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Original Article

Evaluation of pregerminative treatments on seeds of *Euterpe precatoria* Mart. (Huasaí) in the city of Pucallpa-Peru

Evaluación de tratamientos pregerminativos en semillas de *Euterpe precatoria* Mart. (Huasaí) en la ciudad de Pucallpa-Perú

Avaliação dos tratamentos pregerminativos nas sementes de *Euterpe precatoria* Mart. (Huasaí) na cidade de Pucallpa-Peru

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ABSTRACT

In the research, pre-germination treatments applied to *Euterpe precatoria* Mart. seeds were evaluated, with the aim of observing their germination capacity, reducing the average germination time, making germination uniform and increasing the germination value. Eight pre-germination treatments are used: immersion in water at room temperature for 24, 72 and 120 hours; immersion in boiling water for 30, 60 and 90 seconds; scarification with sandpaper and scarification with sandpaper and immersion in water at room temperature for 24 hours at 20% of the cover, as well as one without any treatment. The experimental design was factorial, completely randomized, with four replicates per treatment and 25 seeds per replicate. Analysis of variance ANOVA with Duncan's test was applied to establish differences between treatments. The results showed significant differences value *(P<0.05*) between the pre-germination treatments and the control, with the exception of immersion in water at room temperature for 24 hours. The treatments with the highest percentage of germination were with water at room temperature, where 88, 76, and 62 % germinated respectively, followed by the scarified ones with 44 and 35 %, and the control with 30 % and the treatments with boiling water did not generate any germination. The treatment at room temperature for 72 hours, was the one that showed the best values of average germination time, greater germination uniformity and higher germination value, so it is the best to be applied to improve the germination capacity of *E. precatoria* Mart seeds.

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Keywords: *Euterpe precatoria*; pre-germination; dipping; scarification.

RESUMEN

En la investigación se evaluaron tratamientos pregerminativos aplicados a semillas de *Euterpe precatoria* Mart., con la finalidad de observar su capacidad germinativa, disminuir el tiempo medio de germinación, uniformizar la germinación y aumentar el valor germinativo. Se emplean ocho tratamientos pregerminativos: inmersión en agua a temperatura ambiente por 24, 72 y 120 horas; inmersión en agua hirviendo por 30, 60 y 90 segundos; escarificación con lija y escarificación con lija e inmersión en agua a temperatura ambiente por 24 horas al 20 % de la cubierta, así como una sin ningún tratamiento. El diseño experimental fue factorial, completamente aleatorio, con cuatro repeticiones por tratamiento y 25 semillas por repetición y se aplicó análisis de varianza ANOVA con prueba de Duncan para establecer diferencias entre los tratamientos. Los resultados mostraron diferencias significativas valor *(P<0.05*) entre los tratamientos pregerminativos y el control, a excepción de la inmersión en agua a temperatura ambiente por 24 horas. Los tratamientos con mayor porcentaje de germinación fueron con agua a temperatura ambiente, donde germinaron 88, 76, y 62 % respectivamente, seguidas por los escarificados con 44 y 35 %, y el control con 30 % y los tratamientos con agua hirviendo no generaron germinación alguna. El tratamiento a temperatura ambiente por 72 horas, fue el que mostró los mejores valores de tiempo medio de germinación, mayor uniformidad germinativa y mayor valor germinativo, por lo que es el mejor a ser aplicado para mejorar la capacidad germinativa de las semillas de *E. precatoria* Mart.

Palabras clave: *Euterpe precatoria*; pregerminación; inmersión; escarificación.

