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Original Research

Effect of Mechanical, Chemical and Physical Scarification on the Germination of Brazil Nut Seeds (*Bertholletia excelsa* Bonpl.) in Peru

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ABSTRACT

Bertholletia excelsa Bonpl. (Brazil nut) is an economically important species in the Amazon, whose natural seed production takes between 365 and 545 days, also showing low germination rates. With the aim of reducing the production time of germinated Brazil nut seeds and to optimize the variables associated with the germination process, one factor was evaluatedtype of seed scarification; resulting in one control and four treatments. Fifty seeds per replication and 200 seeds per treatment were evaluated, all sourced from the same Brazil nut plantation, with 10 days of storage. The results revealed that the seeds with complete removal of the seed coat began germination

23 days after sowing and, after 187 days, showed the highest germination potential (96%), germination energy (92.2%), germination speed coefficient (0.49), mean germination time (118 days), and germination rate (0.31). The findings of the study could be used to reduce the germination time of B. excelsa seeds and increase their germination potential. The results obtained on the quality and germination process of Bertholletia excelsa (Amazon nut) seeds allow for more efficient management practices in forest nurseries and reforestation programs, prioritizing seedhead removal as an essential treatment to ensure homogeneous production, with higher values of potential, energy and germination rate, as well as an adequate average germination time.

INTRODUCTION

Bertholletia excelsa Bonpl. (Lecythidaceae), commonly known as "Brazil nut," is a native species of great economic importance in the Amazon region. It is recommended for both reforestation programs in degraded areas and forest plantations, due to its production of multiple products such as timber, seeds, and oil (Costa *et al*., 2009; Santos *et al*., 2017; de Souza *et al*., 2023). It plays an essential role from social, economic, and environmental perspectives, being highly valued in silviculture, agroforestry systems, and the restoration of degraded areas (Da Costa *et al*., 2022). Specifically, the seeds of *B. excelsa* have become one of the most important non-timber forest products in the Amazon rainforest, mainly due to their nutritional benefits (Takeuchi and Egea, 2020; de Souza *et al*., 2022).

However, the seeds of *Bertholletia excelsa* exhibit physiological dormancy, with low, slow, and irregular germination rates (Larrea-Alcázar *et al*., 2018). In this context, the genetic makeup of the species is a determining factor in the morphology and physiology of the germination process (Gonçalves *et al*., 2024). Therefore, studies aimed at increasing seedling production through sexual reproduction of high-quality seedlings are essential for the management, reforestation, and conservation of the species (da Silva *et al*., 2022; Guimarães, de Lima and Ferreira, 2025), especially in the face of growing pressures from exploitation and ecosystem fragmentation within its natural distribution range (Bortolin *et al*., 2020). In this regard, poor germination represents a critical phase in the life cycle and is a limiting factor for ex situ conservation and large-scale cultivation (Thakur *et al*., 2025); it is influenced by factors such as dormancy level, light, temperature, substrate type, moisture, water stress, nitrate levels, light intensity, as well as the position and depth at which seeds are sown in the seedbed (Flórez-Martínez *et al*., 2024; Gomes *et al*., 2024; Otani, Zheng and Kawakami, 2024). Although dormancy is genetically determined, it is also influenced by the maternal environment before and after anthesis. Recent progress in molecular genetics and bioinformatics has greatly enhanced our understanding of the molecular mechanisms underlying seed dormancy and germination in model plants as well as in economically important crop species (Otani, Zheng and Kawakami, 2024).

Around the world, climate change-related abiotic stresses have been found to impact the growth and yield of various crop species. In response, many of these crops have developed different strategies to cope with stress factors like high temperatures and water shortages (Han et al., 2009). Specifically, increased temperatures have

been shown to affect seed germination by reducing viability and vigor, as well as hindering seedling establishment in herbaceous plants, such as rice (Han et al., 2009).

