

Research Article

The Germination and Subsequent Development of Cocoa Seedlings (*Theobroma cacao* L.) Are Subject to Influence From the Pregerminative Methods Employed

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In Peru, cocoa production has increased significantly, thereby establishing a prominent position for this crop in the country's agricultural landscape. The effect of different pregerminative methods on seed germination of cocoa ecotypes and their influence on seedling development in nursery were evaluated. Three cocoa ecotypes were used: INDES 24, INDES 31, and INDES 67, and five pregerminative methods were applied to the seeds: with mucilage, mucilage removal, tegument removal, water immersion, and immersion in *Trichoderma harzianum* solution. Parameters such as germination percentage (GP), mean germination time (GT₅₀), mean germination rate (GR₅₀), and plant growth characteristics in terms of cotyledon height, plant height, number of leaves per plant, and stem diameter were evaluated. The results revealed that the treatments INDES 24 + removal tegument, INDES 67 + removal tegument, INDES 67 + water immersion, and INDES 67 + immersion in *T. harzianum* showed the best GPs of 100% at 72 h. Additionally, the INDES 67 + tegument removal showed the best mean germination time and rate (GT₅₀ and GR₅₀) with 24 h and 8 seeds/24 h, respectively. For growth parameters, INDES 67 + mucilage removal showed the highest values for cotyledon height and the number of leaves per plant with 4 cm and 13.33 leaves/plant; for plant height and stem diameter, the treatments INDES 31 + tegument removal and INDES 24 + mucilage removal obtained the highest average values with 30.21 cm and 6.65 mm, respectively. These findings demonstrate that pregerminative methods significantly impact the germination and growth of cocoa plants. This insight can enhance cocoa propagation practices and improve the success rate of their establishment in the field.

Keywords: germination; mucilage; nursery; pregerminative method; seedling development; tegument; *Theobroma cacao*

1. Introduction

Cocoa (*Theobroma cacao* L.) is a tropical tree that originated approximately in the Pilo-Cene and Miocene era [1, 2]. The use and domestication of *T. cacao* was during the middle Holocene in the upper Amazon between 5000 and 5300 years ago [3–5]. Today, it is cultivated all over the world for its social, economic, and environmental importance; it is also

the raw material for the medical, cosmetic, and chocolate industries [6]. Cocoa-derived products form the economic foundation of several developing countries [7]. Worldwide, 6.7 million hectares are cultivated, producing 4.7 million tons and generating 8.6 million dollars annually [8]. In Peru, the total cocoa production area exceeded 192,747 hectares, ranking tenth in Gross Agricultural Production Value in 2023 [9].

In the cocoa production chain, propagation is a crucial biological process, starting with seed germination to produce nursery seedlings [10, 11]. Although there are vegetative propagation methods such as somatic embryogenesis and propagation by cuttings [12, 13], seed germination is the only method that provides advantages in rooting and ensures plant vigor [14].

In order to produce seedlings with good agronomic characteristics and guarantee their successful establishment in the final field, certain conditions can be manipulated prior to seed germination. These include the presence or elimination of mucilage [15, 16], tegument removal, water immersion, and immersion in beneficial microorganisms such as *Trichoderma harzianum* [17]. Another important factor in the germination and development of seedlings is the ecotype of origin of the seeds, so it is crucial to measure the impact on germination time and subsequent agronomic characteristics [18, 19]. Likewise, the quality of cocoa seedlings in the nursery depends mainly on the variety and physiological quality of the seeds, the latter being a determining factor to ensure homogeneous germination and vigorous growth [20].

In the northern part of Peru, the area under cocoa cultivation has increased significantly in recent years [9], which has driven the mass production of seedlings through botanical propagation. However, seed germination and the development of cocoa seedlings in the nursery continue to be a limiting factor for efficient propagation. In this context, the implementation and adaptation of appropriate pregerminative methods can constitute a practical and accessible alternative for producers, thus improving the efficiency of germination and plant establishment, increasing income, and promoting economic development, thus contributing to poverty reduction and strengthening food security in the northern part of the country. Consequently, this study evaluated the effect of five pregerminative methods (intact seed or with mucilage, mucilage removal, tegument removal, water immersion, and immersion in *T. harzianum*) on the seed germination process and seedling development of three cocoa ecotypes (INDES 24, INDES 31, and INDES 67) from the cocoa germplasm bank from the Amazonas department, Peru.

2. Materials and Methods

2.1. Study Area. The experiment was carried out at the Plant Production Center for Research and Technology Transfer of the Centro Experimental Yanayacu of the Instituto Nacional de Innovación Agraria (INIA), located in the province of Jaén, Peru (Figure 1). The study site is situated at the following geographical coordinates and altitude: 746,447.97 W and 9,372,010.24 S, at 624 m above sea level. The study area has an average temperature of 28 °C and an average annual precipitation of 1200 mm [21].

