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CHARACTERIZATION AND SCARIFICATION OF *NELTUMA* SPP. SEEDS FROM SOIL AND TREES

CARACTERIZACIÓN Y ESCARIFICACIÓN DE SEMILLAS DE *NELTUMA* SPP, PROCEDENTES DEL SUELO Y DE ÁRBOLES

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The carob tree (*Neltuma/Prosopis* spp.), a native species from Piura region (northeast Peru), is of great importance in the socioeconomic and ecological activity of the area. This tree is an important food source for humans and animals. There is little information about its sexual propagation. Therefore, in this study, the carob fruit and seed are characterized and chemical and biological scarification in the germination of carob collected from the soil and tree are evaluated. The evaluated treatments were: T0 (48 h imbibition), T1 (5 % tinner for 1 h), T2 (15 % tinner for 1 h), T3 (25 % tinner for 1 hour), T4 (35 % tinner for 1 hour), T5 (25 % biol for 20 min), T6 (50 % biol for 20 min), T7 (75 % biol for 20 min) and the germination variables: germination percentage, germination speed and germination index and first day of germination. The characterization of the fruit coincides with that reported in other studies on the identification of *Neltuma piurensis*. In soil-derived seeds, treatments T3 and T7 improved GP (62.5 % and 56.5 %), GS (0.87 and 0.75 germinated seeds per day), and GI (5.36 and 4.79). However, the FDG occurred one day after sowing compared to T0.

Key words: *biol, germination indicators, pod and seed biometry, seed scarification, tinner*

El algarobo (*Neltuma/Prosopis* spp.), especie nativa de la región de Piura (noreste de Perú), es de gran importancia en la actividad socioeconómica y ecológica de la zona. Este árbol es una importante fuente de alimento para humanos y animales. Existe poca información sobre su propagación sexual. Por lo tanto, en este estudio se caracteriza el fruto y la semilla de algarrobo, y se evalúa la escarificación química y biológica en la germinación de algarrobo recolectado de suelo y árbol. Se evaluaron los tratamientos: T0 (imbibición a 48 h), T1 (tinner 5 % por 1 h), T2 (tinner 15 % por 1 h), T3 (tinner 25 % por 1 hora), T4 (tinner 35 % por 1 hora), T5 (biol 25 % por 20 min), T6 (biol 50 % por 20 min), T7 (biol 75 % por 20 min) y las variables de germinación: porcentaje de germinación, velocidad de germinación e índice de germinación y primer día de germinación. La caracterización del fruto coincide con lo informado en otros estudios en la identificación de *Neltuma piurensis*. En semillas procedentes del suelo, los tratamientos T3 y T7 mejoraron el PG (62.5 % y 56.5 %), VG (0.87 y 0.75 semillas germinadas por día) e IG (5.36 y 4.79). No obstante, el PDG se dio un día después de la siembra con el T0.

Palabras clave: *biol, biometría de vaina y semilla, escarificación de semillas, parámetros de germinación, tinner*

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Introduction

The carob tree is a tree species native to the Piura region, initially known as *Prosopis pallida* and renamed as *Neltuma piurensis* (L. Vasquez, Escurra and Huaman) CE Hughes and GP Lewis (Hughes *et al.* 2022). Currently, this forest tree belongs to Fabaceae family, subfamily Caesalpinioideae (Hughes *et al.* 2022). The carob tree's distribution in the Americas extends from arid to tropical zones. In Peru, the carob tree is a representative species of the dry forests of the north, extending from the Tumbes region to the north of La Libertad (Rivera *et al.* 2020). The carob tree is of great socioeconomic and ecological interest in the Piura region (Caycho *et al.* 2023). It is used as forage for goats, sheep, and cattle. It is also used as a construction material, in artisanal medicine, and in local cuisine (Depenthal and Meitzner Yoder 2018). The carob fruit is composed of pod (90 %) and seed (10 %) and is used in human and animal feeding (Silva *et al.* 2000 and Zarzosa *et al.* 2021). Given its high nutritional value (carbohydrates, fiber, proteins and sugars) (Ludeña Gutiérrez *et al*. 2021), this fruit has been shown to be a good forage food and to increase the weight of goats (Silva *et al.* 2000) and rabbits (Macías-Rodríguez and Usca-Méndez 2017), in addition to reducing production costs. Several studies have reported that carob trees improve the physicochemical and biological characteristics of arid and semi-arid soils (Santos-Jallath *et al.* 2012 and Depenthal and Meitzner Yoder 2018).

