Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 25 | No. 2 | Article ID D1852 | 2026

Original Research Paper

https://doi.org/10.46488/NEPT.2026.v25i02.D1852

Open Access Journal

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

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Abbreviation: Nat. Env. & Poll. Technol.
Website: www.neptjournal.com

Received: 01-08-2025
Revised: 10-09-2025
Accepted: 27-09-2025

Key Words:

Brazil nut tree
Mechanical scarification
Chemical scarification
Pre-germination treatments

Citation for the Paper:

Ponce-Avila, J.E., Gutiérrez-Collao, J.E., Ángeles-Suazo, J.M., Saldaña-Chafloque, C.F., Pantoja-Medina, B., Ponce-Escobal, R. and Tucto-Ortega, F.Y., 2026. Effect of mechanical, chemical, and physical scarification on the germination of Brazil nut seeds (*Bertholletia excelsa* Bonpl.) in Peru. *Nature Environment and Pollution Technology*, 25(2), D1852. https://doi.org/10.46488/NEPT.2026.v25i02.D1852

Note: From 2025, the journal has adopted the use of Article IDs in citations instead of traditional consecutive page numbers. Each article is now given individual page ranges starting from page 1.

ABSTRACT

Bertholletia excelsa Bonpl. (Brazil nut) is an economically important species in the Amazon, whose natural seed production takes between 365 and 545 days and shows low germination rates. To reduce the production time of germinated Brazil nut seeds and optimize the variables associated with the germination process, one factor was evaluated: type 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 seeds with complete seed coat removal 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 this 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 the "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 because of their nutritional benefits (Takeuchi & 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 a 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 the sexual

reproduction of high-quality seedlings are essential for the management, reforestation, and conservation of the species (da Silva et al. 2022, Guimarães et al. 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 et al. 2024). Although dormancy is genetically determined, it is 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 and economically important crop species (Otani et al. 2024).

Globally, 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, such as high temperatures and water shortages (Han et al. 2009). Specifically, increased temperatures have been shown to affect seed germination by reducing viability and vigor and 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 before 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, and the equipment and infrastructure of the nursery (Delgado-Paredes et al. 2024). Consequently, this information is essential for maximizing planting efficiency (Ticona-Arapa et al. 2024). In this context, the objective of this study was to reduce the production time of germinated Brazil nut seeds and optimize the variables associated with the germination process.

MATERIALS AND METHODS

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" W, Fig. 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%.

Seed Collection

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

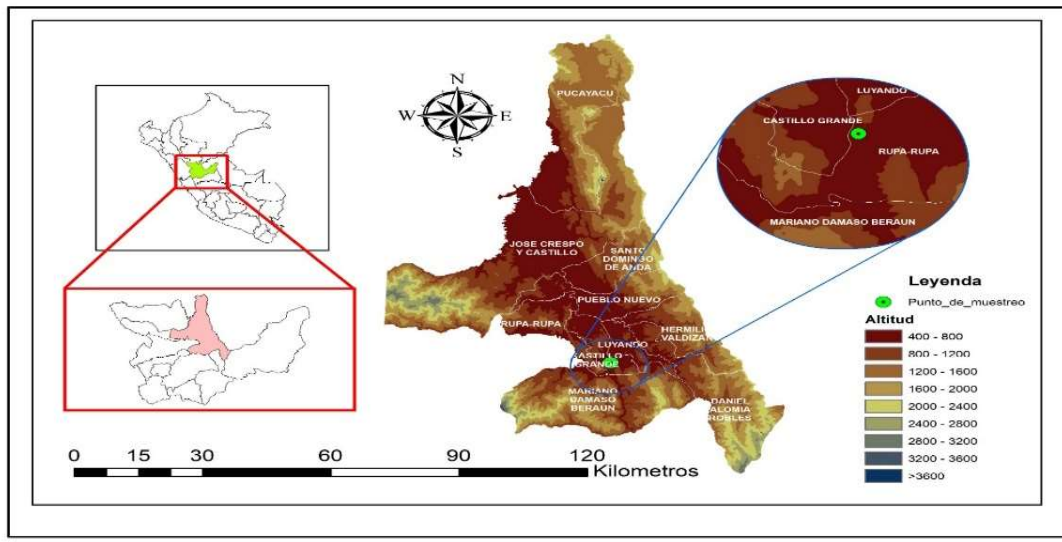

Fig. 1: Location of Tingo María (Peru).

scientific names and the families to which plant species belong.

