of the next generation (Roveri Neto and Paula 2017). In candeia trees, there is high production of seeds of reduced size and without dormancy, which fall into the category of orthodox and, therefore, can be stored for long periods under conditions of low temperature and humidity (Nery *et al.* 2014). It is important to highlight that within the same species, individuals can present different patterns in relation to seed size and ger-

mination capacity (Pereira *et al.* 2017). To better understand these patterns, it is essential to conduct studies at the mother-tree level.

Research of this nature is still incipient in *E. incanus*. Biometric analysis, in addition to detecting the existence of variability within the population and helping to understand the components of this variation (Gonçalves *et al.* 2013), provides insights into aspects of dispersion, seedling establishment, and ecological succession dynamics (Monteiro *et al.* 2016). Additionally, germination tests are useful for understanding the reproductive strategies of the species, distinguishing the physiological quality of seeds produced by different mother trees for propagation and seedling production purposes, and determining the variability present in the population (Roveri Neto and Paula 2017).

Therefore, the objective of this study is to evaluate the biometrics and germination of *E. incanus* seeds from different mother trees to contribute to the valorization, conservation, and cultivation of the species.

METHODS

Study area. This study was conducted in a campo rupestre ecosystem in the municipality of Diamantina, Minas Gerais (figure 1). It is a site undergoing restoration, which, until 2002, served as a solid waste disposal site for the city (Maravilha *et al.* 2023). With approximately 2.15 hectares, the area is home to a population of approximately 350 individuals of *E. incanus* (Santos *et al.* 2017), and is located at coordinates 18° 12' 17" S and 43° 34' 08" W, on the Juscelino Kubitschek Campus (JK) of the Federal University of the Vales do Jequitinhonha e Mucuri (UFVJM), at an elevation of approximately 1,400 m a.s.l. (Maravilha *et al.* 2023).

The predominant soils are litholic neosols and cambisols, associated with the quartzitic and phyllitic rocks of the Espinhaço Supergroup, characterized by low fertility. The terrain ranges from flat to gently undulating, although slopes can reach up to 60° in some places (França *et al.* 2018). According to the Köppen classification, the climate in the region is of the Cwb type, typically tropical, with mild and humid summers between October and April and cooler and drier winters between June and August. The average annual temperature is approximately 18 °C and 19 °C, and the average annual precipitation ranges from 1,250 to 1,550 mm (Alvares *et al.* 2013).

Selection of mother trees, collection and processing of seeds. Ten *E. incanus* mother trees were selected based on phenotypic criteria, such as vigor, good phytosanitary conditions, seed production, and cylindrical trunk (figure 2). The limited number of trees was determined for practical reasons, considering the time, resources, and staffing constraints. In October 2018, fruit collection occurred at the end of the fruiting period (figure 3A). Fully developed fruits, resulting from open pollination and ready for seed



Figure 1. Campo Rupestre area in Diamantina, Minas Gerais, Brazil, where the population of *Eremanthus incanus* is found.
Área de Campo Rupestre en Diamantina, Minas Gerais, Brasil, donde se encuentra la población de *Eremanthus incanus*.