SÍNTESE

Na pesquisa, foram avaliados tratamentos pregerminativos aplicados à *Euterpe precatoria* Mart. sementes, com o objetivo de observar sua capacidade germinativa, reduzindo o tempo médio de germinação, uniformizando a germinação e aumentando o valor germinativo. São utilizados oito tratamentos pré-germinação: imersão em água à temperatura ambiente durante 24, 72 e 120 horas; imersão em água a ferver durante 30, 60 e 90 segundos; escarificação com lixa e escarificação com lixa e imersão em água à temperatura ambiente durante 24 horas a 20 % da cobertura, bem como um sem qualquer tratamento. O desenho experimental foi fatorial, completamente randomizado, com quatro réplicas por tratamento e 25 sementes por réplica. A análise de variância ANOVA com o teste de Duncan foi aplicada para estabelecer diferenças entre tratamentos. Os resultados mostraram diferenças significativas (*P<0,05*) entre os tratamentos de pré-germinação e o controle, exceto pela imersão em água à temperatura ambiente por 24 horas. Os tratamentos com maior percentagem de germinação foram os tratamentos com água à temperatura ambiente, onde germinaram 88, 76 e 62 % respectivamente, seguidos dos escarificados com 44 e 35 %, e o controlo com 30 % e os tratamentos com água a ferver não geraram qualquer germinação. O tratamento à temperatura ambiente durante 72 horas mostrou os melhores valores de tempo médio de germinação, maior uniformidade de germinação e maior valor de germinação, por isso é o melhor a ser aplicado para melhorar a capacidade germinativa das sementes de E. precatoria Mart.

Palavras-chave: *Euterpe precatoria*; prégerminação; imersão; escarificação.

INTRODUCTION

The *Euterpe Precatoria* Mart. (Huasaí) is a palm tree found in the Amazon floodplain and is highly valued for its high nutritional value; studies on its nutritional composition indicate the presence of amino acids, fatty acids, dietary fiber, vitamin A, vitamin C, iron and calcium (Peixoto *et al.,* 2016), Isaza *et al.,* (2014). This palm is naturally found below 2000 m in the firmness forests and along the banks of rivers, in periodically flooded areas that grow from Belize in the north to Brazil and Bolivia in the south (Paniagua-Zambrana, Bussmann and Macía, 2017). León and Saldaña (2011) indicate that these palms are a very important group of plants for the inhabitants of rural populations in the Amazon, because they benefit from their multiple uses. They use the trunks and leaves to build houses, the fibers to make crafts, the fruits and palm heart for food.

In addition to the traditional uses of the plant, it has been studied for its medicinal potential, as in the case of the work of Peixoto *et al.,* (2016), who studied the antioxidant capacity of the fruits of E. precatoria, a property also investigated by Ortega, (2015) and Kang *et al.*, (2012) Likewise, Galotta, Boaventura and Lima, (2008) demonstrated that the fruits of the palm have antioxidant and cytotoxic properties. This shows the importance of the reproduction of the plant, not only to preserve a valuable resource in rural communities, but also as a treatment in medicine.

It has been shown that the germination of *E. precatoria Mart* seeds is slow and uneven, often resulting in low seedling production. Its propagation is done from seeds with a great variation in the germination process, which is also influenced by several factors such as temperature, light and humidity (Xavier *et al.,* 2018). In the search to improve the germination of *E. precatoria*, works have been carried out where pre-germination treatments have been applied by means of hydration (De Souza *et al.*, 2018) and it has been demonstrated that rehydration is favourable because, through it, a higher percentage of germination is achieved. Similarly, León and Saldaña, (2011) investigated the germination potential of *E. precatoria* seeds by subjecting them to hydration, heating and partial wear treatments, obtaining significant differences in the germination percentage with respect to the treatments.

In the present research, the objective was to evaluate the effect of germination treatments on the germination capacity, average germination time, germination uniformity and germination value of E seeds. *precatoria Mart* seeds, subjected to scarification and soaking treatments in cold and hot water, in order to establish the pre-germination treatment that produces a greater germination efficiency, which allows the production of a greater quantity of seedlings, which can be used for future projects of sowing the plant, for the sake of its massification and sustainable use of its properties, both food and building material and medicinal.

MATERIALS AND METHODS

The experiment took place at the Vivero Forestal of the Universidad Nacional de Ucayali (UNU), located at Km. 6000 of the Federico Basadre Highway. Politically it is located in the District of Callaría, Province of Coronel Portillo, Department of Ucayali. The nursery is of a permanent type, with a production capacity of 200 thousand plants per year. Seedbeds and chimes have constructions based on rustic material such as wood and roof of leaves typical of the place. According to the meteorological station of the Universidad Nacional de Ucayali, the annual average climatic conditions in the city of Pucallpa are: average annual temperature of 26.9 ºC, with a maximum

of 36.5 ºC and a minimum of 17.4 ºC; average annual precipitation of 1773 mm; solar brightness of 159.1 h and R.H. 83.8 %.