Evaluation of the Physical Quality of the Seed lLT

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

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

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

where P is the purity percentage, TSW is the total sample weight (g), and IW is 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. The average weight of the eight replicates was calculated and multiplied by 10 to obtain the estimated weight of 1,000 seeds. This 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 each. The samples were dried for 17 ± 1 h at a constant temperature of 103 ± 1°C. After drying, the samples were cooled in a borosilicate glass desiccator (Brand™ 65043) and 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 seed weights. This was determined using Equation 3.

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

Where MC is the moisture content (%), FW is the fresh weight (g), and DW is the dry weight (g).

Research Design

A completely randomized design + 1 control was used, consisting of the proper arrangement of seeds for germination. 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 (Table 1 and Fig. 2).

Treated and untreated seeds (control) were placed under controlled conditions. Notably, 200 seeds were analyzed per treatment, with four replicates and 50 seeds per replicate. The seeds were placed under controlled conditions in germination chambers consisting of metal containers lined with cotton as a substrate. The containers were irrigated with distilled water every 24 h 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. 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 $g.L^{-1}$ of water) to prevent the attack of microorganisms. The light condition was continuous (daily,

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

Treatments	Description
T_1	**Seed coat removal:** This involved 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 germination.
T_3	**Chemical scarification:** The seeds were immersed in 100% sulfuric acid for 4 h, and both the seeds and sulfuric acid were placed in glass containers, with a volume ratio of 2:1 (acid to seeds). The mixture was stirred to ensure that the acid was evenly distributed over the seeds. Finally, the acid was removed, and the seeds were rinsed with water for 10 min.
T_4	**Hot water immersion:** The seeds were soaked in hot water for 5 min; however, the water was previously boiled and then cooled to 70°C, maintaining this temperature for 5 min. After imbibition, the water and seeds were allowed to cool, and the seeds remained soaked for 12 h.
T_5	No treatment

every 24 h) using a fluorescent light as an artificial source similar to sunlight.

Monitoring of Germination Variables

The monitoring of germination variables began 24 h after sowing and continued for seven months, with the recording of five germination response variables. Germination variables were monitored 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 & Black 1995). This 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 number of germinated seeds, and TS indicates the total number of sown seeds.

Germination energy is a variable that expresses the maximum number of seeds that germinate within a given period (Pece et al. 2010). This 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 number of germinated seeds.

The germination rate coefficient was calculated as the sum of the number of germinated seeds divided by the number of days evaluated (Maguire 1962). This was determined using Equation 6.

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

Where GRC is the germination rate coefficient, ni is the number of seeds germinated on the ith day, and ti indicates the days for the germination process on the i-th day required for seeds to complete the germination process (Kader 2005). This 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 that the germination process lasts (González-Zertuche & Orozco-Segovia 1996). This was determined using Equation 8.

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

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

The independence between experimental units was verified to meet the requirements of analysis of variance

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

(ANOVA) (González et al. 2019). Differences in means between treatments were compared using Tukey's test ($p < 0.05$), and the analysis was performed using R software version 3.5.2.

RESULTS AND DISCUSSION

Descriptive Analysis of the Seed Lot Quality

The purity of the *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 had an average moisture content of 24.7%.

Analysis of the Variables of the Germination Process

According to the Kolmogorov–Smirnov test, all the collected data showed a normal distribution ($p > 0.05$). The statistical analysis revealed a highly significant difference between the treatments (Table 2). The experimental model was highly adequate, with high R^2 values for all the variables. The low coefficients of variation in GP, GE, MGT, and GS indicate

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), and Germination Rate (GR), **p < 0.0001.