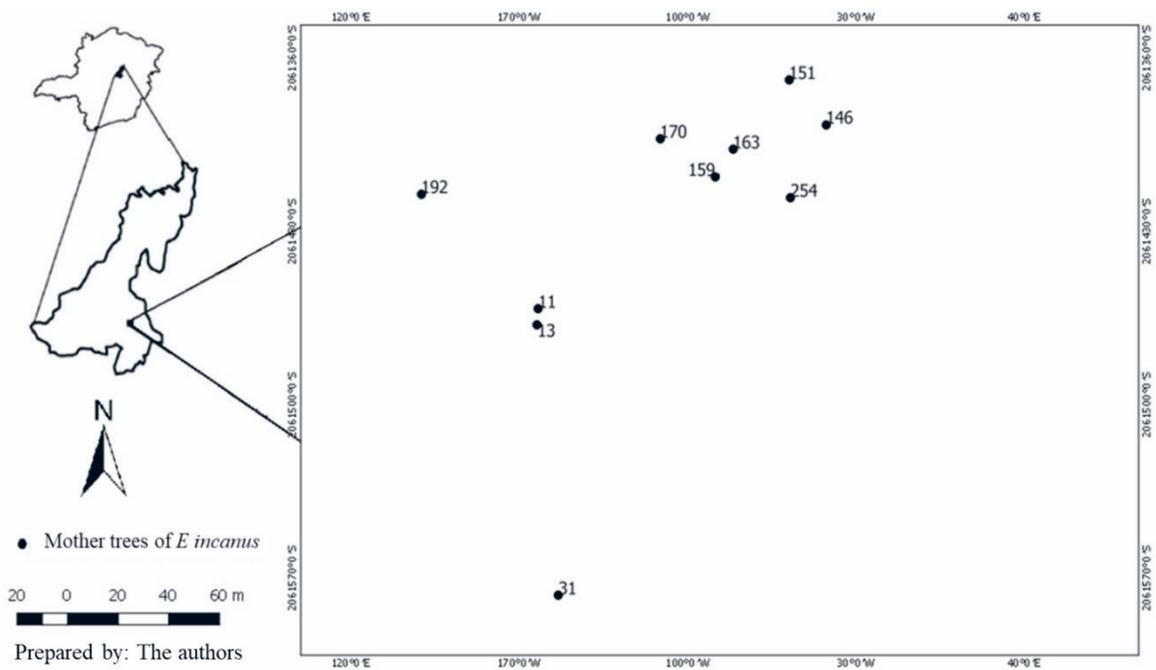


Figure 2. Spatial distribution of mother trees of *Eremanthus incanus* in Diamantina, Minas Gerais.
Distribución espacial de árboles madres de *Eremanthus incanus* en Diamantina, Minas Gerais.

dispersal, were collected. The collection was carried out simultaneously from all mother trees, specifically in the lower part of the canopy.

Once the collection was completed, the material was transported to the Integrated Center for Propagation of Forest Species (CIPEF), part of the Department of Forestry Engineering (DEF), at the JK Campus of UFVJM. At CIPEF, the seeds were manually processed using a fine-mesh sieve to macerate the capitula (figure 3B) and a blower to remove impurities (figure 3C). The newly collected and processed seeds (figure 3D), without any prior storage, were then used for biometric and germination experiments.

The botanical material of the species comprises the database of the Jeanine Felfili Dendrological Herbarium (HDJF-UFVJM), whose specimen has registration HDJF - 3988.

Seed biometrics. A random sampling approach was adopted to perform biometric measurements, consisting of 100 seeds for each studied mother tree. The seeds were manually distributed on millimeter paper in four repetitions of 25 units each (figure 4).

Images were captured using a digital camera to determine the length and width of seeds. For length, measurements were taken from the base to the apex, whereas the width was measured in the middle region. All measurements were processed using the Anati Quanti 2.0 software (Aguar *et al.* 2007). Additionally, the individual weights of 100 seeds were determined using an analytical balance with an accuracy of 0.0001 g.

Seed germination. Initially, the seeds from each mother tree were disinfected using the fungicide Cuprocarb 500® at a concentration of 1 g L⁻¹ for 15 min (figure 5A). Subse-



Figure 3. Collection and processing process of *Eremanthus incanus* seeds. A) Fruit collection directly from the mother trees, B) Maceration of the capitula using a sieve, C) Blower for impurity removal, D) Processed seeds ready for use.

Proceso de recolección y procesamiento de semillas de *Eremanthus incanus*. A) Recolección de frutos directamente de los árboles madres, B) Maceración de los capítulos utilizando un tamiz, C) Soplador para la eliminación de impurezas, D) Semillas procesadas listas para su uso.