In recent decades, this species has been threatened by unsustainable anthropogenic activity such as agricultural expansion, urban sprawl, climate change, and stress from biotic and abiotic factors. Because of this, it has been categorized as a vulnerable species, according to Supreme Decree Nº. 043-2006-AG, which categorizes threatened species of wild flora (Organismo de Supervisión de los Recursos Forestales y de Fauna Silvestre 2013). Currently, there is limited research focused on sexual propagation through carob seeds. Therefore, it is necessary to explore scarification strategies that increase seed germination (Asif *et al.* 2020) and technical information on the propagation of this species.

Seed germination is the initial phase of plantlets development, which begins with water absorption, giving rise to a sequence of metabolic processes until the emergence of the radicle (Huang *et al.* 2020, Xu *et al.* 2023 and Ruesta-López *et al*. 2024). A Good initial seed development favors the adaptability and initial vigor of plantlets and directly influences on crop yield (Vieira *et al.* 2019 and Morales Pizarro *et al.* 2023). The main form of propagation of carob tree is through seeds (sexual form), which have a very rigid testa (Ewens *et al.* 2022), which is why different scarification (stratification) strategies have been used: mechanical, thermal and chemical, which favor seed germination (Bhansali 2010). Based on the above, the objective of this study was to characterize the carob fruit and seed and evaluate chemical and biological scarification in the germination of carob seeds collected from the soil and the tree

Materials and Methods

The study was carried out in the laboratory of the Academic Department of Plant Morphophysiology, belonging to the National University of Piura, Peru, with coordinates 5°11'40.2" S, 80°37′58.2" W at 30 m o.s.l. (figure 1). The research was conducted from November 2023 to January 2024.

Plant material: In the collection of seeds, healthy, vigorous trees were selected from the Pariñas district, Talara province, Piura Region, located at 4°31'26.6" S, 81°12'26.2" W, at 15 m o.s.l. (figure 1). The fruits were collected based on two criteria: criterion 1 (directly from the tree) and criterion 2 (fallen on the soil).

Characterization of fruits and seeds: Fruit characterization was performed on 50 pods (fruits). The following were evaluated: length (L), width (W), and weight (W). For seed characterization, a total of 100 seeds were selected. Their L, W, W, and shape were determined.

The shape of the seed was determined by the relation between the width (W) and length (L): W/L>1 (flattened or ovoid), L/W=1 (spherical), W/L<1 (elongated or oblong), using the scale proposed by Peña-Castillo *et al.* (2023).

Products used: It was worked with commercial Thinner products (toluene, methyl acetate, methyl alcohol, 2-butoxyethanol and 4-methylpentan-2-one; Peruvian Chemical Products Corporation S.A. Av. César Vallejo 1851 - El Agustino Lima - Peru) and biol or biofertilizer was provided by the Department of Plant Morphophysiology, prepared using the technique of Chanduví-García *et al*. (2023).

Seed scarification treatments: The seeds were disinfected with 70 % alcohol for five seconds. Then, they were subjected to various scarification treatments: soaking in distilled water for 48 h (T0), 5 % tinner for 1 h (T1), 15 % tinner for 1 h (T2), 25 % tinner for 1 h (T3), 35 % tinner for 1 h (T4), 25 % biol for 20 min (T5), 50 % biol for 20 min (T6) and 75 % biol for 20 min (T7). Later, the seeds were sown in Petri dishes containing sterile paper towels moistened with distilled water. Each Petri dish contained 20 carob seeds and formed a repetition. Seed germination was evaluated for 15 d at 25±2 °C using the following variables:

Germination percentage (GP): The GP was determined using the formula proposed by Brown and Mayer (1988), where GP = (total germinated seeds/total sown seeds) * 100. Seeds were considered germinated when they had 2 mm of radicle length.

Germination speed (GS): It was calculated using the formula proposed by Maguire (1962), where GS = number of germinated seeds / germination time.