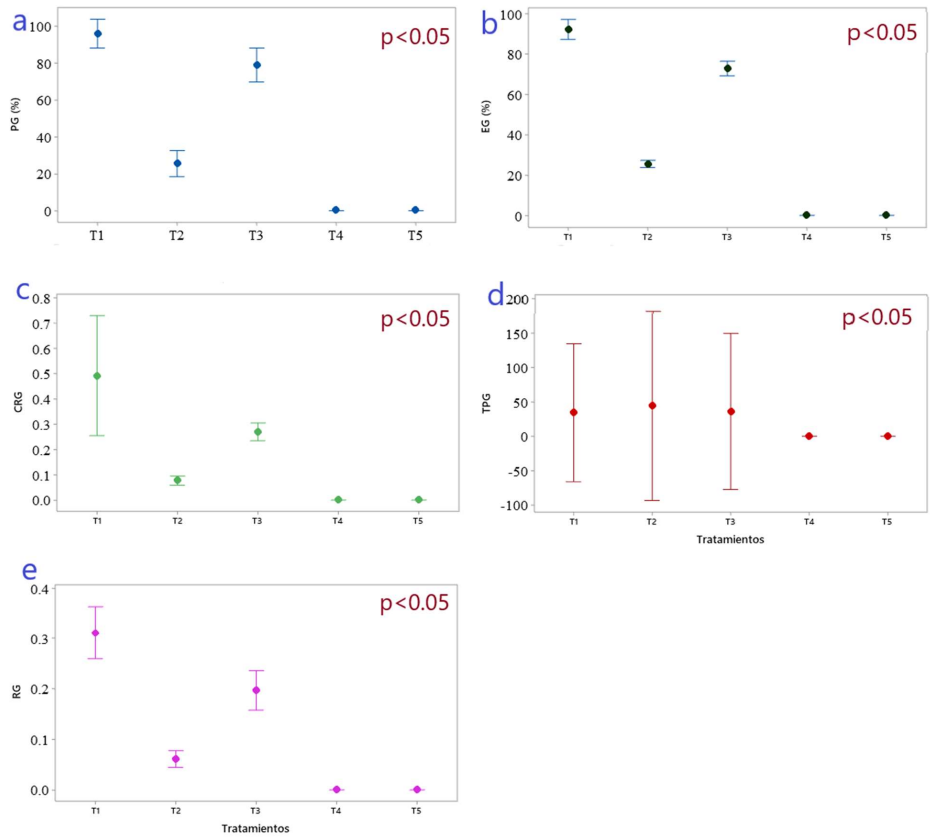

Fig. 3: Boxplot and ANOVA-Tukey at a 5% significance level for a GP, b. GE, c. GRC, d. MGT, and e. GR across five treatments (T_1, T_2, T_3, T_4, and T_5).

high precision in the data, whereas the high coefficient of variation in GRS may reflect greater natural biological variability or sensitivity to the treatments.

The Germination Potential (GP) (Fig. 3a) in the five treatments analyzed showed 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) ($p < 0.05$) showed 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 showed 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) ($p < 0.05$) showed maximum and minimum values in T_2 (180) and T_4–T_5 (0), respectively. Finally, the Germination Rate (GR) (Fig. 3e) ($p < 0.05$) showed maximum and minimum values in T_1 (0.37) and T_4–T_5 (0.0), respectively.

On the other hand, Fig. 4 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 to 145] and GRC values ranging from [0.35 to 0.50]. 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.

Likewise, some parameters showed high correlations (Fig. 5). A very strong positive correlation was observed between GP and GRC, GE and GRC, GE and GR, GRC and GR, GP and GE, and GP and GR ($r > 0.9$). However, the correlations between MGT and GP, GE, GRC, and GR were low ($r < 0.3$), indicating a weak dependence between these variables.

discussion

5.7% of the seeds showed damage, such as mechanical injury or pathogenic contamination (Hurtado et al. 2020). The values of the mean, variance, standard deviation, and coefficient of variation indicate the 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 fell within the optimal