Therefore, the germination process and the morphological and physiological quality of seedlings in their initial stage-prior to being transplanted to the field depend largely on the origin and position of the seed within the fruit, the production methods used, the type of substrate, management during production, environmental conditions, as well as the equipment and infrastructure of the nursery (Delgado-Paredes *et al*., 2024). Consequently, this information is essential to maximize planting efficiency (Ticona-Arapa *et al*., 2024). In this context, the objective was to reduce the production time of germinated Brazil nut seeds and to optimize the variables associated with the germination process.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted at the Forest Seed Certification Laboratory of the National Agrarian University of the Jungle, Tingo María, Huánuco, Peru; coordinates 09°18'00" south latitude and 76°01'00" west longitude (Figure 1). According to data obtained from the José Abelardo Quiñones Meteorological Station located in the city of Tingo María, geographically situated at 9°18'54" south latitude and 75°59'40.2" west longitude the area has an average temperature of 24.3°C, an average annual rainfall of 3,200 mm, and an average relative humidity of 87%.

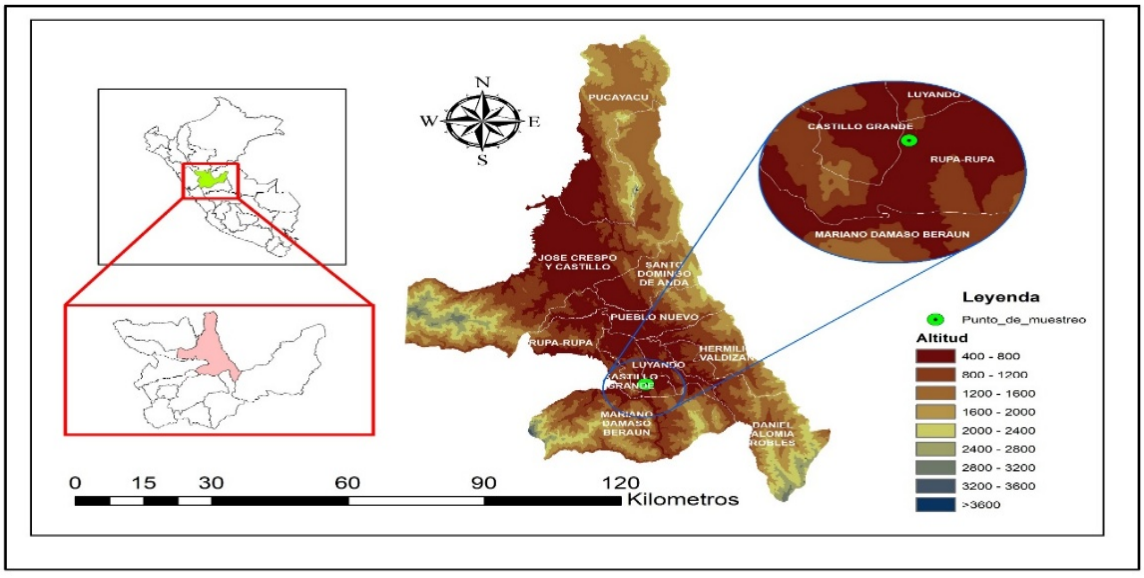

Fig. 1: Location of the city of Tingo María (Peru).

2.2. Seed collection

A batch of 3,000 seeds was obtained through direct harvesting from plantations in the city of Puerto Maldonado, Madre de Dios region, Peru. Additionally, the correct scientific name of the species was verified using a database provided by Tropicos (2022), which contains information on scientific names and the families to which plant species belong.

2.3. Evaluation of the physical quality of the seed lot

It was evaluated through the physical purity test, the determination of the weight of 1,000 seeds, and the moisture content, in accordance with the standards established by ISTA (2017). In addition, analysis of variance was performed, as well as the calculation of standard deviation and coefficient of variation to assess the consistency of the data.

The physical purity test was determined by manually separating pure seeds from impure ones, classifying them based on their physical integrity and morphological characteristics. Subsequently, both fractions were weighed using a Henkel BQ1001 digital analytical balance, in accordance with the procedures established by ISTA (2017). It was determined using Equation 1:

$$P = \left(\frac{TSW - IW}{TSW}\right) \times 100 \quad \ldots(1)$$

Where the variable P represents the percentage of purity; TSW indicates the total sample weight (g); and IW represents the impurity weight (g).