The plant materials used in this research were 900 fruits of *E. precatoria Mart*. Other materials and tools were: scale, stove, water sandpaper No. 240, copper oxychloride (cupravit fungicide), seedbed, substrate 1:2:1 sand, agricultural soil and organic matter (decomposed sawdust).

The work was done with 10 kg of seeds collected from four trees in the Curimaná area. The fruits were collected from the father tree with the following characteristics: average age 15 years, between 12.5 to 15 cm, height between 20 and 25m; it was found associated with "Pona", "Unguragui". The collected fruits were moved to the study site and spread on a table for drying at room temperature under shade. Then the fruit was manually shelled to separate the seeds from the fruit, using wooden and stone tools. 900 seeds were selected manually, taking into account the shape and size of the seeds, discarding the smaller and deformed seeds.

The following characteristics of the seeds were measured

Seed quality: quality seeds and defective seeds (mesocarp remains in the fruit) were weighed. The following formula was used to calculate the percentage of seed quality (Mancera *et al.*, 2007) (Equation 1).

$$\%P = \frac{Pp}{Pt} \times 100 \qquad (1)$$

Where:

%P=Percentage of quality.
Pp=Quality seed weight.
Pt=Total sample weight (quality seed weight + weight of defects).

Number of seeds per kilogram: for the calculation of this variable, 10 samples of 100 seeds each were taken at random and weighed. The weight per 100 seeds was then averaged. To determine the number of seeds per kilogram, the formula (Equation 2).

$$N^{o}\text{of seeds} = \frac{1000 \times 100}{\text{average weight of 100 seeds}} \qquad (2)$$

Moisture content of seeds: to determine the moisture content of the seeds, 5 random samples of 10 seeds were taken and weighed, then dried in the oven at a temperature of 103 ± 2 ºC until their weight became constant. For the calculation of moisture content, the following formula was applied as described by Rojas and Aristizábal (2012) (Equation 3).

$$\%H = \frac{(P_i - P_f)}{P_i} \times 100 \qquad (3)$$

Where:

H=Percentage of humidity of the sedes.
Pi=Seed initial weight.
Pf=Final weight of the sedes.

After the characterization of the seeds, they were disinfected with the use of copper oxychloride (cupravit) to avoid the attack of fungi, taking into account the following

- The seeds that were not soaked before planting were treated with copper oxychloride powder (cupravit), in the case of treatment 1 (control) 8 scarification with sandpaper.
- Seeds treated with liquid were immersed in copper oxychloride solution (cupravit) at a concentration of 5 g/litre for 5 minutes.

Nine pre-germination treatments were established, which are described in Table 1.

Tabla 1. - Description of the treatments applied

Treatments	Description
T1	Witness, no treatment was applied.
T2	Immersion of seeds in water at room temperature for 24 hours
T3	Immersion of seeds in water at room temperature for 72 hours
T4	Immersion of seeds in water at room temperature for 120 hours
T5	Immersion of seeds in boiling water for 30 seconds
T6	Immersion of seeds in boiling water for 60 seconds
T7	Immersion of seeds in boiling water for 90 seconds
T8	Scarification with water sandpaper N° 240, 20 % of the area of the cover of the seed.
T9	Scarification with water sandpaper No. 240, 20 % of the area of the seed cover and immersion in water at room temperature for 24 hours

The seedbed of the UNU's Vivero Forestal was conditioned and disinfected. It was conditioned, removing the substrate and the shed was built, using semi-shelled palm leaves. For the disinfection of the substrate, a cupravit solution (5 g/l)/m2 was prepared and then applied to the entire area of the bed. After disinfecting the seedbed and carrying out the treatments, the seeds were seeded at a distance of 7x7 cm. Each treatment was seeded with four repetitions, with 25 seeds per repetition. The germination count was made during 105 continuous days.

The experimental design was unifactorial, completely randomized (Kuehl, 2001), with four replicates per treatment and 25 seeds per replicate where:

Factor: pre-germination treatments.

