

## Osmopriming increases jussara palm seed germination

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### ABSTRACT

Jussara palm (*Euterpe edulis* Mart.) is an economically significant palm due to the extraction of the palm heart and acai fruits. However, both extractions reduce the ability of the plant to recover in nature. Thus, it is essential to understand and improve the palm propagation methods. Osmoconditioning is a technology that maximizes seed germination rates and seedling production. In this context, this study aimed to evaluate the effect of different potassium nitrate concentrations on the jussara palm seed germination process. The experiment was set in a completely randomized design. The treatments consisted of five concentrations of KNO<sub>3</sub> (0 - control, 1, 2, 3, 4, and 5 g L<sup>-1</sup>). Four replications were used per treatment, with 25 seeds each. Data collection occurred daily, considering seeds that emitted the coleoptile as germinated until germination stabilized for all treatments. The Germination Speed Index (GSI) was evaluated, and the germination percentage (%) was determined at the end of the experiment. It was found that priming with KNO<sub>3</sub> had a significant effect (p<0.05) on all evaluated characteristics. The seeds of the jussara palm showed a linear response with increasing concentrations for germination percentage. Osmoconditioning with KNO<sub>3</sub> promotes an increase in the percentage and speed of germination of jussara seeds.

**Keywords:** *Euterpe edulis* Mart.; Arecaceae; Potassium nitrate; Osmoconditioning.

### Osmopriming aumenta a germinação de sementes da palmeira juçara

A palmeira juçara (*Euterpe edulis* Mart.) é uma espécie de grande importância econômica devido à extração do palmito e dos frutos do açaí. No entanto, ambas as extrações reduzem a capacidade da planta de se recuperar na natureza. Assim, é fundamental entender e aprimorar os métodos de propagação da palma. O osmocondicionamento é uma tecnologia que maximiza as taxas de germinação de sementes e produção de mudas. Nesse contexto, este estudo teve como objetivo avaliar o efeito de diferentes concentrações de nitrato de potássio (KNO<sub>3</sub>) no processo de germinação de sementes de palmeira juçara. O experimento foi montado em um delineamento inteiramente casualizado. Os tratamentos consistiram em cinco concentrações de KNO<sub>3</sub> (0 - controle, 1, 2, 3, 4 e 5 g L<sup>-1</sup>). Foram utilizadas quatro repetições por tratamento, com 25 sementes cada. A coleta de dados ocorreu diariamente, considerando as sementes que emitiram o coleóptilo como germinadas até a germinação estabilizar para todos os tratamentos. Foi avaliado o Índice de Velocidade de Germinação (GSI) e ao final do experimento foi determinado o percentual de germinação (%). Verificou-se que o priming com KNO<sub>3</sub> teve efeito significativo (p<0,05) em todas as características avaliadas. As sementes de juçara apresentaram resposta linear com o aumento das concentrações para porcentagem de germinação. Osmocondicionamento com KNO<sub>3</sub> promove aumento na porcentagem e velocidade de germinação em sementes juçara.

**Palavras-chave:** *Euterpe edulis* Mart.; Arecaceae; Nitrato de potássio; Osmocondicionamento.



## 1. Introduction

*Euterpe edulis* Mart. is a species belonging to the Arecaceae family, also known as jussara palm, and occurs in the Atlantic Forest biome. This palm is well known for producing two economically important products, the palm heart and the açai fruit. However, it does not regenerate after the palm heart extraction, meaning that the extraction leads to plant death. On the other hand, the fruit harvest occurs annually, providing highly nutritious, antioxidant, and anthocyanin-rich açai pulp (Baptista et al., 2021; Pereira et al., 2022).

Indiscriminate harvest of fruits reduces the natural re-occurrence of jussara palms by decreasing seed dispersal. Several frugivorous birds feed on the berries, and with reduced food availability, these birds move away, thus decreasing natural dispersal (Leite et al., 2012; Carvalho et al., 2021). The increased interest in palm heart products has reduced the native populations of this species. Therefore, there is a need for restoration in the areas where the palm heart is collected to ensure the continuity of production (Brasil, 2017).

As seed germination is the propagation method of jussara palm, the germination is indifferent to light and has a high germination rate in the temperature range of 25-30 °C. The presence of the mesocarp reduces seed germination (Aguilar et al., 2017). Understanding the germination processes of seeds enables their use, whether as ornamental plants or for forest restoration. However, developing technologies that facilitate seed production, quality, commercialization, and conservation is challenging, particularly for palm seeds, which are recalcitrant and may have dormancy (Beckmann-Cavalcante et al., 2012; Ferreira et al., 2021).