The weight of 1,000 seeds was determined from eight replicates of 100 pure seeds each. In each replicate, 100 seeds were weighed using a Henkel BQ1001 digital analytical balance. Then, the average weight of the eight replicates was calculated, and this value was multiplied by 10 to obtain the estimated weight of 1,000 seeds. It was determined using Equation 2:

$$\text{Weight of 1,000 seeds} = \sum \text{weight of 8 replicates} \quad \ldots(2)$$

The moisture content was determined using the oven-drying method with a Thermo Fisher Scientific F30428C device. For this, 30 seeds were selected and distributed into two replicates. The samples were subjected to drying for 17 ± 1 hours at a constant temperature of 103 ± 1 °C. After drying, the samples were cooled in a borosilicate glass desiccator (Brand™ 65043) and subsequently weighed using a Henkel BQ1001 digital analytical balance (capacity: 1 kg; precision: 0.01 g). The moisture content was calculated on a fresh weight basis by comparing the initial and final weights of the seeds. It was determined using Equation 3:

$$MC = \left(\frac{FW - DW}{FW}\right) \times 100 \quad \ldots(3)$$

Where MC represents the moisture content percentage; FW indicates the fresh weight (g); and DW indicates the dry weight (g).

2.4. Research design

A completely randomized design + 1 control was used, consisting of the proper arrangement of seeds for the germination process. Four treatments were evaluated: T_1: seed coat removal, T_2: scarification of the seed coat, T_3: immersion in sulfuric acid, and T_4: immersion in hot water, each with four replicates of 50 seeds each (Tab. 1 and Fig. 2).

Table 1: Pre-germination treatments applied to the seeds of *Bertholletia excelsa* Bonpl.

Treatments	Description

T_1	**Seed coat removal:** This consisted of removing the seed coat using a Kamasa 6" Universal plier, completely detaching the seed coat without causing damage to the endosperm.
T_2	**Seed coat perforation:** Approximately 10% of the seed coat was removed using a Kamasa 6" Universal plier by applying slight pressure to the middle part of the seed, causing the seed coat to crack without damaging the endosperm. This was done to allow the entry of oxygen and water, thereby promoting the initiation of the germination process.
T_3	**Chemical scarification:** The seeds were immersed in 100% sulfuric acid for 4 hours; both the seeds and the sulfuric acid were placed in glass containers, with a volume ratio of 2:1 (acid to seeds). The mixture was stirred to ensure the acid was evenly distributed over the seeds. Finally, the acid was removed, and the seeds were rinsed in water for 10 minutes.
T_4	**Hot water immersion:** The seeds were soaked in hot water for 5 minutes; however, the water was previously boiled and then cooled to 70°C, maintaining this temperature for 5 minutes. After imbibition, the water and seeds were allowed to cool, and the seeds remained soaked for a period of 12 hours.
T_5	No treatment

Fig. 2: a. Seed coat removal. b. Seed coat perforation. c. Immersion in sulfuric acid. d) Immersion in hot water .

The treated seeds, along with the untreated seeds (control), were placed under controlled conditions. It is important to note that 200 seeds were analyzed per treatment, with 4 replicates and 50 seeds per replicate. The seeds were placed under controlled conditions in germination chambers, which consisted of metal containers lined with cotton as a substrate. The containers were irrigated with distilled water every 24 hours to provide the

moisture required to induce the germination process (at temperatures of 25°C and relative humidity between 80% and 90%) under a short photoperiod. The substrate was kept saturated throughout the seven-month evaluation period. Additionally, the cotton was replaced every 30 days to keep the seeds free from pathogens during the evaluation period. The seeds were disinfected with Benomyl® and FujiOne® at 0.3% (3 g/L of water) in order to prevent the attack of microorganisms. Also the light condition was continuous (daily every 24 hours) using a florescent as an artificial source similar to sunlight.

2.5. Monitoring of germination variables

The monitoring of germination variables began 24 hours after sowing and continued for seven months, with the recording of five germination response variables. The monitoring of germination variables was carried out simultaneously, and a seed was considered germinated when the radicle emerged from the seed coat and began to elongate.

Germination potential is a variable expressed as a percentage that indicates the proportion of seeds that have germinated relative to the total number of seeds sown (Bewley and Black, 1995). It was determined using Equation 4:

$$\mathbf{GP} = \left(\frac{\mathbf{GS}}{\mathbf{SS}}\right) \times \mathbf{100} \quad \ldots(4)$$

Where GP represents the germination potential (%); GS indicates the total germinated seeds; and TS indicates the total sown seeds.