Factor levels: treatment variants (T1, T2, T3, T4, T5, T6, T7, T8, T9).

Experimental unit: plot with 25 seeds.

Response variable: germination of seeds.

Percentage of germination (% G): all seeds germinated by treatment during the time of the experiment were counted and the percentage of germination was calculated (Equation 4).

$$\%G = \frac{GA}{M} \times 100 \qquad (4)$$

Where:

%G=Germination percentage.
GA=Germination accumulated until the last evaluation.
M=Quantity of seeds sown.

Average Germination Time (GMT): was calculated using the equation shown by Martinez, Miranda and Magnitskiy, (2012) (Equation 5).

$$TMG = \frac{Spg}{Sg} \qquad (5)$$

Where:

TMG= Medium Term Germination Time.
Spg=Sum of mean points per single germination.
Sg=Sum of germinated seeds.

(Equation 6)

$$Spg = \frac{(T_1+T_0)}{2} \times G_1 + \frac{(T_2+T_1)}{2} \times G_2 + \ldots + \frac{(T_e+T_{e-1})}{2} \times G_e \qquad (6)$$

Germination Uniformity (UG): to evaluate the Germination Time Typical Deviation (DTG) was determined.

Germinative Value (GV): For its determination, the formula described by Barone, Duarte and Luna, (2016) was used (Equation 7).

$$VG = \frac{\frac{G_1}{T_1} + \frac{G_2}{T_2} + \ldots\ldots + \frac{G_e}{T_e}}{M} \qquad (7)$$

Where:

VG=Germination value or Maguire index.
Gi=G1 + G2 +G3 +. . . + Ge.
Gi=Simple nth evaluation germination.
Ti = T1 + T2 + ... + Te
*Ti=Time elapsed from so*wing to the ith evaluation
M=Quantity of seeds sown.

Previously, a Shapiro-Wilk test was carried out to analyse the assumption of normality of the data, obtaining a statistic of 0.924 and a P-value of 0.0715. The results of the normality test indicate that it cannot be discarded that the data fit a normal distribution with a 95 % confidence level, so Analysis of Parametric Variance (ANOVA) with Duncan's test at a significance level α=0.05 was used. The calculations were performed with the statistical package StatgraphicsCenturion XVII.

RESULTS AND DISCUSSION

The development of the proposed experimental design generated the results shown in Table 2, where the germination percentages obtained with the pre-germination treatments are observed (Table 2).

Table 2. - Percentage of *E. precatoria Mart* germination under the effect of nine pre-germination treatments

Repetitions	Treatments								
	T1	T2	T3	T4	T5	T6	T7	T8	T9
1	32	60	80	76	0	0	0	36	32
2	36	68	92	80	0	0	0	48	36
3	24	64	92	72	0	0	0	40	28
4	28	56	88	76	0	0	0	52	44
Average	30	62	88	76	0	0	0	44	35
Coefficient of variation (%)	17,2	8,3	6,4	4,3	0	0	0	16,6	19,5

The Table 2 shows that with the treatments T5, T6 and T7 there was no germination of the seeds, despite the care and similar handling that was applied to the whole experiment, which makes them not suitable for the study, so they were discarded (Table 2).

A higher percentage of germination is observed with the T3 treatment, in which the seeds were rehydrated for 72 h in cold water, a result that contrasts with that reported by León and Saldaña, (2011), who show that all *E. precatoria* seeds treated by immersion in cold water showed germination percentages higher than 90 %. The same authors reported germination percentages for untreated seeds and partially worn out seeds greater than 80 %, a result that is also contrary to what was observed in the present research. It should be taken into account that, in the case of the cited research, the seeds were obtained from the region of Loreto in Peru and subjected

to different conditions than those established in the present research, which could influence the differences observed.

De Souza *et al.,* (2018) showed results of germination percentage of *E. precatoria* pretreated by soaking for 72 h of 61.7 %, lower value than the one obtained in the present investigation, which is similar to the one of seeds treated by soaking for 24 h in water at room temperature. This corroborates that the germination potential of the seeds does not depend exclusively on the treatments applied, but also on the origin and quality of the seeds, since the authors cited worked with seeds obtained from the forest nursery of the Universidad Federal de Amazonas in Manaus, Brazil.