Different technologies of pre-germination treatments are often used to increase, accelerate, and uniformize germination rates. Osmoconditioning is one of these technologies, which involves immersing the seeds in an osmotic solution for a specific time and temperature, where the osmotic potential of the solution regulates the amount of water to be absorbed. Thus, the reduction in water potential in the medium accompanies an increase in the osmotic pressure of the solution (Ribeiro et al., 2015; Johnson and Puthur, 2021).

Potassium nitrate ( $KNO_3$ ) is an osmotic agent, a solution that stimulates germination and has been used by several authors (Gallon et al., 2018; Boroujerdnia et al., 2020; Ciacka et al., 2022). The mechanism of action of potassium nitrate is to act in electron reception, reduce the nitrite form in the seeds, reoxidize NADPH, increase NADP utilization, reduce the dehydrogenase of the pentose phosphate cycle, and help overcome seed dormancy (Marcos Filho, 2015).

The application of this technology allows for maximizing seed germination. Thus, using osmoconditioning for jussara seed germination can be

an alternative option to increase seed germination rates and seedling production. In this context, this study aimed to evaluate the effect of different potassium nitrate concentrations on the jussara palm seed germination process.

## 2. Material and Methods

The experiment was conducted during May and June 2022 at the Horticultural Seed Laboratory, located in the Department of Agricultural Sciences, Plant Production Sector, of the College of Agricultural and Veterinary Sciences (FCAV) at São Paulo State University (UNESP), Campus of Jaboticabal, SP, Brazil.

The fruits of *E. edulis* were harvested from three plants cultivated in the Experimental Nursery of Ornamental and Forest Plants at FCAV/UNESP, Jaboticabal (coordinates: 21°15'2"S, 48°16'47"W, and 600 meters above sea level). The climate in the Jaboticabal region is subtropical, classified as Cw-type (humid tropical with a dry winter and rainy summer), with minimum, average, and maximum temperatures of 19.8 °C, 24.5 °C, and 32.5 °C, respectively (Köppen, 1948). After harvesting, the fruits were placed in plastic boxes and transported to the laboratory.

The fruits were depulped in the laboratory by manually rubbing them against a stainless-steel sieve (6 mm mesh size) under running water to remove residues. The diaspores (seeds with adhered endocarp) were then subjected to asepsis by soaking them in a 2% sodium hypochlorite solution for 10 minutes, followed by rinsing with running water.

Following depulping, the seed moisture content was determined. Two subsamples of 10 seeds each were taken, and the moisture content was determined using the oven-drying method at  $105 \pm 3$  °C for 24 hours (Brasil, 2009). The water content found was 19.7%. A completely randomized experimental design was adopted.

The treatments consisted of five concentrations of  $KNO_3$  (0 - control, 1, 2, 3, 4, and 5 g L<sup>-1</sup>). Four replications were used per treatment, with 25 seeds each.

On the evaluation of the priming effect, the seeds were pre-soaked in distilled water (hydropriming - control) or  $KNO_3$  solutions (osmopriming) using transparent plastic seed germination boxes ("gerbox") measuring 11 × 11 × 3 cm. Fifty seeds were placed in each box, along with 50 mL of each solution. The boxes were closed to prevent solution evaporation and placed in a Biochemical Oxygen Demand (BOD) germination chamber for 24 hours at 25 °C under an 8-hour photoperiod. After the pre-soaking period, the excess solution was removed with distilled water, and the seeds were air-dried on a bench until they reached their initial

moisture content or a similar value. To restore the water content to a level close to its initial value, before applying the treatments, the samples from each treatment were initially weighed on a precision scale (SHIMADZU®, model AY220) with an accuracy of 0.001 g. Following the conditioning period, successive weighing was performed until the samples reached a mass equivalent to that recorded before conditioning.

After drying, the seeds were distributed in transparent plastic boxes ("gerbox") containing medium-grain expanded vermiculite as the substrate, maintained at 100% of its water-holding capacity. The boxes with the seeds were wrapped in transparent plastic bags and placed in a BOD chamber with alternating temperatures of 20-30 °C (Beckmann-Cavalcante et al., 2012) and an 8-hour photoperiod. The germination chambers had four daylight white fluorescent lamps, each of 20 watts (ELETROLAB®, model EL202/4).

Data collection occurred daily, considering seeds that emitted the coleoptile as germinated until germination stabilized for all treatments. The positions of the boxes were randomly changed during each evaluation to maintain randomness in the experiments.