Germination energy is a variable that expresses the maximum number of seeds germinated within a given period (Pece *et al*., 2010). It was determined using Equation 5:

$$\mathrm{GE} = \left(\frac{\mathrm{MáxGS}}{\mathrm{GS}}\right) \times 100 \quad \ldots(5)$$

Where GE represents germination energy (%); MaxGS indicates the Maximum number of germinated seeds; and GS indicates the total germinated seeds.

The germination rate coefficient is the sum of the number of germinated seeds divided by the number of days evaluated (Maguire, 1962). It was determined using Equation 6:

$$\mathrm{GRC} = \sum\left(\frac{\mathrm{ni}}{\mathrm{ti}}\right) \quad \ldots(6)$$

Where GRC represents germination rate coefficient; ni indicates the number of seeds germinated on the *i*-th day; and ti indicates time in days for the germination process on the *i*-th day.

The mean germination time is a variable that refers to the average time required for seeds to complete the germination process (Kader, 2005). It was determined using Equation 7:

$$\mathrm{MGT} = \frac{\sum(\mathrm{ni}\times\mathrm{ti})}{\sum \mathrm{ni}} \quad \ldots(7)$$

Where MGT represents mean germination time; ni indicates the number of seeds germinated on the i-th day; and ti indicates time in days for the germination process on the i-th day.

Germination speed is a variable that relates the number of germinated seeds to the number of days the germination process lasted (González-Zertuche and Orozco-Segovia, 1996). It was determined using Equation 8:

$$GR = \sum \left(\frac{ni}{t}\right) \quad \ldots(8)$$

Where GR represents germination rate; ni indicates the number of seeds germinated on the i-th day; and t indicates time in days that the germination process lasted.

The independence between experimental units was verified to meet the requirements of the analysis of variance (ANOVA) (González *et al*., 2019). Differences in means between treatments were compared using Tukey's test ($p < 0.05$); the analysis was performed using R software, version 3.5.2.

3. RESULTS OR RESULTS AND DISCUSSIONS

3.1. Descriptive analysis of the seed lot quality

The purity of *B. excelsa* seeds was 94.3%. The mean weight of 1,000 seeds was 8,887.5 g, with a variance of 11.2, a standard deviation of 3.4, and a coefficient of variation of 0.4. The samples recorded an average moisture content of 24.7%.

3.2. Analysis of the variables of the germination process

According to the Kolmogorov–Smirnov test, all collected data showed a normal distribution ($p > 0.05$). The statistical analysis reported a highly significant difference between treatments (Tab. 2). The experimental model was highly adequate, with high R^2 values for all variables. The low coefficients of variation in GP, GE, MGT, and GS indicate high precision in the data, while the only high coefficient of variation in GRS may reflect greater natural biological variability or sensitivity to the treatments.

Table 2: Analysis of variance of the variables in the germination process

Source of variation	df	GP	GE	GRC	MGT	GS
		p-valor	p-valor	p-valor	p-valor	p-valor
Total	19					
Treatments	4	<0,0001***	<0,0001***	<0,0001***	<0,0001***	<0,0001***
Error	15					
C.V		9,79	4,70	40,38	6,28	16,56
R^2		0,99	1,00	0,91	1,00	0,98

Germination Potential (GP), Germination Energy (GE), Germination Speed Coefficient (GSC), Mean Germination Time (MGT), Germination Rate (GR), **$p < 0.0001$.

The Germination Potential (GP) (Fig. 3a) in the five treatments analyzed shows significant differences (ANOVA-Tukey test, $p < 0.05$), with the highest and lowest values observed in T_1 (102%) and T_4–T_5 (0%),

respectively. Likewise, the Germination Energy (GE) (Fig. 3b) ($p < 0.05$) shows maximum and minimum values in T_1 (98%) and T_4–T_5 (0%), respectively. The Germination Rate Coefficient (GRC) (Fig. 3c) ($p < 0.05$) also shows maximum and minimum values in T_1 (0.72) and T_4–T_5 (0.0), respectively. Similarly, the Mean Germination Time (MGT) (Fig. 3d) ($p < 0.05$) shows maximum and minimum values in T_2 (180) and T_4–T_5 (0), respectively. Finally, Germination Rate (GR) (Fig. 3e) ($p < 0.05$) shows maximum and minimum values in T_1 (0.37) and T_4–T_5 (0.0), respectively.