2.2. Biological Material. The biological material used in this study included the cocoa ecotypes INDES 24, INDES 31, INDES 67, and the fungus *T. harzianum* Rifai (AP2M1-C1). The cocoa ecotypes were sourced from the germplasm bank at the Instituto para el Desarrollo Sustentable de Ceja de Selva

(INDES-CES), affiliated with the Universidad Nacional Toribio Rodríguez de Mendoza (UNTRM). This facility is situated in the rural settlement of Naranjos Alto, Cajaruro District, Utcubamba Province, Amazonas (792,277.7 W 9,364,086.8 S). The selection of these ecotypes was based on their outstanding agronomic characteristics, particularly their high yields and sensory quality in the case of INDES 24 and INDES 31, according to Oliva and Maicelo [22]. On the other hand, ecotype INDES 67 was selected for its outstanding genetic attributes, including its evolutionary history and level of heterozygosity [2, 23]. The *T. harzianum* fungus used in this study was commercially acquired from the Servicio Nacional de Sanidad Agraria (SENASA) since this fungus species favors the absorption of nutrients such as iron, phosphorus, and nitrogen; it also improves seed germination [24].

2.3. Application of Pregerminative Methods. The cocoa seeds were obtained from healthy and physiologically mature pods. They were extracted from the middle third of the pod on the same day of harvest. The seeds of each ecotype underwent five pregerminative treatments: intact seed or with mucilage (no method was performed), mucilage removal, tegument removal, water immersion, and immersion in *T. harzianum* (Figure 2). The mucilage was removed from the cocoa seeds by abrasion with sand and wood sawdust, in accordance with López and Gil [25]. To extract the tegument, the mucilage was first removed; then, the tegument was manually stripped off. The treatments for seeds with mucilage, without mucilage, and without tegument were directly placed on a germination bed made of washed river sand sterilized by solarization. The treatment of seeds immersed in water was performed by immersing intact seed in sterile distilled water (SDW) until 100% germination was observed. The application of *T. harzianum* was done by immersing intact seeds in a solution of SDW + *T. harzianum* at a concentration of 1×10^8 conidia/mL; according to Bettiol et al. [26] and Campos et al. [27], the concentration of conidia was calculated using a Neubauer chamber. Ten seeds from each ecotype were assessed for each pregerminative method. The germination process was monitored every 24 h until 100% germination, indicated by the emergence of the radicle in all seeds, was achieved for each treatment.

2.4. Growth Conditions. The substrate used consisted of agricultural soil, river sand, and rice straw in proportions of 2:1:1. All substrates were sterilized at 121 °C for 30 min. The sterile substrate was placed in 7 × 12-inch polyethylene bags. The germinated seeds were sown using the indirect method, placing one seed per bag. Ten germinated seeds were sown. The bags with the seeds were coded, and five central plants from each treatment were evaluated. During the evaluation period, the plants were sheltered under Raschel mesh providing 50% shade. Cultural practices, including manual watering and weed removal, were carried out regularly. To manage pests and fungi, thiodicarb and copper sulfate pentahydrate were applied as needed.