Germination index (GI): It was calculated with the formula proposed by Scott *et al.* (1984), where:

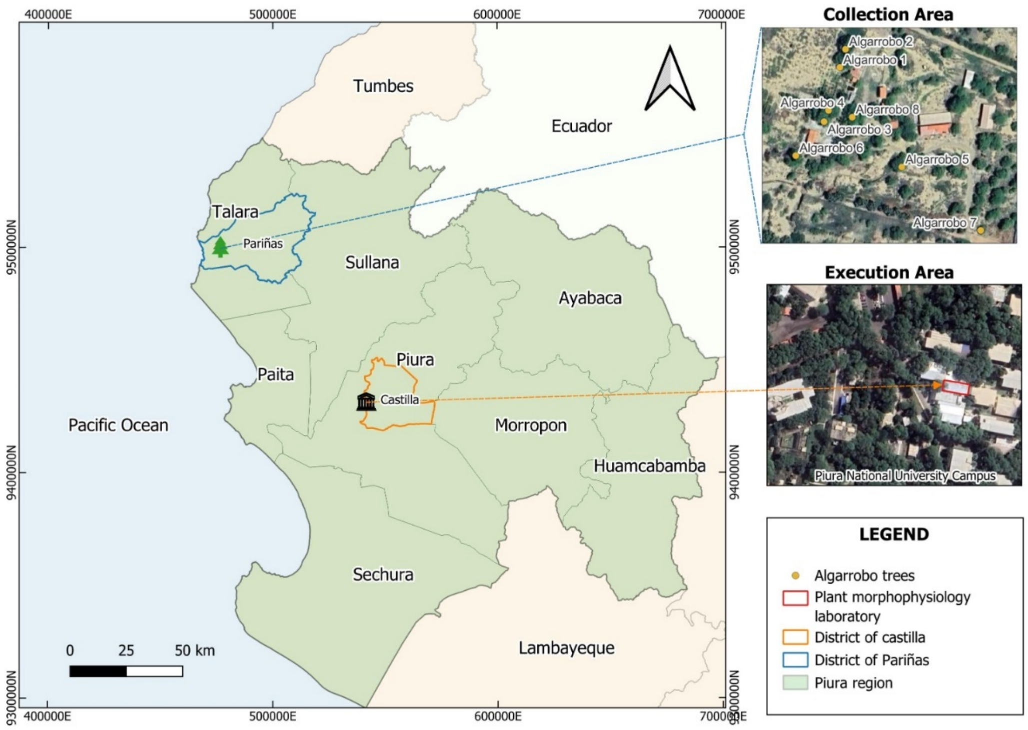

Figure 1. Carob harvesting and processing area.

GI = ∑ (the number of germinated seeds on the nth day * number of days after sowing) / total number of seeds sown.

First day of germination: The germination start time was evaluated using the method proposed by Gutiérrez-Gutiérrez *et al.* (2022).

Statistical analysis: For the characterization of fruits and seeds, the arithmetic mean (mean), standard deviation (SD) and coefficient of variation (CV) were used. The study was carried out in a completely random design (CRD) with eleven treatments and five repetitions per treatment. The obtained values were analyzed using ANOVA (analysis of variance) and Tukey's multiple comparison of means test ($P \leq 0.05$).

Results

Characterization of fruit and seed: The carob fruit (pod) had a length of 19.12 cm, a width of 12.10 mm, and a fruit weight of 7.58 g. The seed showed a length of 6.62 mm, width of 2.39 mm, weight of 0.05 g and the L/W ratio was 0.36 (table 1).

Germination variables: In the GP (tree), T4 with 32.5 % was significantly higher than the other treatments, followed by T3 with 23.75 % (table 2). In the GP (soil), T3 exceeded T0 by 20 %, being significantly higher than the other treatments (table 2). The highest GP was from seeds, whose fruits were collected from the soil compared to those harvested directly from the tree.

In the GS (tree), T4 with 0.43 germinated seeds per day significantly exceeded the other treatments (table 2), and in the GS (soil), the treatments did not has significant differences, with values between 0.5 to 0.87 germinated seeds per day (table 2). The highest GS values were obtained from fruits collected from the soil.

Table 1. Biometric description of fruits and seeds of carob tree Neltuma piurensis (L. Vasquez, Escurra & Huaman) CE Hughes & GP Lewis.