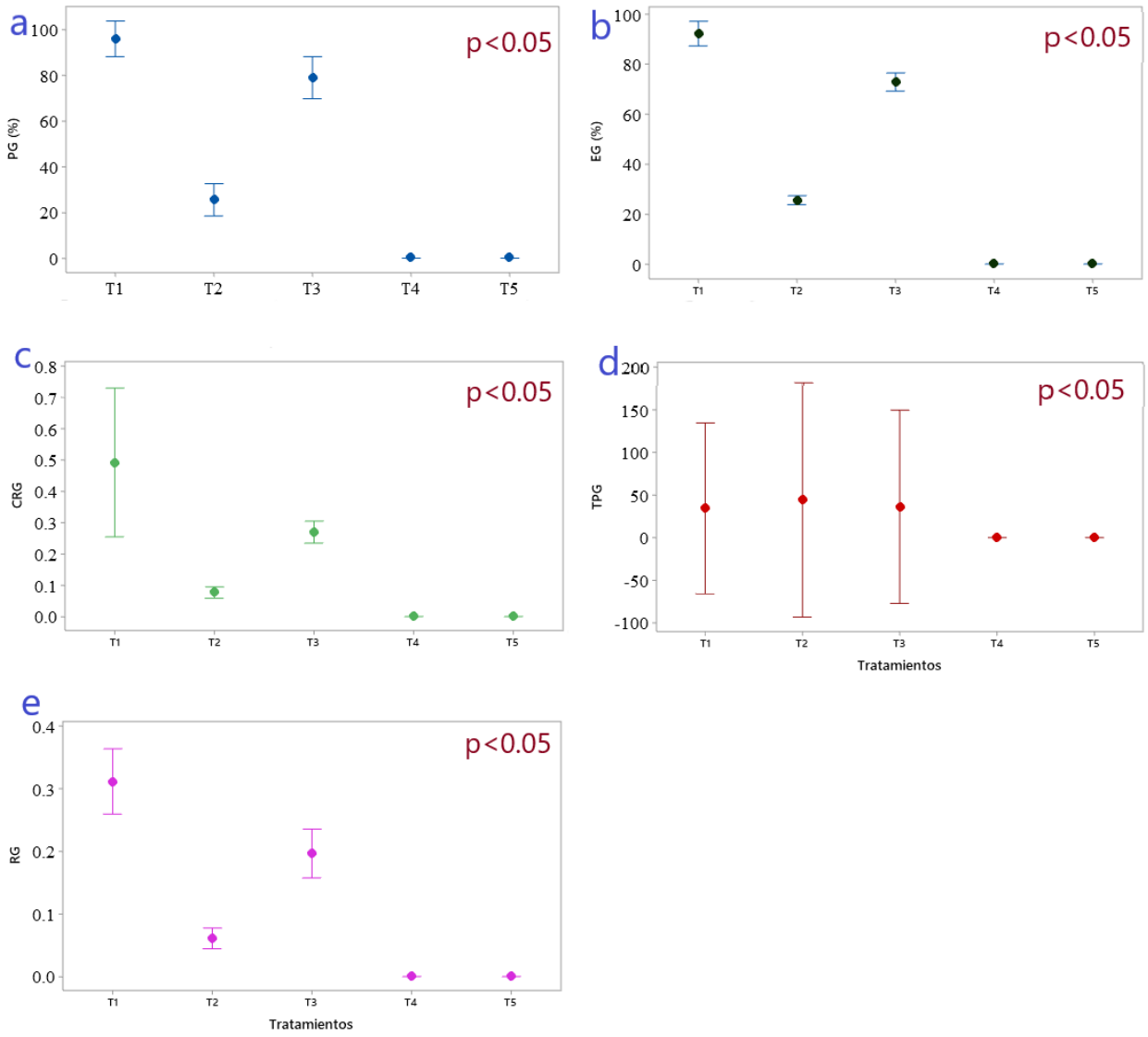

Fig. 2: Boxplot and ANOVA-Tukey at a 5% significance level for a. GP, b. GE, c. GRC, d. MGT, and e. GR across 5 treatments (T_1, T_2, T_3, T_4, and T_5).