The Germination Speed Index (GSI) was calculated using the formula established by Maguire (1962), based on the daily counts of germinated seeds, and the results were expressed in days. At the end of the experiment, the germination percentage (%) was determined using the formula proposed in the Regras para Análise de Sementes (Rules for Seed Analysis) (Brasil, 2009).

The data were subjected to analysis of variance (ANOVA). When significance was observed, polynomial regression analysis at a 5% level ( $p < 0.05$ ) was conducted to determine the behavior of the variables according to the increasing concentrations. The significant equations with the highest coefficient of determination ( $R^2$ ) were selected. The germination percentage data were previously transformed using the arcsine square root transformation  $(x/100)^{1/2}$  for statistical analysis. The statistical software used was AgroEstat® version 1.1.0.711 (Barbosa; Maldonado Júnior, 2015). Germination distribution graphs over time were also created.

### 3. Results and Discussion

It was found that priming with  $KNO_3$  had a significant effect ( $p < 0.05$ ) on all evaluated characteristics. The seeds of *Euterpe edulis* Mart. showed a linear response with increasing  $KNO_3$  concentrations for germination percentage (Figure 1A). The highest germination percentage was achieved with the dose of 5 g L<sup>-1</sup> of  $KNO_3$ , while the lowest was

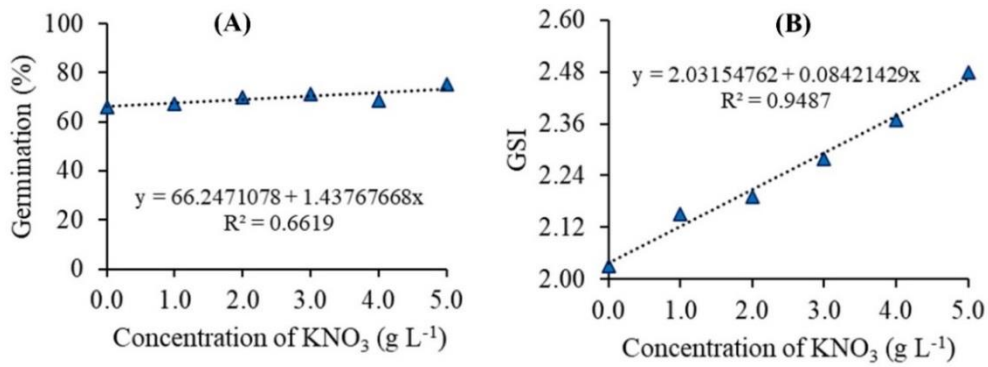
observed in the control treatment (hydropriming), with percentages of 73.43% and 66.34%, respectively.

This technique induces molecular mechanisms during seed imbibition with potassium nitrate ( $KNO_3$ ), where nitrate ions are transformed into nitric oxide (NO). This transformation leads to a decrease in abscisic acid (ABA) levels while simultaneously increasing the activity of gibberellins (GA), thereby facilitating the breaking of dormancy and promoting germination (Ciacka et al., 2022). Furthermore,  $KNO_3$  plays a crucial role in maintaining redox balance by reoxidizing NADPH, which is essential for various metabolic pathways, including the pentose phosphate pathway. This process provides the necessary energy and biosynthetic precursors for germination (Waqas et al., 2019). Additionally, the osmotic properties of  $KNO_3$  regulate water absorption, safeguarding seeds from potential damage and ensuring consistent germination (Marcos Filho, 2015; Ciacka et al., 2022). These combined mechanisms enhance seed germination, seedling vigor, and crop establishment, even under stressful conditions (Johnson and Puthur, 2021).

The regression analysis revealed a linear relationship between  $KNO_3$  concentration and the germination speed index (GSI) (Figure 1B). Seeds treated with 5 g L<sup>-1</sup> of  $KNO_3$  exhibited the highest GSI value (2.45), indicating faster germination than the control treatment (2.03), which showed slower germination. The result suggests that osmoconditioning with  $KNO_3$  accelerates germination. Faster germination is advantageous as it reduces the exposure time of seeds to potentially unfavorable environmental conditions, thus enhancing seedling establishment (Marcos Filho, 2015).

Based on this context, the results indicate that priming was successful, as the germination percentage achieved was 20.74% higher than the previous literature results without using this technique for the same species, as visualized by Aguiar et al., 2017. Furthermore, the GSI values observed in this study were 20.69% higher than those reported in previous studies on *Euterpe edulis* using other techniques, such as mechanical scarification (Beckmann-Cavalcante et al., 2012), fruit preconditioning (with and without water immersion), and various methods of pulp removal (Cursi and Cicero, 2014).