Description	Variable	Sample	Mean	SD	CV, %
Fruit	Pod length, cm	50	19.12	4.22	22.07
	Pod width, mm	50	12.10	1.43	11.81
	Pod weight, g	50	7.58	1.73	22.79
Seed	Seed length, mm	100	6.62	0.50	7.56
	Seed width, mm	100	2.39	0.22	9.29
	Seed weight , g	100	0.05	0.01	22.29
	Width/ length	100	0.36	0.03	9.30

Table 2. Analysis of germination variables of carob seeds Neltuma spp.

Treat*.	GP		GS		GI		FGD	
	Tree	Soil	Tree	Soil	Tree	Soil	Tree	Soil
T0	12.5±2.89[cd]	52.5±6.45[ab]	0.23[ab]	0.74[a]	0.75[cd]	4.17[a]	1.5[a]	1.0[a]
T1	15±4.08[bc]	43.75±2.5[ab]	0.21[ab]	0.61[a]	1.28[bcd]	4.0[a]	3.0[ab]	3.75[ab]
T2	15±7.07[bc]	41.25±8.54[ab]	0.23[ab]	0.64[a]	1.49[bc]	3.34[a]	7.75[c]	6.25[b]
T3	23.75±6.29[ab]	62.5± 9.57[a]	0.32[ab]	0.87[a]	2.33[ab]	5.36[a]	3.75[ab]	3.25[ab]
T4	32.5±5[a]	36.25±11.09[b]	0.43[a]	0.5[a]	3.41[a]	3.36[a]	3.5[ab]	4.5[b]
T5	2.5±2.89 [d]	41.25±16.0[ab]	0.17[b]	0.55[a]	0.19[d]	3.48[a]	1.5[a]	4.25[ab]
T6	15.0±4.08[bc]	50.0±7.07[ab]	0.25[ab]	0.69[a]	0.98[cd]	4.86[a]	3.5[ab]	3.75[ab]
T7	15.0±4.08[bc]	56.25±12.5[ab]	0.22[ab]	0.75[a]	1.29[bcd]	4.79[a]	4.5[b]	3.00[ab]
P.Value	0.0351	0.0000	0.0228	0.0928	0.0000	0.0547	0.0000	0.0049

In the GI (tree), T4 with 3.41 obtained the highest GI, being superior to the other treatments. The GI (soil) treatments did not has significant differences with values between 3.34 to 5.36 (table 2), which showed that the highest GI is obtained from the samples extracted from the soil.

In the FGD (tree), the T0 and T5 treatments germinated in a shorter time by 1.5 days (table 2). However, the FGD (soil), T0 germinated in 1 day, being its germination significantly faster, showing that the FGD was in seeds from the soil zone.

Discussion

In the characterization of fruits and seeds are agree with the research carried out by Prokopiuk *et al.* (2000) in the identification of *Prosopis pallida*, currently renamed *Neltuma piurensis* (L. Vasquez, Escurra & Huaman) CE Hughes & GP Lewis (Hughes *et al.* 2022). However, the seed width was smaller at 2.39 mm (table 1), showing that the environment influences on the carob genotype. The seed's L/W ratio indicates an oblong shape (Peña-Castillo *et al.* 2023).

Germination variation is influenced by the collection area, as well as the fruit (pod) maturity, sugar content, and storage time (Ludeña Gutiérrez *et al.* 2021). Likewise, Henciya *et al.* (2017) mention the presence of phytochemicals: alkaloids, flavonoids, terpenes, tannins, phenolic compounds (quinones) in fruits and seeds of *Neltuma/Prosopi* spp., which are in higher concentrations in freshly harvested fresh fruits, which influences on seed germination. However, dried pods used in animal feeding should not exceed 40 % of phytochemicals substances of those freshly harvested (Ruiz-Nieto *et al.* 2020). De Sousa Leite *et al.* (2018) show that the physiological maturity of the seed directly influences on the germination and vigor of the seed.