On the other hand, Fig. 3 shows the behavior of GP, GE, and GR as a function of MGT and GRC, where in Fig. 3a, it can be observed that the maximum GP value is associated with MGT values ranging from [100 – 160] and GRC values ranging from [0.3 – 0.6]. Likewise, in Fig. 3b, it can be observed that the maximum GE value is associated with MGT values ranging from [100 – 145] and GRC values ranging from [0.35 – 0.50].

And in Fig. 3c, it can be observed that the maximum GR value is associated with MGT values ranging from [120 – 140] and GRC values ranging from [0.35 – 0.45]; indicating that optimizing germination potential, energy, and speed depends on the short period in which the highest number of seeds germinate, with optimal results observed in treatment T_1.

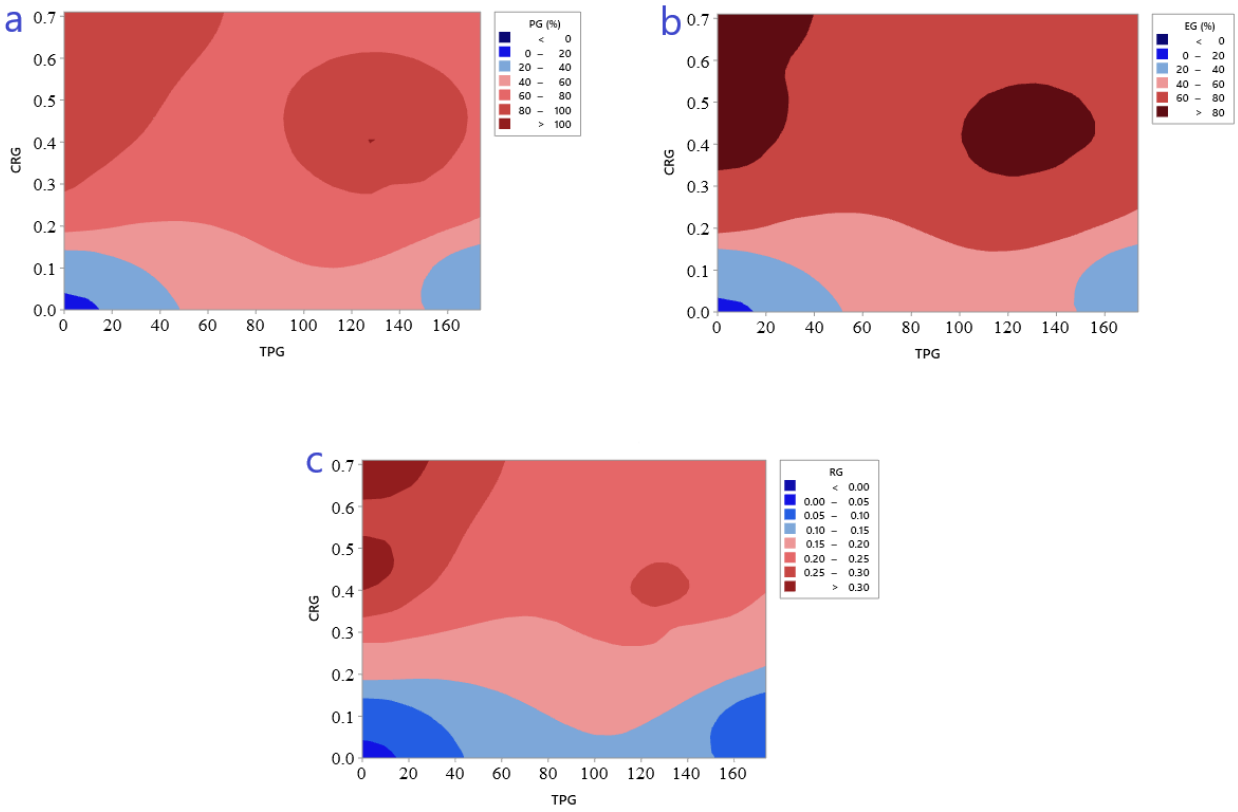

Fig. 3: Spectral variation of a. GP, b. GE, and c. GR as a function of GRC and MGT.