The effect of the temperature to which the seeds were submitted was determinant. It was observed that the pre-treatments where the seeds were soaked in boiling water did not promote the germination of the seeds but, on the contrary, inhibited it. In their research, Xavier *et al.,* (2018) showed that temperature is fundamental for the germination of *E. precatoria*, concluding that, at higher temperatures, lower percentages of germination are obtained, which coincides with what was observed in the present research. The negative effect of treatment with boiling water was also reported by Flores-Córdova *et al.,* (2016), who used seeds of fodder species and weeds in their research and obtained that the treatment of immersion in hot water can produce damage to the embryo, because it can be aggressive which caused non-germination.

It is also observed that the treatments by scarification presented lower percentages of germination compared to the treatments by hydration. This treatment has been previously studied for seeds of other species and it has been proven that its effectiveness depends on the seed (Martínez, Orozco and Martorell, 2006; Ortiz-Timoteo *et al.,* 2018).

When applying the analysis of variance, it was observed that the P-value is less than the established significance *(P-value=0.0000< 0.05*), which indicates that the treatments significantly influence the results obtained for the response variable (% germination).

To examine which measures of the treatments are significantly different from others, the Duncan's mean difference test was applied (Table 3).

Table 3. - Results of Duncan's multiple range analysis

Treatments	Cases	average	Homogeneous Groups
T1	4	30,0	A
T9	4	35,0	A
T8	4	44,0	B
T2	4	62,0	C
T4	4	76,0	D
T3	4	88,0	E

Averages with a common letter are not significantly different *(p > 0,05)*

It is only observed that T9 shows no difference with respect to T1 (control); the rest of the treatments show higher germination percentage values and with significant differences with respect to T1. It can therefore be said that scalding the seeds does not generate a significant difference in the percentage of germination with respect to seeds that have not been treated; although statistically T8 is different from T1, its value is lower than treatments based on cold water immersion. The Figure 1 shows the growth curves obtained for each of the treatments (Figure 1).

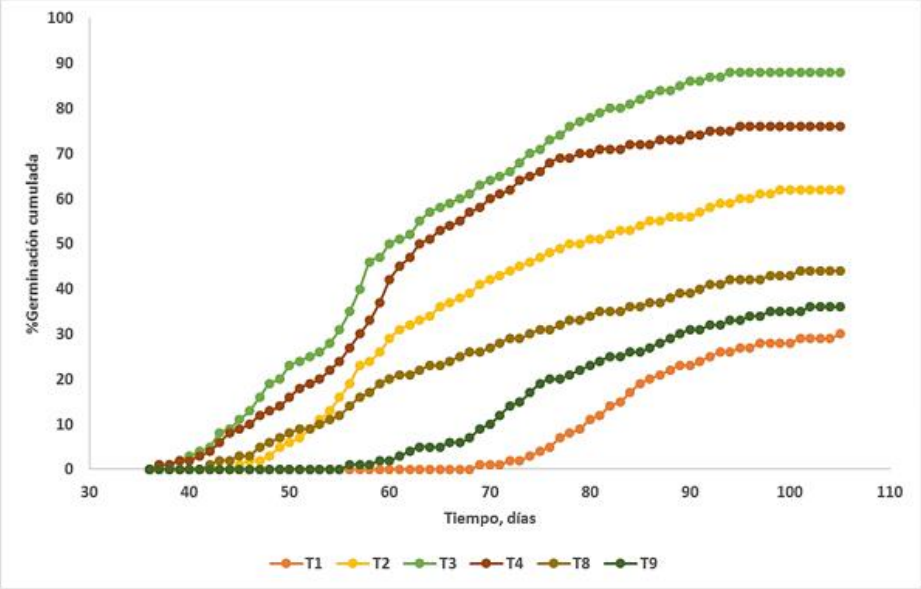

Figure 1. - Germination curves of *E. precatoria* seeds as a function of treatments

It is observed that the seeds treated with T3 and T4 began to germinate in a shorter time, compared to the others, being T1 where a longer period of time was obtained without observing any germination. This corroborates the effectiveness of pre-germination treatments, since even T9, which had no statistical difference with T1, allows the seeds to start germinating in a shorter time.