Regarding the results of this research, seeds from the soil showed the highest GP with chemical scarification with Thinner at 25 % (T3) and biological with Biol at 75 % (T7). These results are consistent with Abdala *et al.* (2020) in that chemical scarification based on sulfuric acid for three minutes had the highest GP compared to physical and thermal scarification. The effect of chemicals alters, degrades and softens the seed coat composed of hydrophobic and lignified substances (macrosclereids) present in the seeds of *Neltuma/Prosopis* genus. This lignified layer is responsible for preserving, protecting and preventing the imbibition of the seed. In addition, De Sousa Leite *et al.* (2018) increased the GP of *Bromelia laciniosa* seeds to 83 % with acetone for 90 min in mature seeds and to 60 % in immature seeds compared to the control with 40 % and 35 %, respectively. Similarly, these authors reduced the germination days to 15 (mature seed) and 25 (immature seed), days after sowing (DAS), compared to a control that began germination at 45 and 55 DAS, respectively. Rivera Curi *et al.* (2020) obtained the highest GP with 33.3 % in *P. pallida* seeds, treated with 100 % acetone for 10 min, being higher than the control with a GP of 30 %.

Carob seeds treated with biol significantly improved GP. These results are consistent with those described by Ruesta-López *et al*. (2024) in passion fruit (*Passiflora edulis* S.) seeds treated with 5 % biol, increasing GP to 80 % during 12 h of imbibition. However, at 24 h the GP was lower with 8.33 %. According to Chanduví-García *et al.* (2023), Galecio-Julca *et al.* (2023) and Morocho-Romero *et al*. (2024), the biol has carboxylic acids, humic acids and microorganisms, which weaken the seed coat and increase the GP. Utello *et al.* (2023) show that heat treatments reduce the growth of embryonic roots compared to chemical or mechanical treatments performed on seeds.

The highest germination speed is in seeds extracted from the soil with treatments based on Thinner at 25 % (T3) and Biol at 75 % (T7). These products weaken the seed layer of carob seed, which facilitates seed imbibition or hydration; activating various metabolic processes that initiate seed germination and gas exchange (Batista Sánchez *et al.* 2017 and Utello *et al.* 2023). Ruesta-López *et al.* (2024) mention that passion fruit seeds exposed to 5 % biol for 12 h, increase the GS to 1.5 germinated seeds per day, surpassing the control. The positive effect of the biostimulant (biol) significantly influences on cell multiplication, promoting the development and emergence of the radicle (Mendivil-Lugo *et al.* 2019). The highest GI was obtained with seeds collected from the soil with chemical treatments T3 (tinner 25 %) and organic treatments T6 (biol 50 %) and T7 (biol 75 %). These results coincide with those reported by Abdala *et al.* (2020), indicating that chemical scarification (sulfuric acid) improves the germination speed index (GSI). On the other hand, Utello *et al.* (2023) report that mechanical scarification (sanding) was superior to chemical (sulfuric acid) and thermal scarification, improving the uniformity and spontaneity of the germination process, being a key factor in the propagation stage in the nursery. Coa *et al.* (2014) mention that the combined effect of mechanical scarification and imbibition at 12 and 24 h of the seeds, improved the GI compared to chemical treatments (sulfuric acid and muriatic acid) and without scarification with imbibition. On the other hand, Ruesta-López *et al.* (2024) improved the GI in passion fruit seeds treated with 5 % biol.

The FDG was lower at T0 (48 h imbibition) in seeds from the soil (1 d) and from the tree (1.5 d). The results of the study differ from those obtained by Abdala *et al.* (2020), who show that chemical scarification (sulfuric acid) accelerates the beginning of germination. Morales Pizarro *et al.* (2023) show that the imbibition time and the concentration of stimulants influences on the FDG. Similarly, Ruesta-López *et al.* (2024) accelerated the FDG by imbibition of seeds for 12 h and 24 h. However, when passion fruit seeds were treated with higher concentrations of biol, the FDG was delayed.

Conclusions

The morphometric characteristics of the fruit and seeds coincide with those from *Neltuma piurensis* genus. However, the seed width shows to be larger than those described for this species. Seed maturity is a key factor in seed germination of *Neltuma* spp. genus. The chemical pre-germination treatment with Tinner (T3), followed by the organic treatment with biol (T7), improved the germination variables GP (62.5 % and 56.25 %), GS (0.87 and 0.75 germinated seeds per day) and GI (5.36 and 4.79) and the FDG was given a DAS with T0, the best values were showed by seeds from the soil.

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