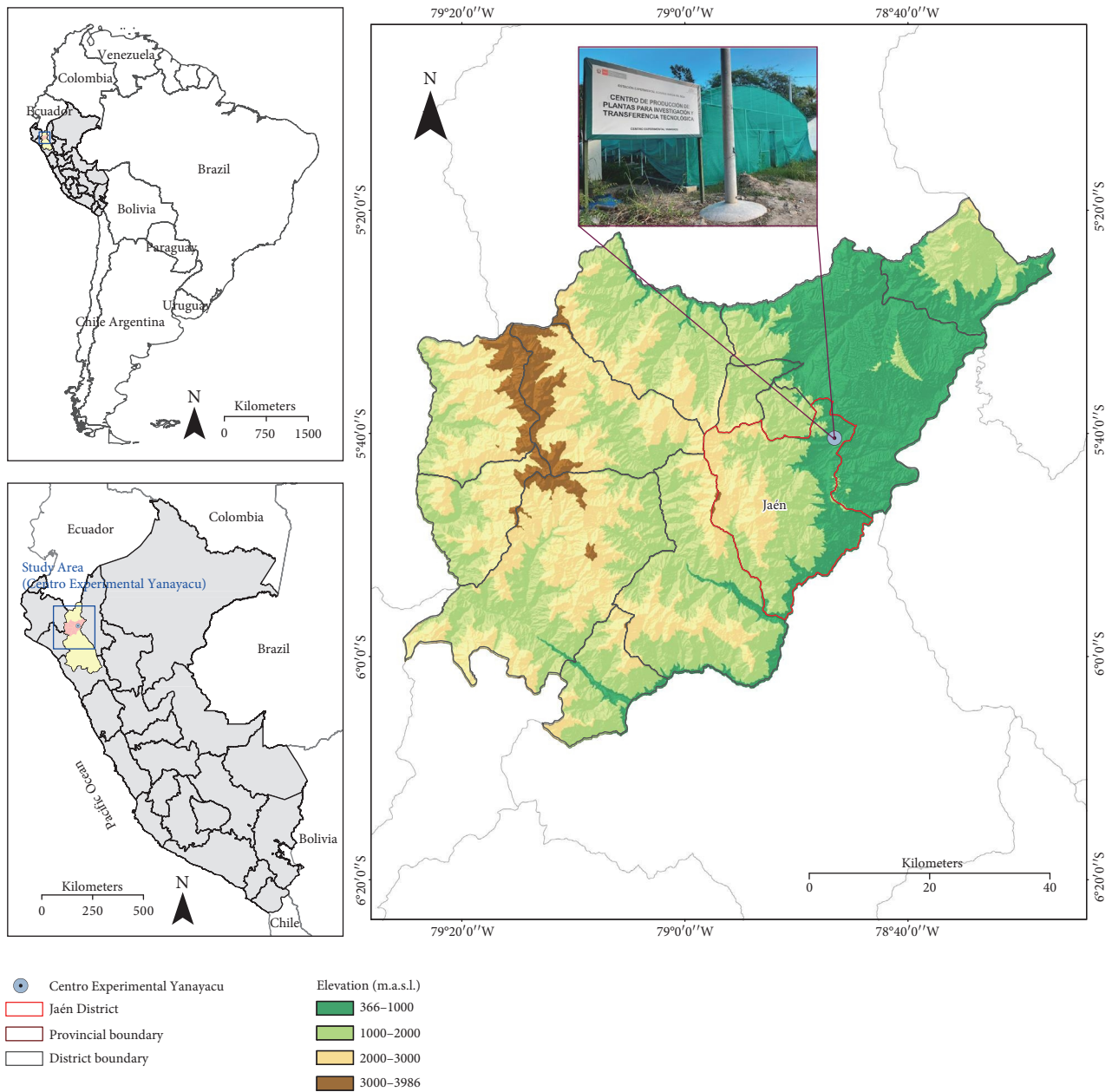


FIGURE 1: Geographical location of the Center for Plant Production for Research and Technology Transfer at Centro Experimental Yanayacu, Province of Jaén, Cajamarca.

2.5. *Experimental Design.* A completely randomized design (CRD) with a 3×5 factorial arrangement was used: three ecotypes of *T. cacao* L. and five pregerminative methods, resulting in 15 treatments from the combination of factors, with three repetitions each (Table 1).

2.6. *Parameters Evaluated.* The parameters evaluated during the germination process were (a) germination percentage (GP) [28], mean germination time (GT_{50}) [29, 30], and mean germination rate (GR_{50}) [31], calculated using Equations (1)–(3), respectively. A total of 450 seeds were evaluated in the study, with 10 seeds per treatment.

$$GP = \frac{N^\circ \text{ of germinated seeds}}{\text{Total } N^\circ \text{ of seeds}} \times 100, \quad (1)$$

$$GT_{50} = t_i + \frac{(N/2 - n_i)(t_j - t_i)}{n_j - n_i}, \quad (2)$$

where N =final number of emergences, n_i and n_j =the cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.



FIGURE 2: Initial stages of cocoa seed germination under five pregerminative methods.

TABLE 1: Ecotypes of *Theobroma cacao* L. and pregerminative methods.

Ecotypes	Pregerminative methods
INDES 24	With mucilage
INDES 24	Mucilage removal
INDES 24	Tegument removal
INDES 24	Water immersion
INDES 24	Immersion in <i>T. harzianum</i>
INDES 31	With mucilage
INDES 31	Mucilage removal
INDES 31	Tegument removal
INDES 31	Water immersion
INDES 31	Immersion in <i>T. harzianum</i>
INDES 67	With mucilage
INDES 67	Mucilage removal
INDES 67	Tegument removal
INDES 67	Water immersion
INDES 67	Immersion in <i>T. harzianum</i>

$$GR_{50} = \sum_{i=1}^k n_i/t_i, \quad (3)$$

where n_i = number of seeds germinated in the i th data acquisition, t_i = time of the i th data acquisition, and k = time of duration of the germination test.

The parameters assessed during plant growth included (a) cotyledon height at 8 days after sowing (DAS), measured from the base of the stem to the middle part of the cotyledon with a millimeter ruler; (b) plant height, measured from the

base of the stem to the apical part of the plant with a millimeter ruler; (c) number of leaves, by directly counting the leaves formed on the entire plant; and (d) stem diameter, measured 2 cm from the base of the stem. Variables b, c, and d were evaluated biweekly for 3 months, and the data from the last evaluation were used for the analysis. A total of 225 plants were evaluated, with five plants per treatment.

2.7. Data Analysis. The data obtained from agronomic variables such as cotyledon height, plant height, number of leaves, and stem diameter were subjected to the Shapiro–Wilk normality test and Levene’s test for homogeneity of variance. An analysis of variance (ANOVA) and Fisher’s LSD multiple comparison test were then performed. These tests were conducted at a 5% significance level using the Infostat/Professional statistical software, version 2018p. The germination process data were used to estimate the mean germination time (GT_{50}) and mean germination rate (GR_{50}) for each treatment. GT_{50} and GR_{50} were estimated by logistic regression using SAS statistical software [32].

3. Results

3.1. GP. The statistical analysis showed highly significant differences ($p < 0.001$) among the evaluated treatments (Figure 3). GPs ranged from 0% to 100% at 72 h. The treatments INDES 24 + tegument removal, INDES 67 + tegument removal, INDES 67 + water immersion, and INDES 67 + immersion in *T. harzianum* exhibited the highest germination rates, all reaching 100%. Conversely, the treatments that did not show germination were INDES 31 + with mucilage and INDES 31 + water immersion.