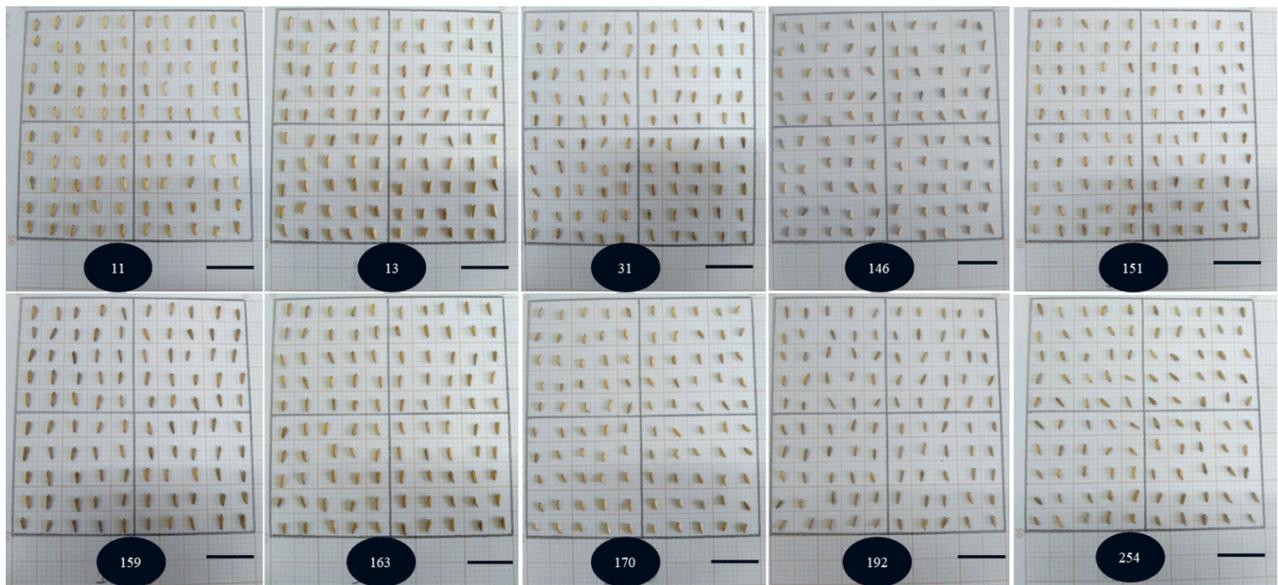


Figure 4. Seeds of *Eremanthus incanus* originating from ten mother trees, photographed on graph paper for subsequent measurement using Image-Pro Plus® software. Bars = 1 cm.

Semillas de *Eremanthus incanus* procedentes de diez árboles madres, fotografiadas en papel milimetrado para su posterior medición utilizando el software Image-Pro Plus®. Barras = 1 cm.

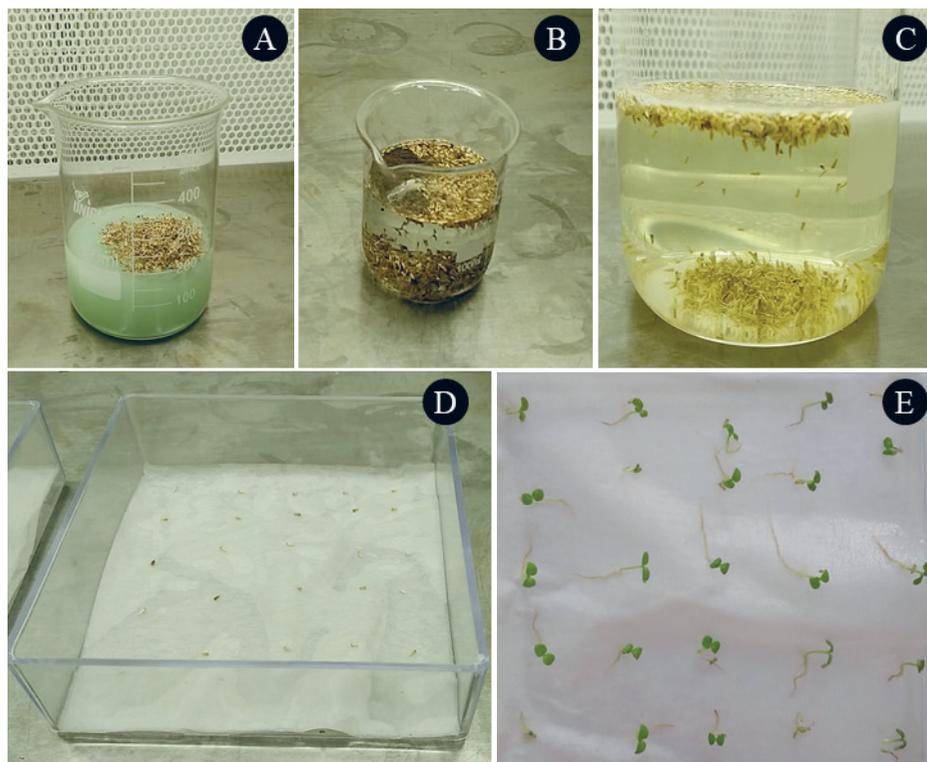


Figure 5. Disinfection of *Eremanthus incanus* seeds in fungicide (A), 70 % alcohol (B), and 2.5 % sodium hypochlorite (C). Seeds arranged in a gerbox (D) and observed after germination (E).