From the results of the germination distribution of the seeds over time and the germination peaks (Figure 2), it can be observed that there was a slight variation in the germination patterns. Germination began on the sixth day, and stabilization occurred on the twenty-sixth day for all treatments. It is important to note that this behavior ensures that some seeds can germinate over time, even if there are unfavorable conditions for germination at any given time.



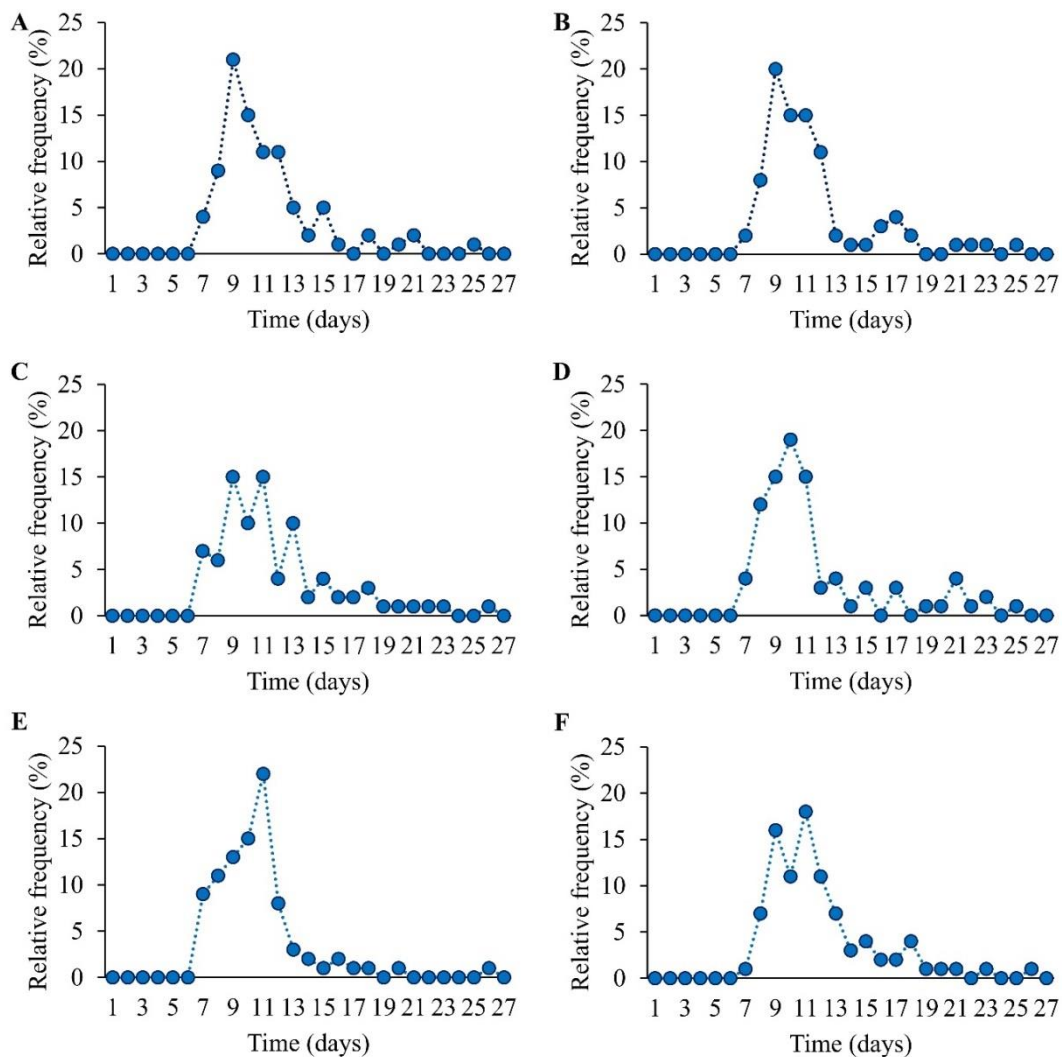
**Figure 1.** Germination percentage (A) and germination speed index - GSI (B) of *Euterpe edulis* seeds according to the  $\text{KNO}_3$  concentration in the seed priming. Jaboticabal, SP, 2022.

However, the longer the number of days for the seedling to emerge and remain in the initial stages of development, the greater the vulnerability to environmental conditions (Marcos Filho, 2015).