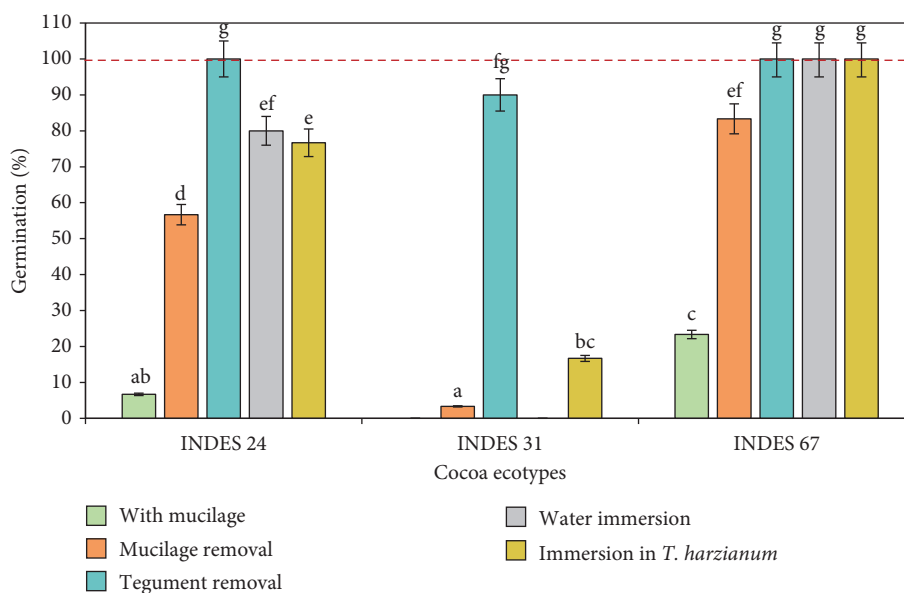


FIGURE 3: Seed germination percentage of three cocoa ecotypes subjected to five pregerminative methods at 72 h. Significant differences between means are denoted by different letters, according to Fisher’s LSD test ($p \leq 0.05$).

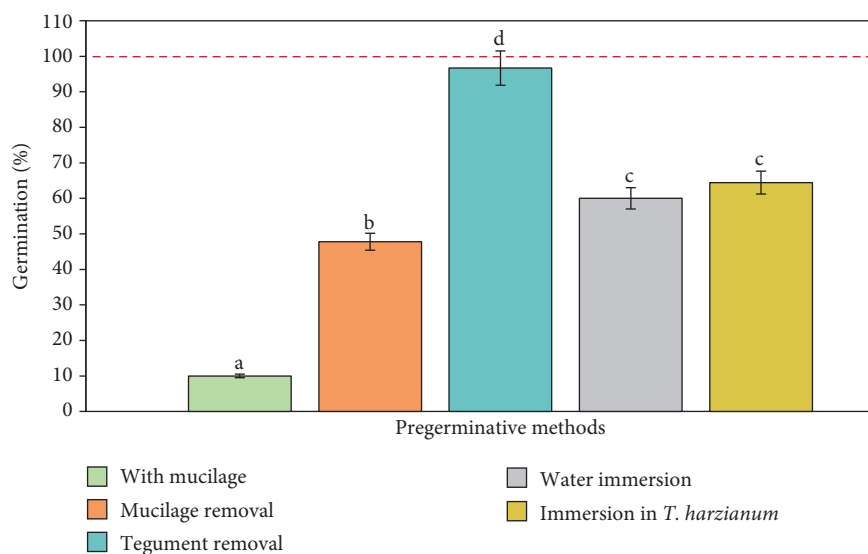


FIGURE 4: Germination percentage of cocoa seeds under five pregerminative methods at 72 h. Significant differences between means are denoted by different letters, according to Fisher’s LSD test ($p \leq 0.05$).

The removal of the tegument was the pregerminative method that obtained the highest value in all the ecotypes, while seeds with mucilage showed a lower value (Figure 3).

In Figure 4, the average GP for the five pregerminative methods evaluated, regardless of cocoa ecotype, is shown. All pregerminative methods had a positive effect on the seeds. There were significant differences ($p < 0.001$) among the pregerminative methods after 72 h of application. The method without tegument showed the highest GP at 96.67%, while the method with mucilage showed the lowest GP at 10%.

Figure 5 shows the percentage of seed germination for three cocoa ecotypes evaluated at 72 h. The Fisher’s LSD test revealed three statistically different groups (group a, b, and c);

as well as the ANOVA showed highly significant differences ($p < 0.001$) among the cocoa ecotypes. Ecotype INDES 67 showed the highest GP with 81.33%, followed by INDES 24 with 64%, while INDES 31 showed the lowest GP with 22%.