Likewise, some parameters show high correlations (Fig. 4). A very strong positive correlation is observed between GP – GRC, GE – GRC, GE – GR, GRC – GR, GP – GE, and GP – GR ($r > 0.9$). However, correlations between MGT and GP, GE, GRC, and GR are low ($r < 0.3$), indicating a weak dependence between these variables.

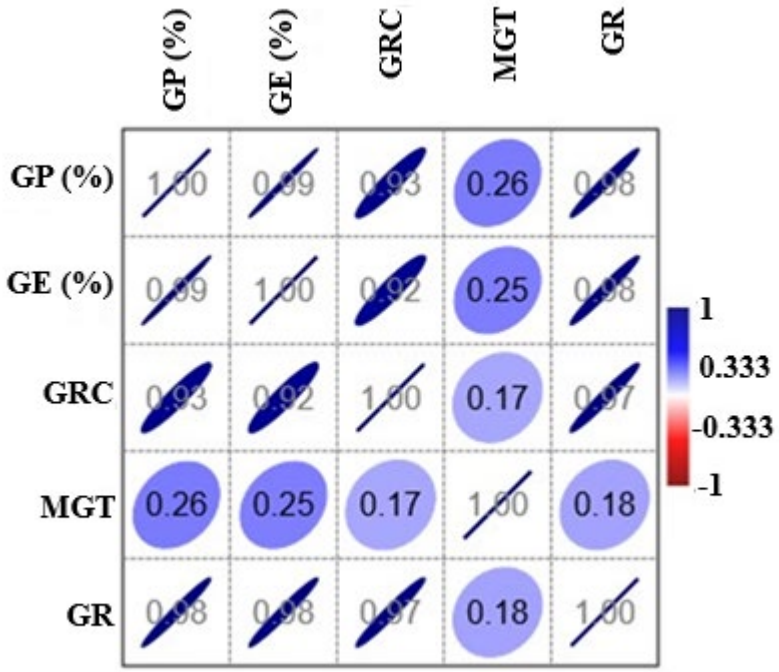

Fig. 4: Pearson correlation matrix for the variables GP, GE, GRC, MGT, and GR.

4. DISCUSSION

5.7% of the seeds showed damage, such as mechanical injury or pathogenic contamination (Hurtado et al., 2020). The values of mean, variance, standard deviation, and coefficient of variation indicate high uniformity of the seed lot, representing a key morphological characteristic associated with the ability to produce more vigorous seedlings (Rodríguez, 2008). Likewise, based on the average moisture content of the lot, the seeds fall within the optimal range of 22 to 26%, which is considered adequate for the species (Kainer et al., 1999b).

Germination of *B. excelsa* seeds under natural conditions can take between 12 and 18 months (Zuidema, 2003), due to seed dormancy, which involves physiological latency (Ahmed and Omran, 2024), and physical dormancy caused by the hard seed coat that restricts gas exchange and water imbibition (Kainer *et al*., 1999b; Loffler *et al*., 2022; Williams *et al*., 2024). Consequently, the research applied eco-friendly pregerminative treatments as an ecological technology to promote the germination process of *B. excelsa* (Ureta-Leones *et al*., 2021). However, T_4 (hot water immersion) and T_5 (control) did not show any significant results.

T_1 produced significantly higher values in GP, GE, GRC, and GR, and a lower MGT, statistically differing from the other treatments. This confirms that higher values indicate greater seed vigor (Pece *et al*., 2010), reflecting good physiological quality and higher energy (Espitia *et al*., 2016). In contrast, studies on the thick seed coat of *B. excelsa* indicate that it limits water uptake and gas exchange, thereby imposing physical dormancy (Hernández *et al*., 2021; Magnitskiy and Plaza, 2007).