Desinfección de semillas de *Eremanthus incanus* en fungicida (A), alcohol al 70 % (B) e hipoclorito de sodio al 2,5 % (C). Semillas dispuestas en una gerbox (D) y observadas después de la germinación (E).

quently, three rinses were performed using autoclaved distilled water, and the seeds were transferred to 70 % alcohol solution for 30 s (figure 5B). After this step, the seeds were immersed in a 2.5 % active chlorine sodium hypochlorite solution with five drops of commercial detergent Tween 20 for 20 min for every 100 mL of solution (figure 5C). During this stage, seeds were selected using the densimetric method, keeping only those that were submerged. Finally, six rinses of the seeds were performed with autoclaved distilled water, completing the disinfestation and preparation of the seeds.

After disinfestation, seeds from each mother tree were distributed onto two previously sterilized germitest paper sheets, moistened with distilled water, and placed in acrylic germination boxes sterilized with 70 % alcohol (figure 5D). The germination boxes were kept in *Biochemical Oxygen Demand* (BOD) germination chambers at an alternating temperature of 20-30 °C and a photoperiod of 10 h of light and 14 h of darkness (Tonetti *et al.* 2006). During the light periods, the temperature was set to 30 °C, and during the dark periods, it was set to 20 °C.

The experiment was set up in randomized blocks with 10 treatments (mother trees) and four repetitions (BOD incubators), each containing 25 seeds. The number of germinated seeds was evaluated on alternate days, starting from the first day after test installation, using the criterion of primary root protrusion (figure 5E). When necessary, the germitest paper was moistened with distilled water to maintain a favorable humidity environment. The assessments were concluded when the germination rate remained constant. The following variables were calculated from the

collected data: germination percentage and Germination Speed Index (GSI), the latter being calculated according to the formula presented by Maguire (1962).

Data analysis. Initially, the collected data were subjected to the Shapiro-Wilk test to check for normality of residuals and the Bartlett test to assess homogeneity of variances. Once the assumptions were met, an analysis of variance (ANOVA) was conducted. In cases where significant differences between treatments were detected, means were grouped using the Scott-Knott test at a significance level of 5 %. Analyses were performed using R software (R Core Team 2021) with the assistance of the ExpDes.pt package, version 1.2.0 (Ferreira *et al.* 2021).

RESULTS

Seed biometrics. Statistically significant differences ($P < 0.05$) were observed among the mother trees for all the evaluated biometric characteristics (table 1). The seeds showed an average length ranging from 1.89 (mother tree 151) to 2.47 mm (mother tree 11), average width ranging from 0.55 (mother tree 192) to 0.81 mm (mother tree 11), and an average weight ranging from 0.0051 (mother tree 146) to 0.0113 g (mother tree 159). For seed length and weight, five groups were formed, and for width, the mother trees were separated into four groups.

Seed germination. The germination of *E. incanus* seeds began on the third day after the installation of the test for 90 % of the mother trees. Except for mother tree 192, which star-

Table 1. Average values of the characteristics length, width, and weight of seeds from ten *Eremanthus incanus* mother trees.

Valores promedio de las características longitud, ancho y peso de las semillas de diez árboles madres de *Eremanthus incanus*.

Mother trees	Length (mm)	Width (mm)	Weight (g)
11	2.47 a	0.81 a	0.0101 b
159	2.44 a	0.69 b	0.0113 a
13	2.43 a	0.72 b	0.0112 a
163	2.35 b	0.72 b	0.0072 d
31	2.14 c	0.64 c	0.0062 e
254	2.07 c	0.74 b	0.0102 b
146	2.06 c	0.69 b	0.0051 e
192	1.99 d	0.55 d	0.0088 c
170	1.93 e	0.64 c	0.0082 d
151	1.89 e	0.60 c	0.0060 e
CV (%)	2.62	4.84	8.99

Averages followed by the same letter in the column do not differ from each other according to the Scott-Knott test at 5 % significance. CV (%): experimental coefficient of variation.