Figure 6 shows the curve of the germination process of seeds of three cocoa ecotypes subjected to five pregerminative methods, this germination behavior was evaluated every 24 h until 192 h. Seeds treated with the tegument removal method were the best in all ecotypes. Thus, ecotypes INDES 24 and INDES 67 treated with the tegument removal method showed 100% germination at 72 h; also, INDES 67 with the water immersion and *T. harzianum* immersion methods showed 100% germination at 72 h. The seeds that took longer time

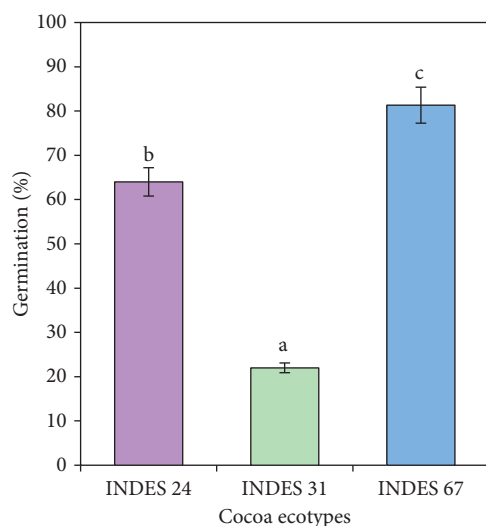


FIGURE 5: Germination percentage of three cocoa ecotypes at 72 h. Significant differences between means were denoted with different letters, according to Fisher's LSD test ($p \leq 0.05$).

to obtain 100% germination were recorded in intact seeds with mucilage, in all ecotypes. Hence, INDES 31 with mucilage required the longest duration for complete germination, taking 192 h. This was followed by INDES 24 and INDES 67 with mucilage, which achieved 100% germination in 120 h.

3.2. Mean Germination Time and Germination Rate (GT_{50} and GR_{50}). The results indicated that pregerminative methods significantly influence the germination behavior of seeds from the three cocoa ecotypes, as assessed by the mean germination time GT_{50} and mean germination rate VR_{50} . The treatments that required the shortest time to reach 50% germination (GT_{50}) were INDES 67 + tegument removal, INDES 24 + tegument removal, and INDES 67 + water immersion; likewise, the treatments that recorded the highest speeds to reach 50% germination (GR_{50}) were INDES 31 + with mucilage and INDES 31 + mucilage removal (Table 2). Conversely, the treatments that required longer times to reach 50% germination (GT_{50}) were INDES 31 + with mucilage and INDES 31 + mucilage removal; also, the treatments INDES 67 + tegument removal, INDES 67 + mucilage removal, and INDES 31 + tegument removal showed lower speeds to reach 50% germination (GR_{50}) (Table 2).

3.3. Growth Parameters. Table 3 shows the plant growth parameters of all treatments. All the variables evaluated show statistically significant differences at 90 DAS (Table 3). Regarding cotyledon height, the best treatment was INDES 67 + mucilage removal; on the contrary, INDES 31 + with mucilage showed the lowest value. The highest values for plant height were recorded in INDES 31 + tegument removal and INDES 31 + water immersion with 30.21 cm and 29.39 cm, respectively; in contrast, INDES 67 + with mucilage and INDES 24 + with mucilage reported the lowest values of 18.94 cm and 21.01 cm, respectively. Regarding the number of leaves per plant, INDES 67 +

mucilage removal and INDES 67 + immersion in *T. harzianum* showed higher average values of 13.33 and 13.27 leaves per plant. For stem diameter, the best treatments were INDES 24 + mucilage removal and INDES 24 + water immersion, which were the best treatments with average values of 6.65 mm and 6.61 mm, respectively; in contrast, the lowest stem diameter was observed in INDES 67 with mucilage, measuring 4.36 mm.

4. Discussion

4.1. Effect of Pregerminative Methods on Germination. The results showed that the use of intact seeds with mucilage prolonged the onset and cessation of germination compared to the other pregerminative methods, which evidences an inhibitory effect of mucilage on the speed and uniformity of the germination process. This observation is consistent with previous studies that indicate that cocoa mucilage can act as a physical and chemical barrier that hinders water imbibition and radicle emergence [18, 33] due to mucilaginous and enzymatic compounds that alter the metabolic activity of the seed [34]. Likewise, Bhatt et al. [35] pointed out that mucilage can inhibit the onset of seed germination; therefore, its removal can significantly improve this process [36].

However, although our study confirms this negative effect on germination, mucilage should not be interpreted exclusively as an obstacle to germination. In ecological contexts, it has been shown that mucilage in seeds of wild species related to cocoa contributes to moisture retention and improves adherence to the substrate [37]. Therefore, the apparent negative impact of mucilage under controlled conditions does not contradict its benefits in natural environments but rather reveals an evolutionary adaptation whose functionality may depend on the ecological context and not necessarily on the agronomic context. This functional duality of mucilage allows us to reconcile the findings of this study with the existing ecological and evolutionary literature.

Regarding the tegument removal method, this was the best in all the ecotypes, since 100% germination was obtained in a range of 72–120 h, while the intact seed method obtained 100% germination in a range of 120–192 h; this difference was marked by the presence and absence of the tegument in the seeds, since all seeds were viable. This statement aligns with previous reports describing the tegument as a structural barrier protecting the seed [38, 39] that limits the entry of water and oxygen [40, 41]. It was also observed that treatments with water immersion and application of *T. harzianum* also favored germination, which is consistent with evidence that imbibition facilitates the activation of hydrolytic enzymes and that *Trichoderma* improves nutrient availability [42, 43], produces phytohormones such as auxins, and reduces oxidative stress during germination [44, 45].