Although pronounced germination peaks were observed, seed germination was irregular, reinforcing the comments made by Cursi and Cicero (2014) that this

species has low, slow, and uneven germination.

The action mechanism of potassium nitrate ( $\text{KNO}_3$ ) acts on the electron reception, reducing nitrite levels within the seeds, reoxidizing NADPH, and increasing the availability of NADP for the reduction of the pentose phosphate cycle dehydrogenases, assisting in overcoming seed dormancy (Marcos Filho, 2015).



**Figure 2.** Germination distribution over 27 days of a lot of 100 *Euterpe edulis* seeds under the effect of priming. Hydroconditioned seeds (A) and osmoconditioned at concentrations of 1 (B), 2 (C), 3 (D), 4 (E), and 5 (F)  $\text{g L}^{-1}$  of  $\text{KNO}_3$ . Jaboticabal, SP, 2022.

Its action may also be related to its role in hormonal balance within the seeds, reducing germination inhibitors such as abscisic acid (Gashi et al., 2012; Golmohammadzadeh et al., 2015). Nitrates are substances that release nitric oxide (NO). The NO in  $\text{KNO}_3$  is an endogenous signaling molecule that plays a fundamental role in germinating seeds from different species (Pereira et al., 2020). NO is also capable of reducing the effects of seed aging, overcoming seed dormancy, and increasing tolerance to various abiotic stresses such as water stress, salinity stress, and heavy metal stress (Silva et al., 2019; Ciacka et al., 2022; Kataria et al., 2022).

Based on the results obtained for both analyzed characteristics, a beneficial effect of priming can be observed, as the germination percentage and GSI values were higher in the seeds with osmoconditioning than in the control treatment (Figure 1). This can be explained by the fact that with increasing concentrations of  $\text{KNO}_3$  in the solution, there was an increase in osmotic pressure and reduced water absorption by the seeds (Waqas et al., 2019). Therefore, the more negative the water potential of the system, the less available water there will be. As a result, the seed can only extract water from the environment if it has a more negative water potential than the system. Otherwise, water loss from the seed to the environment will occur (Santos Junior and Silva, 2020). Consequently, the seeds that absorbed less water due to the negative potential caused by higher  $\text{KNO}_3$  concentrations were likely favored in their germination process. However, for most species, high levels of soluble salts can reduce the substrate's water potential, decreasing the seeds' water absorption capacity and inhibiting germination due to the osmotic and toxic effects of the salt (Silva et al., 2021).

Furthermore, it should be noted that *Euterpe edulis* seeds had a high water content at the time of harvest (%), and the pre-soaking performed as a conditioning technique in the experiment may have directly affected the germination process. According to Marcos Filho (2015), seed soaking in water can harm seeds due to rapid water uptake, which can cause injuries and hinder aeration. Ribeiro et al. (2015) observed a reduction in the germination percentage of *Euterpe edulis* seeds when immersed in water for 48 hours, as water absorption beyond a certain level led to seed metabolism without radicle protrusion, resulting in seed deterioration.

For *Roystonea regia* (Kunth) O.F. Cook, also belonging to the Arecaceae family, the rehydration process negatively influenced the germination speed index and the average germination time of the seeds (Ferreira et al., 2021). According to Boroujerdnia et al. (2020), the seed priming technique with  $\text{KNO}_3$  has also been beneficial for seed germination in palm species

(*Phoenix dactylifera* L.). These findings support that  $\text{KNO}_3$  priming can be a valuable tool for increasing seedling production and promoting sustainable management of this species.

#### 4. Conclusions

Osmoconditioning with potassium nitrate ( $\text{KNO}_3$ ) significantly improved the germination percentage and germination speed index of *Euterpe edulis* seeds at the concentration of 5 g L<sup>-1</sup>. This technique offers a practical and efficient method to enhance seed germination, which is critical for the propagation and restoration of *E. edulis* populations.

#### Authors' Contribution

Thiago Souza Campos: Conceptualization, data curation, formal analysis, investigation, methodology, software, visualization and writing-original draft. Antonio Maricélio Borges de Souza: Conceptualization, investigation, methodology, visualization and writing-original draft. André Caturelli Braga: Data curation, investigation, methodology, visualization. Guilherme Rodrigues Vieira: Formal analysis, investigation, visualization and writing-original draft. Vânia Maria Pereira: Investigation, software, visualization, writing-review and editing and translation. Kathia Fernandes Lopes Pivetta: Funding acquisition, methodology, project administration, resources, supervision, validation and writing review and editing.

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