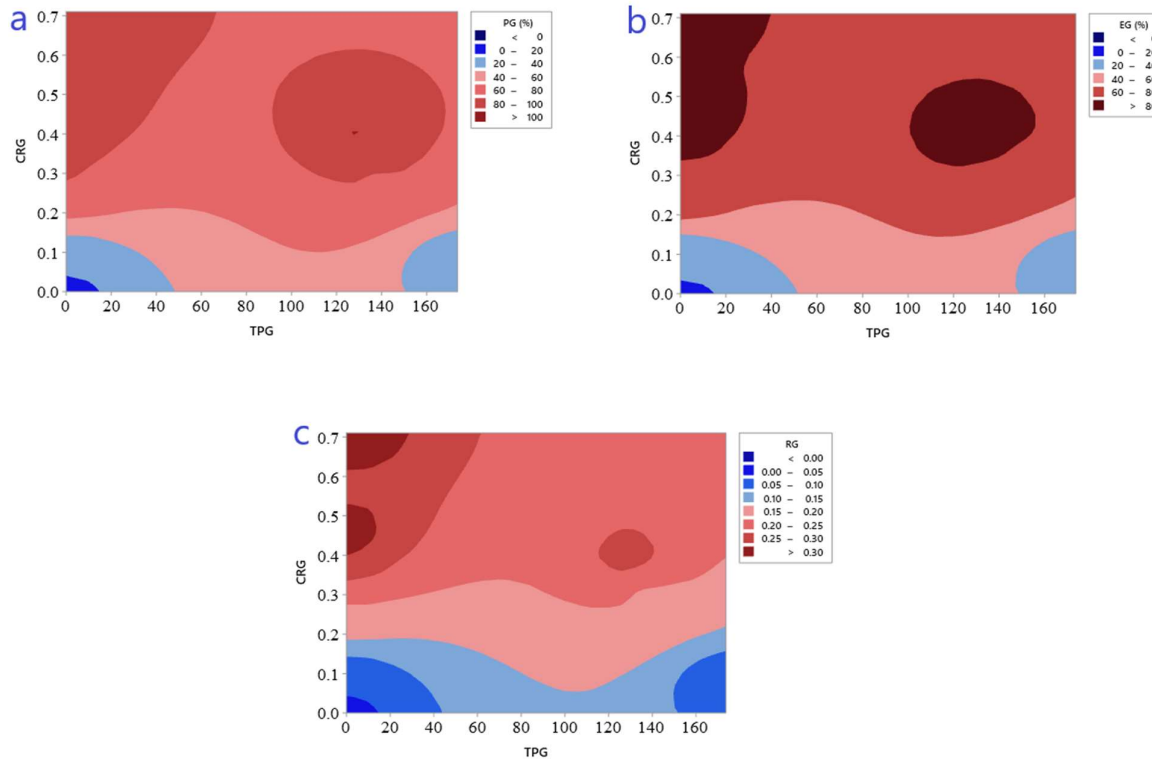

Fig. 4: Spectral variation of a. GP, b. GE, and c. GR as a function of GRC and MGT.

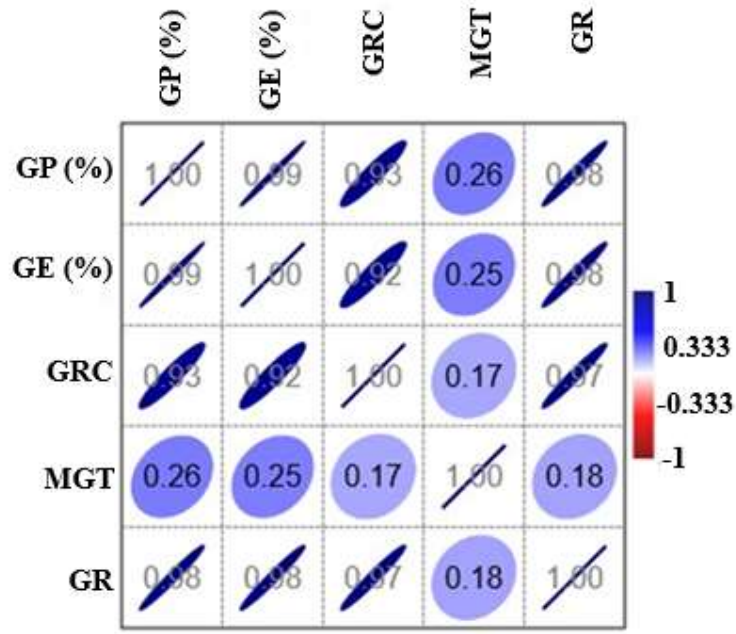

Fig.5: Pearson correlation matrix for GP, GE, GRC, MGT, and GR.

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) because of seed dormancy, which involves physiological latency (Ahmed & 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, eco-friendly pregerminative treatments have been applied as an ecological technology to promote the germination process of *B. excelsa* (Ureta-Leones et al. 2021). However, the T_4 (hot water immersion) and T_5 (control) groups did not show any significant results.

T_1 produced significantly higher GP, GE, GRC, and GR values and a lower MGT than the other treatments. This confirms that higher values indicate greater seed vigor (Pece et al. 2010), reflecting good physiological quality and higher energy content (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 & Plaza 2007).

With T_1, germination began 23 days after sowing and continued for a total of 187 days, proving to be more effective than 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 by 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 was 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 when large quantities of reproductive structures are involved (Sanabria et al. 2004). The optimal treatment was administered in the following order: T1>T3>T2>T4>T5 for PG, RG, EG, and CRG. In contrast, T_3 yielded significantly good results, with no internal tissue damage caused by 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 h 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, 1999b).

CONCLUSIONS

Brazil nut seeds (*Bertholletia excelsa* Bonpl.) 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). In contrast, the control group did not show any germinated seeds during the 210-day evaluation period. Additionally, seeds immersed in hot water did not germinate over the same period. The findings of this study 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 have influenced their quality. The physical properties of seed germination, such as seed size and shape, as well as resistance to environmental stress, significantly influence environmental conservation, as they directly affect the natural regeneration of ecosystems. Seeds with prolonged dormancy can wait for suitable conditions to germinate, avoiding losses due to extreme climate conditions. Germination properties can also help mitigate or adapt to climate change (higher temperatures and 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 the production of seedlings. 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 apply to projects for the conservation, restoration, and sustainable use of *B. excelsa*, providing a scientific basis for improving the efficiency of the species' propagation, thus contributing to the sustainability of Amazonian forests.

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