Our results confirm, extend, and in some respects qualify the findings of previous research. While previous studies identified individual effects of mucilage, tegument, and *Trichoderma* use, this research compares in an integrated manner five pregermination methods on three cocoa ecotypes, demonstrating that tegument removal is the most

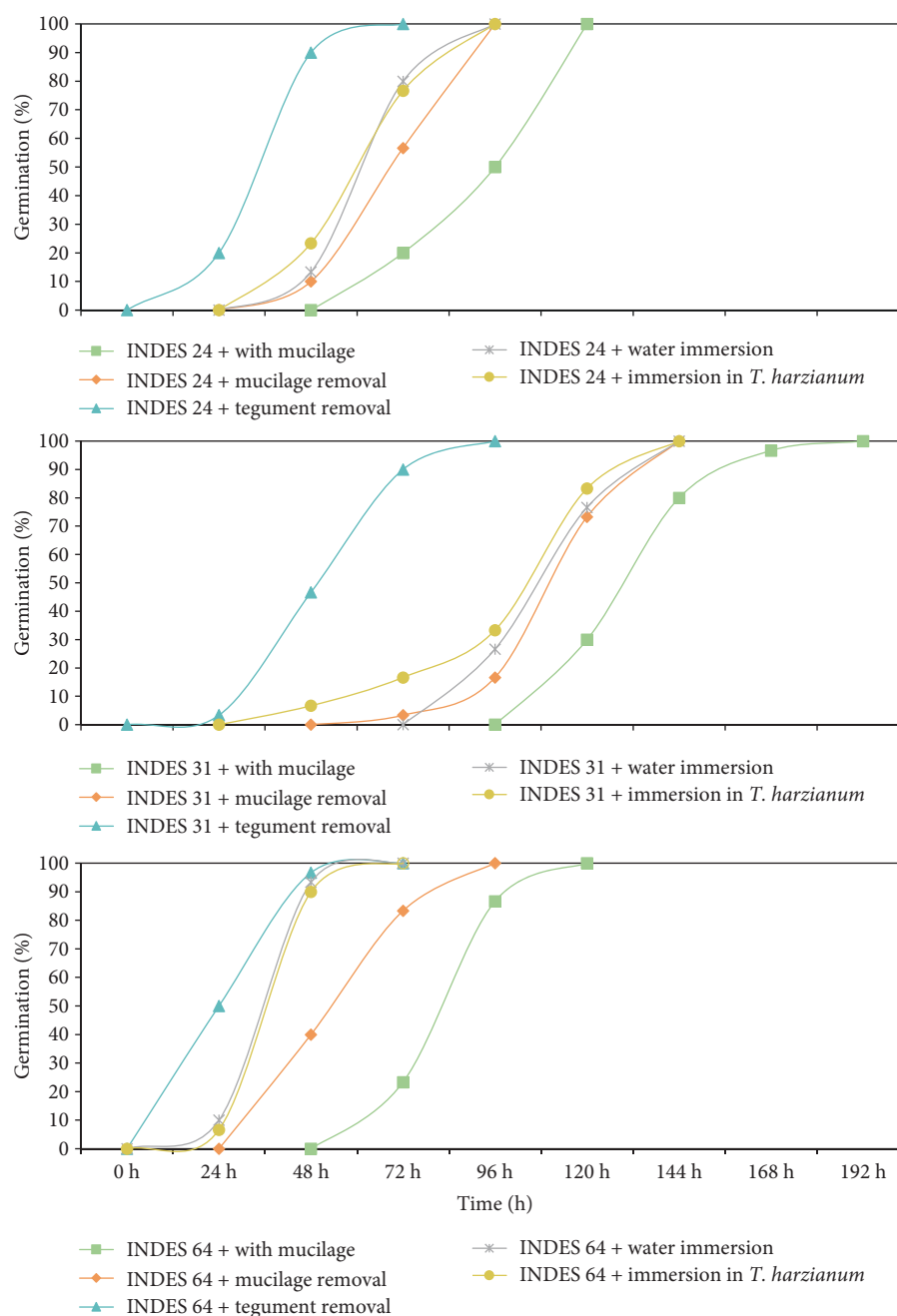


FIGURE 6: Cumulative germination percentage (mean ± SD) of three cocoa ecotypes under five pregerminative methods during the evaluation. Letters denote significant differences (Fisher’s LSD, $p \leq 0.05$).

effective treatment for improving germination rate and percentage. However, this advantage should not be interpreted as universal, since, in natural systems, the presence of mucilage may confer key adaptations for survival, raising future opportunities to investigate these mechanisms from an ecological, physiological, and evolutionary perspective, especially in wild and phylogenetically close cocoa species.

4.2. Growth Parameters. Regarding the growth parameters evaluated, there were notable differences between our results and those reported in previous studies.