The trends observed in the germination curves show a typical expected behavior, compared with those obtained by other authors such as Rodríguez, Adam and Duran, (2008) in their work on germination and seed viability. Likewise, Torres-Osorio, Aranzazu-Osorio and Carbonell-Padrino, (2015) show germination curves of soybeans, which also behave in a similar way to what has been observed in the present research.

The Table 4 shows the result of the calculation of the different parameters established for the investigation, according to the type of pre-germination treatment applied.

Table 4. - Parameters calculated for seeds according to the treatments applied

Treatments	TMG (days)	UG (days)	VG (%)
T1	83,7	8,5	0,36
T2	65,5	14,1	0,98
T3	60,9	14,0	1,51
T4	60,4	12,7	1,30
T8	66,2	16,3	0,70
T9	76,5	11,6	0,46

TMG: Average Germination Time; UG: Germination Uniformity; VG: Germination Value

The average germination time indicates the average time from the start of germination to the last seed germinated, plus the time of resistance to germination. It can be seen from table 4 that the T1 treatment has an average germination time of 83.7 days; during this time the germination percentage was between 15 and 17 %, this being the treatment with the highest TMG. The T3 and T4 treatments represented the shortest average germination time with 60.9 and 60.4 days respectively. In the case of T3, between 50 and 51 % of seeds germinated during this time, which represented the highest yield. From all this it can be said that the treatments T3 and T4 had lower average germination times and presented higher percentages of germination. This indicates that water accelerates the germination process, which is consistent with what has been obtained in other research (De Souza, Jacobi and De Aquino, 2007; León and Saldaña, 2011; De Souza *et al.,* 2018; Villar, Marcelo and Baselly, 2018).

Germination Uniformity (GU) indicates how uniform the germination is in each of the treatments. This means that the T1 treatment had a value of 8.5 days, which indicates that in the range of 75.2 to 92.2 days (17 days) approximately 22 seeds germinated. This low percentage of germination in a short period of time indicates that 1 or 2 seeds germinated daily, for the reason that these seeds, at the time of the almacigado, did not have the appropriate moisture content. The value obtained for seeds that were immersed in water at room temperature for 72 hours (T3), was the treatment with the highest percentage of germination, of 14 days, which indicates that of the total of seeds germinated in this treatment 58 did it in a time interval of 46.9 to 74.9 days (28 days).

Also, T8 represented the treatment with the highest UG with 16.3. This means that, of 44 seeds that germinated with this treatment, 28 did so in a time interval of 49.9 to 82.5 days (32.6 days). It can be said then that, the seeds without any treatment (T1) present smaller range of time (17 days), compared with the other pre-germinative treatments, which present ranges of greater times; the T1 is the less uniform in its germination and presenting low percentage of germination and the T3 showed the greater uniformity in its germinative process. The results are consistent with the germination response observed by De Souza *et al.,* (2007) and those of León and Saldaña (2011), where the previous hydration of the seeds generates a greater uniformity in germination. The uniformity and germination capacity of seeds pre-treated by immersion in water was also reported by Navarro *et al.,* (2010), who reached conclusions consistent with those of the present research.

In relation to the results of the Germination Value (GV), which is an indication of the average quantity of seeds that germinated per day expressed as a percentage, it is observed that the seeds immersed in water at room temperature for 72 hours (T3) have a germination value of 1.51 %, higher than the germination values of the other treatments. This indicates that, for each day, 1,51 seeds germinate. Furthermore, this treatment applied to *E. precatoria Mart*. seeds improves the germination rate (germination value) per day, because the water softens the integument and endosperm of the hard seeds, as stated by Sánchez *et al.,* (2005); Pérez *et al.,* (2016) and González-Amaya *et al*., (2018).

The best pre-germination treatments of *E. precatoria Mart.* seeds were those of immersion in water at room temperature for 72, 120 and 24 hours. Likewise, these treatments were the ones with the longest average germination times, where those immersed for 72 and 120 hours stood out with 60.9 % and 60.4 % respectively. Similarly, the treatment by immersion in water at room temperature for 72 hours produced the most uniform germination, with a germination uniformity of 14.0 days and a higher germination value of 1.5 %.

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