With T_1, germination began 23 days after sowing and continued for a total of 187 days, proving to be more effective compared to the use of smoke as a germination stimulant for Brazil nut seeds. In the latter method, germination was advanced to 61 days after sowing following the application of a water–smoke solution produced from burning *Cecropia* wood at a 1:250 dilution (Ferraz *et al*., 2013). However, there are also reports from Brazil indicating that storing *B. excelsa* seeds for a period of 5.5 months, soaking them for two hours to remove the shell without using fungicide, and placing them in sterilized coarse sand can lead to germination beginning as early as 14 days and completing by 90 days. However, the germinative potential (GP) achieved under these conditions is lower than that recorded in this study (Müller, 1981; Müller, 1982; Kainer *et al*., 1999a; Kainer *et al*., 1999b). This supports the idea that manual scarification is a reliable method for achieving high GP percentages (Nascimento *et al*., 2022); however, it is not practical to use when large quantities of reproductive structures are involved (Sanabria *et al*., 2004). Also indicate that the optimal treatment is given in this order T1>T3>T2>T4>T5 for PG, RG EG and CRG. On the other hand, T_3 reported significantly good results, with no internal tissue damage caused by the sulfuric acid, as the seeds have a hard seed coat (Guzmán-Pozos *et al*., 2018). Likewise, the GP obtained with this treatment was higher than the germination potential reported when soaking the seeds for 24 hours and removing the seed coat with fungicide (78%). However, a significant difference was reported in the germination process, as with that treatment germination began after one month and was completed within five months (Müller, 1981; Müller, 1982; Kainer *et al*., 1999a; Kainer *et al*., 1999b).

5. CONCLUSIONS

Brazil nut seeds (*Bertholletia excelsa* Bonpl.) subjected to seed coat removal showed the highest average values for Germination Potential (GP) (96%), Germination Energy (GE) (92.2%), Germination Speed Coefficient (GRC) (0.49), Germination Rate (GR) (0.31 seeds/day), and the lowest Mean Germination Time (MGT) (118.02 days). On the other hand, the control group did not show any germinated seeds during the 210-day evaluation period. Additionally, seeds immersed in hot water also showed no germination over the same period. The findings of this research will contribute to reducing the germination time of Brazil nut seeds and optimizing the variables associated with the germination process.

However, due to increased demand and climate change, advances in improving the mechanical and physical properties of seeds influence their quality. The physical properties of seed germination have a significant influence on environmental conservation, as seed size and shape, as well as resistance to environmental stress, directly affect the natural regeneration of ecosystems. Seeds with prolonged dormancy can wait for suitable conditions to germinate, avoiding losses due to extreme climates. Germination properties can also help mitigate or adapt to climate change (higher temperatures, less water). In the field, this information translates into the possibility of establishing standardized pre-germination and sowing protocols, reducing seed losses and maximizing seed batch yields. Furthermore, the strong positive correlation between variables such as germination potential, germination energy, and germination speed coefficient demonstrates that rapid indicators can be used to predict germination success, facilitating early decision-making in seedling production. On the other hand, the

low correlation values with average germination time suggest that it is not a determining factor in the initial evaluation, allowing nursery growers to prioritize other more important parameters. In summary, the findings of this study are applicable to projects for the conservation, restoration, and sustainable use of B. excelsa, providing a scientific basis for improving the efficiency of the species' propagation and, thus, contributing to the sustainability of Amazonian forests.

6. PATENTS

There are no patents resulting from this work.

Author Contributions: For research articles with multiple authors, include a brief paragraph outlining each author's contributions using the following format: "Conceptualization, J.E.P.A, J.E.G.C, J.M.A.S, C..F.S.C, B.P.M, R.P.E and F.Y.T.O; methodology, J.E.P.A, J.E.G.C, J.M.A.S, C.F.S.C, B.P.M, R.P.E and F.Y.T.O; software, J.E.P.A and J.E.G.C; validation, J.E.P.A and J.E.G.C.; formal analysis, J.E.P.A and J.E.G.C.; investigation, J.E.P.A, J.E.G.C, J.M.A.S, C.F.S.C, B.P.M, R.P.E and F.Y.T.O.; data curation, J.E.P.A and J.E.G.C.; writing—original draft preparation, J.E.P.A, J.E.G.C, J.M.A.S, C..F.S.C, B.P.M, R.P.E and F.Y.T.O; writing—review and editing, J.E.P.A, J.E.G.C, J.M.A.S, C..F.S.C, B.P.M, R.P.E and F.Y.T.O; visualization, J.E.P.A, J.E.G.C, J.M.A.S, C..F.S.C, B.P.M, R.P.E and F.Y.T.O.; supervision, J.E.P.A and J.E.G.C. All authors have read and agreed to the published version of the manuscript."

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