The highest cotyledon height obtained in our study was recorded in the INDES 67 ecotype with 4 cm at 8 DAS [46], who recorded an average value of 114.54 ± 6.94 mm in the CCN-51 variety; this difference could be attributed to the variety studied and the time of evaluation, since growth in the early stages may vary according to environmental conditions and the time elapsed since sowing.

In contrast, the greatest plant height obtained in our experiment was recorded in seeds of ecotype INDES 31 without tegument with 30.21 cm after 3 months of sowing, being higher than that described by Nogueira et al. [45] who

TABLE 2: Mean values and variation range (VR) of mean germination time (GT₅₀) and mean germination rate (GR₅₀) of cocoa seeds for all treatments evaluated.

Treatments	GT ₅₀ (h)		GR ₅₀ (seeds/24 h)	
	Mean	VR	Mean	VR
INDES 24 + with mucilage	105.54	(99.36–113.09)	9.98	(9.43–10.32)
INDES 24 + mucilage removal	68.85	(66.68–71.35)	10.00	(9.60–10.50)
INDES 24 + tegument removal	32.23	(30.78–33.76)	8.73	(8.00–10.20)
INDES 24 + water immersion	60.61	(58.99–62.26)	9.29	(9.00–9.60)
INDES 24 + immersion in <i>T. harzianum</i>	58.90	(57.09–60.80)	9.00	(8.50–9.50)
INDES 31 + with mucilage	129.16	(127.42–130.96)	10.13	(9.77–10.46)
INDES 31 + mucilage removal	109.71	(103.40–117.73)	10.11	(9.40–10.60)
INDES 31 + tegument removal	49.10	(47.22–50.97)	8.50	(8.40–8.70)
INDES 31 + water immersion	106.77	(104.96–108.65)	9.92	(9.20–10.55)
INDES 31 + immersion in <i>T. harzianum</i>	99.79	(79.78–150.53)	9.73	(9.60–9.80)
INDES 67 + with mucilage	81.50	(79.87–83.17)	10.10	(9.12–11.10)
INDES 67 + mucilage removal	81.11	(76.88–85.37)	8.30	(7.80–8.70)
INDES 67 + tegument removal	24.00	(22.81–25.09)	8.00	(7.20–8.40)
INDES 67 + water immersion	33.74	(32.36–35.15)	9.07	(8.00–10.80)
INDES 67 + immersion in <i>T. harzianum</i>	34.90	(33.42–36.42)	9.40	(8.00–11.40)

TABLE 3: Mean values and variation range (VR) of cotyledon height (cm), plant height (cm), number of leaves per plant, and stem diameter (mm) of seedlings of different ecotypes subjected to different pregerminative methods.

Treatments	Cotyledon height (cm)		Plant height (cm)		Number of leaves per plant		Stem diameter (mm)	
	$p = 0.0012$		$p = 0.0008$		$p = 0.0009$		$p = 0.0003$	
	$f = 4.46$		$f = 4.74$		$f = 4.62$		$f = 5.45$	
	Mean	VR	Mean	VR	Mean	VR	Mean	VR
INDES 24 + with mucilage	0.94 ^a	(0.67–1.31)	21.01 ^{ab}	(20.42–21.80)	10.27 ^a	(9.40–11.00)	5.25 ^b	(4.82–5.48)
INDES 24 + mucilage removal	3.41 ^{de}	(2.74–3.82)	23.65 ^{cd}	(21.94–25.46)	12.13 ^{b-e}	(12.00–12.20)	6.65 ^f	(6.46–6.78)
INDES 24 + tegument removal	2.29 ^{bc}	(0.51–3.90)	28.61 ^{gh}	(28.44–28.78)	11.53 ^b	(10.80–12.20)	5.60 ^{bc}	(5.34–5.91)
INDES 24 + water immersion	3.05 ^{cde}	(2.86–3.40)	28.55 ^{gh}	(26.92–30.25)	11.93 ^{bcd}	(11.80–12.20)	6.61 ^f	(6.37–7.07)
INDES 24 + immersion in <i>T. harzianum</i>	2.34 ^{bc}	(2.09–2.70)	28.46 ^{fgh}	(27.40–29.92)	12.00 ^{b-e}	(11.60–12.40)	5.98 ^{cde}	(5.78–6.25)
INDES 31 + with mucilage	0.88 ^a	(0.56–1.17)	26.82 ^{efg}	(26.10–27.32)	13.07 ^{ef}	(12.40–13.80)	6.49 ^{ef}	(6.40–6.59)
INDES 31 + mucilage removal	1.33 ^{ab}	(0.97–1.58)	28.61 ^{gh}	(26.92–29.80)	13.07 ^{ef}	(12.00–14.00)	6.51 ^{ef}	(6.14–7.05)
INDES 31 + tegument removal	2.25 ^{bc}	(1.36–3.47)	30.21 ^h	(28.62–31.76)	12.87 ^{c-f}	(12.60–13.40)	6.21 ^{def}	(5.71–6.64)
INDES 31 + water immersion	1.34 ^{ab}	(1.00–1.74)	29.39 ^h	(28.72–30.54)	12.27 ^{b-f}	(11.00–13.60)	6.14 ^{def}	(5.98–6.41)
INDES 31 + immersion in <i>T. harzianum</i>	1.07 ^a	(0.61–1.38)	29.34 ^h	(26.60–31.52)	12.53 ^{b-f}	(11.60–13.20)	6.35 ^{def}	(6.13–6.72)
INDES 67 + with mucilage	3.12 ^{de}	(2.59–3.60)	18.94 ^a	(17.80–20.06)	9.87 ^a	(9.40–10.40)	4.36 ^a	(4.18–4.61)
INDES 67 + mucilage removal	4.00 ^e	(3.84–4.28)	25.40 ^{de}	(24.06–27.18)	13.33 ^f	(13.00–14.00)	5.84 ^{cd}	(5.38–6.44)
INDES 67 + tegument removal	2.70 ^{cd}	(2.21–3.40)	22.15 ^{bc}	(21.90–22.48)	11.80 ^{bc}	(11.20–12.40)	5.24 ^b	(5.03–5.47)
INDES 67 + water immersion	3.41 ^{de}	(3.12–3.62)	26.04 ^{def}	(24.78–28.32)	12.93 ^{def}	(12.20–13.40)	6.02 ^{cde}	(5.79–6.24)
INDES 67 + immersion in <i>T. harzianum</i>	2.30 ^{bc}	(1.81–2.93)	23.83 ^{cd}	(21.98–26.10)	13.27 ^f	(13.00–13.40)	5.93 ^{cd}	(5.64–6.14)
VC (%)	27.67		5.67		5.56		5.47	