Las medias seguidas por la misma letra en la columna no difieren entre sí según la prueba de Scott-Knott al 5 % de significancia. CV (%): Coeficiente de variación experimental.

ted the germination process on the fifth day, all other mother trees had at least one germinated seed. Over the evaluation period, mother tree 11 stood out in relation to the others, both in relation to the initial and final germination rates, as well as the time elapsed to reach maximum germination. In just seven days, a germination rate of 96 % was observed for the seeds from this mother tree. Germination lasted up to nine days for mother tree 159, 11 days for mother trees 146 and 254, 13 days for mother trees 13, 151, and 163, and 15 days for mother trees 31, 170, and 192 (figure 6).

At the end of 17 days, when germination counts were concluded, significant differences ($P < 0.05$) were observed among the mother trees. In the set of 10 mother trees studied, the average germination rate was 64.8 %, and according to the Scott-Knott test (figure 7), three groups were formed. Mother trees 11, 13, 163, and 146 comprised the group with the highest averages, with germination values ranging from 81 % to 96 %. The intermediate group was composed of 31, 170, 192, and 254 mother trees, with averages ranging between 58 % and 71 %. Finally, mother trees

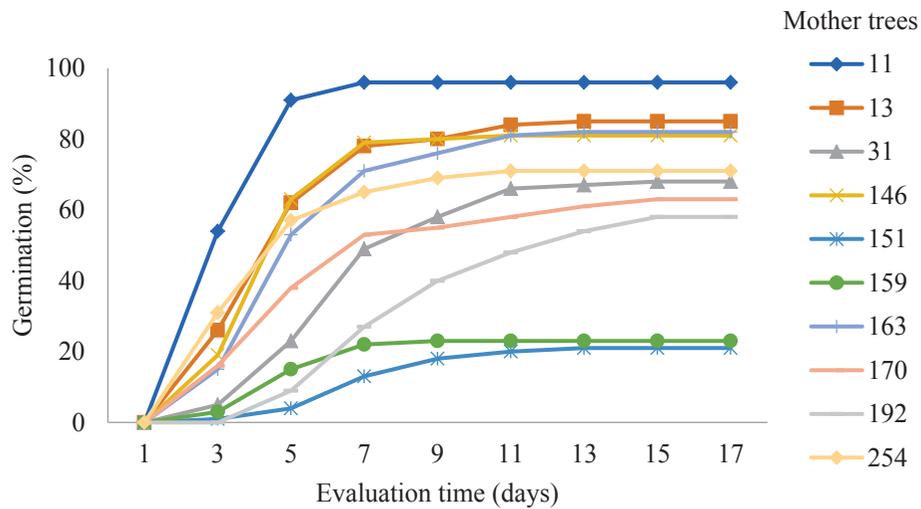


Figure 6. Percentage of seed germination of ten *Eremanthus incanus* mother trees, during 17 days.

Porcentaje de germinación de semillas de diez árboles madres de *Eremanthus incanus* durante 17 días.

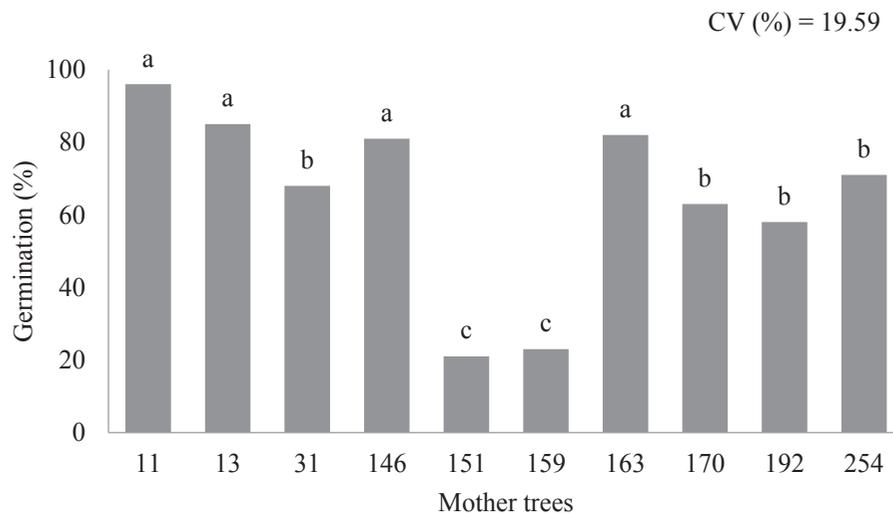


Figure 7. Percentage of seed germination of ten *Eremanthus incanus* mother trees, 17 days after the installation of the test. Averages followed by the same letter do not differ from each other by the Scott-Knott test at 5 % significance. CV (%): Experimental coefficient of variation.