Note: The superscript letters represent the results of the separation of means. Treatments sharing the same superscript letter do not differ significantly, while treatments with different superscript letters show significant differences according to Fisher's LSD test ($p \leq 0.05$).

Abbreviations: VC, variation coefficient; VR, variation range.

reported a maximum value of 28.8 cm in cocoa seedlings treated with *Trichoderma* isolates at 3 months of sowing. Similarly, Chavez-Jalk et al. [47] recorded 29.83 ± 0.50 cm as a maximum height in the CCN-51 variety, a value that is also below that obtained in our study. This difference could be influenced by the pregerminative method used in our study, since the extraction of the seed tegument accelerates

the onset of germination and seedling development. On the other hand, Cortés-Patiño et al. [48] reported 35.5 cm in clone IMC-67, which indicates that the differences between these studies may be influenced by the genetic material used. In our case, INDES group ecotypes are characterized by vigorous growth and early production [49], which could explain the superior performance in plant height.

The highest mean values of the number of leaves per plant were recorded in seeds without mucilage in all ecotypes, which were also higher compared to previous studies. Nogueira et al. [45] found averages of 9.5 and 9 leaves per plant after 3 months of evaluation using *Trichoderma*. For their part, Chavez-Jalk et al. [47] reported values of 12.00 ± 3.46 and 11.00 ± 2.65 , while Gutiérrez et al. [50] observed a maximum of 10.5 leaves per plant in cocoa seedlings. The superiority observed in our study could be due to the pregerminative method used, which stimulates the speed of germination and favors the development of the plant; consequently, it favors the appearance of leaves in the early stages of growth.

Regarding stem diameter, the values recorded were higher than those reported by Chávez et al. [51], who found a maximum of 4.69 ± 0.5 mm in EEET-558 + CCN-51 hybrid cocoa seedlings. Likewise, Chavez-Jalk et al. [47] reported maximum values of 5.85 ± 0.49 mm and 5.19 ± 0.98 mm in fine aroma native cocoa plants, both lower than those obtained in our study. These results confirm that the pregerminative treatment applied, which consists of removing the seed tegument and soaking in water, promotes rapid seed germination and stem thickening in the early stages of cocoa seedling development.

Overall, the results obtained for plant height, number of leaves, and stem diameter exceeded those reported in previous studies, suggesting that the experimental conditions and the pregerminative treatments used contributed favorably to the initial development of cocoa seedlings. In addition, the variability among studies reinforces the importance of genetic material and management practices in the growth of cocoa plants.

This research provides valuable information on pregerminative methods applied to cocoa seeds, which show high potential for improving the botanical propagation of the crop. Furthermore, these methods represent environmentally sustainable alternatives, as they do not generate toxic waste. However, evaluating a limited number of ecotypes and not considering physiological variations at different genetic origins could influence the response of the pregermination methods used. Furthermore, no economic or operational analyses were included to estimate the feasibility of implementing these methods on a production scale. Therefore, future research should expand the evaluation to a larger number of genetic materials and include cost-benefit analyses, using the findings of this study as a basis for advancing the design of more efficient propagation strategies.

5. Conclusions

The results of this study demonstrate that pregerminative methods applied to cocoa seeds significantly influence germination and initial seedling development.

The removal of the tegument was consolidated as the most effective treatment to accelerate germination, while the presence of mucilage delayed this process. Likewise, pregerminative treatments that included immersion in water and *T. harzianum* promoted vigorous growth and reflected in better values of plant height, number of leaves, and stem

diameter. These findings indicate that pregerminative methods can be effective tools, economically accessible to farmers and with low environmental impact to optimize the botanical propagation of cocoa nursery crops.

Data Availability Statement

The original contributions presented in the study are included in the article, and further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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