Porcentaje de germinación de semillas de diez árboles madres de *Eremanthus incanus*, 17 días después de la instalación del test. Medias seguidas por la misma letra no difieren entre sí según la prueba de Scott-Knott al 5 % de significancia. CV (%): Coeficiente de variación experimental.

151 and 159 formed the group with the lowest averages, presenting only 21 % and 23 % germination, respectively.

Regarding the Germination Speed Index (GSI), the mother trees were classified into four groups (figure 8). Mother tree 11 stood out as an isolated group with the highest average (6.5). The second group comprised mother trees 13, 146, 163, and 254, whose GSI values varied between 4.1 and 4.7. The third group was formed by mother trees 31 and 170, with mean GSI of 2.7 and 3.2, respectively. The fourth group included mother trees 151, 159, and 192, with a mean GSI ranging between 0.8 and 1.8.

DISCUSSION

During the maturation process, the seeds undergo complex development that involves both growth and accumulation of reserves in order to reach the characteristic size and mass of the species (Sripathy and Groot 2023). This process is critical to ensure that the seed is ready to germinate and establish a new plant. However, it is essential to highlight that the size and mass of seeds can vary considerably between individuals of the same species in different years of collection, as well as within the same individual (Bispo *et al.* 2017, Leão *et al.* 2018).

In the present study, the separation of *E. incanus* mother trees into distinct groups based on the evaluated biometric characteristics highlights the complexity and adaptation of seeds to environmental conditions, availability of resources, and genetic factors that surround them (Rosa *et al.* 2019). In addition, the mother trees selected in this study are naturally regenerated, and there is no information about their age, which may partly explain the phenotypic variation found (Souza *et al.* 2015).

With regard to germination, in the Rules for Seed Analysis (RAS) There are no test specifications for candeia species in the Rules for Seed Analysis. Thus, the temperature and photoperiod in which the BODs were regulated are in agreement with Tonetti *et al.* (2006), who found that 10 h of light at 30 °C and 14 h of darkness at 20 °C are the ideal conditions for seed germination of *Eremanthus erythropappus* (DC.) Macleish, a species closely related to *E. incanus* at the genus level. In general, seeds of tree species native to Brazil have optimal germination temperatures between 20 °C and 30 °C, which may vary according to the thermal conditions found in their region of origin (Galindo *et al.* 2012). In addition, species that have not undergone intense domestication have a higher germination rate when exposed to alternating temperatures (Mondo *et al.* 2010).

The beginning of radicle protrusion (3rd day after installation of the test) and the time elapsed until germination reached a constant value (17 d) were similar to the results obtained by Tonetti *et al.* (2006). The observed variation in the final percentage of germination (21-96 %) reinforces the influence of genetic and environmental factors. One possible reason for the low production of viable seeds in the Asteraceae family is the presence of inflorescences with multiple capitula and several flowers per capitula, which can lead to a scarcity of maternal resources, resulting in the abortion of ovules and seeds (Marzinek and Oliveira 2019). This redirection or cancellation of maternal investment can be interpreted as an adaptive strategy in response to the limits imposed by the available resources (Lloyd 1980). Another relevant factor to consider is the possibility of self-incompatibility in species of the genus *Eremanthus* (Velten

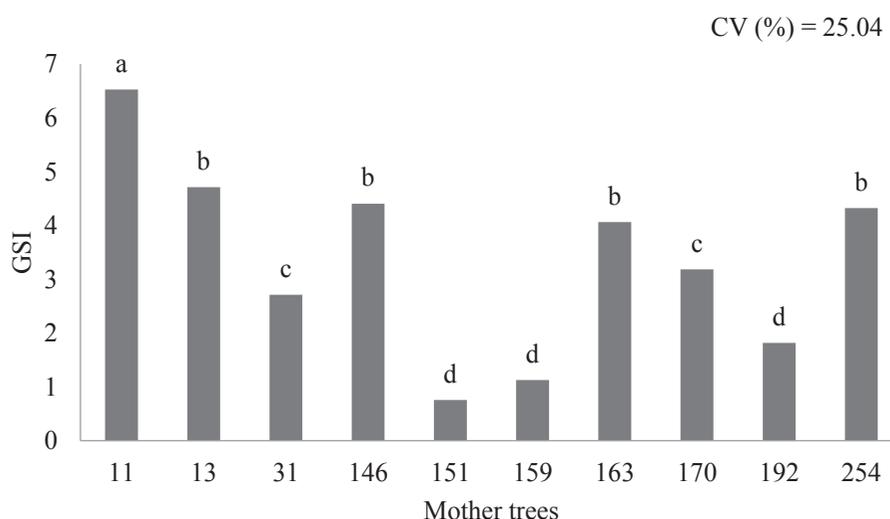


Figure 8. Germination Speed Index (GSI) of the seeds from ten *Eremanthus incanus* mother trees. Averages followed by the same letter do not differ from each other by the Scott-Knott test at 5 % significance. CV (%): Experimental coefficient of variation.

Índice de Velocidad de Germinación (IVG) de las semillas de diez árboles madres de *Eremanthus incanus*. Medias seguidas por la misma letra no difieren entre sí según la prueba de Scott-Knott al 5 % de significancia. CV (%): Coeficiente de variación experimental.

and Garcia 2005). This characteristic can interfere with successful fertilization and, consequently, seed formation, contributing to the low viability observed in some mother trees.

Our study investigated the phenotype of a small and geographically limited population in which the mother trees were close to each other. This proximity can lead to reproductive challenges such as limited cross-pollination, resulting in increased inbreeding and, consequently, reduced genetic diversity, seed viability, and vigor (Price *et al.* 2021). Considering that the environment in question is in the process of recovery, the area where *E. incanus* seeds are collected can influence the routes of dispersers and pollinators, directly affecting the reproduction of the species (Meira Junior *et al.* 2017, Santos *et al.* 2019).

The final percentage of germination and Germination Speed Index (GSI), although they are different measures, are related and provide valuable information about seed quality. In general, the mother trees with the highest final germination percentages also obtained the highest GSI values. This result suggests a strong correlation between the speed at which seeds initiate germination and their effective germination capacity. Fast germination is a crucial indicator of seed vigor, and the higher the GSI, the greater the probability that the seedling will be more successful in its initial development, and consequently, in its survival and later growth (Marcos-Filho 2015).

Our findings contribute significantly to our understanding of the complexities involved in the development and reproduction of *E. incanus* seeds. This information has broad practical potential and can be applied in various ways to establish strategies for valorization, conservation, and cultivation of the species, as well as to guide future research in the field of seed technology and production of seedlings of native forest species, especially those found in the Campos Rupestres of the Cerrado biome.

One practical strategy derived from these findings is the careful selection of mother plants, considering biometric characteristics and performance in germination, which can serve as a starting point for genetic breeding programs and production of high-quality seedlings (Pimenta *et al.* 2023, Mustafa *et al.* 2024) Furthermore, exploring methods that can enhance the growth and survival of *E. incanus* seedlings, especially under adverse conditions both in greenhouse and field settings, is a promising approach. One such approach is the utilization of mutualistic symbionts such as arbuscular mycorrhizal fungi (AMF). Previous studies, such as that of Pagano *et al.* (2010), emphasize the importance of AMF in Brazil's high-altitude vegetation and suggest that restoration programs using *E. incanus* may be facilitated by inoculating these microorganisms.

Additionally, maintaining germplasm banks and restoring degraded areas with the species can significantly contribute to the conservation of genetic diversity and restoration of ecosystem services (Maravilha *et al.* 2023,

Mustafa *et al.* 2024). Environmental education also plays a crucial role in this context by promoting awareness of the importance of conserving *E. incanus* and fostering synergy among governmental bodies, local communities, researchers, and industries in initiatives to valorize and cultivate the species (Ardoin *et al.* 2020).

CONCLUSIONS

The mother trees of *E. incanus* exerted significant effects on the biometric variables, seed germination rate, and Germination Speed Index. Understanding these effects represents a relevant first step in understanding the species and its potential, with important implications for its conservation and sustainable use. It is noteworthy that there are still considerable gaps in our understanding of these processes, especially regarding variability among populations from different geographical regions. Therefore, it is fundamental that future research explore the biometric characteristics and seed germination in specimens from other locations.

AUTHOR CONTRIBUTIONS

LFM: Conceptualization, Formal analysis, Investigation, Methodology, Writing - first draft, Writing - review and editing; MT: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing - review and editing; DPM: Writing - review and editing; NAM: Writing - review and editing; JSCF: Writing - review and editing; JFG: Writing - review and editing.

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