

## Electrical conductivity and osmotic conditioning with polyethylene glycol in storage *Caryocar brasiliense* seeds

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### ABSTRACT

The propagation of *Caryocar brasiliense* (pequi) is still little known. This study aimed to evaluate different osmotic conditions with polyethylene glycol (PEG) in the imbibition process and in the germination of pequi seeds, in addition to verifying the electrical conductivity to evaluate the viability of these seeds. The experiment was conducted with freshly dispersed pyrenes and with pyrenes stored for 90 days. The electrical conductivity of the aqueous solution was measured every six hours for up to 48 hours, using five replications with 20 seeds. The extracted seeds were placed in PEG solutions with osmotic potentials: 0.0; -1.0; -2.0; -3.0; -4.0 MPa, monitored at intervals of 12 hours to 60 hours, plus additional treatment with dry seeds without any type of imbibition. The design was completely randomized with four replications of 25 seeds for germination and four replications of ten seeds for monitoring water content during imbibition. Germination percentage, germination speed index (GSI), percentage of dead and live seeds at 40 days were evaluated. In seeds with longer storage time, the electrical conductivity was higher. Treatments that were not submitted to PEG showed higher GSI, lower mortality percentage and higher germination percentage with 40% and 37%, respectively. The electrical conductivity test was adequate.

**Keywords:** Pequi, Germination, Storage, Prime, Neotropical Savanna.

### Condutividade elétrica e condicionamento osmótico com polietilenoglicol em sementes de *Caryocar brasiliense* armazenadas

#### RESUMO

A propagação de *Caryocar brasiliense* (pequi), ainda é pouco conhecida. Objetivou-se avaliar diferentes condições osmóticas com polietilenoglicol (PEG) no processo de embebição e na germinação de sementes de pequi, além de verificar a condutividade elétrica para avaliar a viabilidade dessas sementes. O experimento foi conduzido com pirênios recém-dispersos e com pirênios armazenados por 90 dias. A condutividade elétrica da solução aquosa foi medida a cada seis horas por até 48 horas, usando cinco repetições com 20 sementes. As sementes extraídas foram colocadas em soluções de PEG com potenciais osmóticos: 0,0; -1,0; -2,0; -3,0; -4,0 MPa, monitorados com intervalo de 12 horas a 60 horas, mais o tratamento adicional com sementes secas sem nenhum tipo de embebição. O delineamento foi inteiramente casualizado com quatro repetições de 25 sementes para germinação e quatro repetições de dez sementes para monitoramento do teor de água durante a embebição. Avaliou-se a porcentagem de germinação, índice de velocidade de germinação (IVG), porcentagem de sementes mortas e vivas aos 40 dias. Nas sementes com maior tempo de armazenamento, a condutividade elétrica foi maior. Os tratamentos que não foram submetidos ao PEG apresentaram maior IVG, menor percentual de mortalidade e maior percentual de germinação com 40% e 37%, respectivamente. O teste de condutividade elétrica foi adequado.

**Palavras-chave:** Pequi, Germinação, Armazenamento, Prime, Bioma Cerrado.



## 1. Introduction

The *Caryocar brasiliense* Camb. (“pequiizeiro,” “pequi tree”) is a fruit tree native to the Brazilian Cerrado biome (neotropical savanna), of extreme importance for farmers in this biome, mainly in the north of Minas Gerais and traditional communities that collect and sell its fruits. The fruit is exploited by extractivism, generates income and occupation for several families, with collection, processing, and marketing. In addition to the fresh fruit, pequi is processed and marketed in the form of liqueur, oils for gastronomic purposes, soap, pequi flour, cream, preserved almonds and pequi pulp, chestnuts, syrups, ice cream, and sweets (Faria-Machado et al., 2015; Pinto et al., 2016; Guedes et al., 2017).

The degree of response to germination as a function of water stress varies between seeds of different species (Pereira et al., 2014), and that of the pequi tree was hitherto unknown. Low water content can improve seed conservation, as is the case of orthodox seeds, tolerant to the desiccation process, which allows the maintenance of embryonic cell viability, due to specific mechanisms to maintain the state of metabolic stillness. Thus, the ideal range of water content is around 4 to 7% to 15 to 20% depending on the species (Carvalho and Nakagawa, 2012; Rajjou et al., 2012). The water content of pequi tree seeds can drop from 40% to 7% in just 20 days of storage in places with an average temperature of 27°C (Sousa et al., 2017a).

The success in germination and establishment of the species will depend mainly on the physiological quality of the seed (Finch-Savage and Bassel, 2016). The integrity of the membranes is estimated by the test evaluating the effect of the extravasation of cellular solutes on the electrical conductivity of an aqueous solution (Brasil, 2009). Several types of tests allow the evaluation of seed vigor for the various cultivated species, but the electrical conductivity test stands out for its simplicity, quick results, and low cost. This test has not yet been carried out for pequi tree seeds.

The germination process begins with the entry of water into the seeds, resuming metabolic activity; however, dehydration and rehydration during seed development and germination are associated with high levels of oxidative stress, resulting in damage to DNA and proteins, leading to a loss of seed vigor and viability (Finch-Savage and Bassel, 2016). When using pequi tree seed without the endocarp, the imbibition process accelerates (Sousa et al., 2017a); however, the damaged DNA must be repaired during germination (Rajjou et al., 2012). Some osmotic agents are used to control the water content level in the seed and to estimate the resistance to water stress in seeds, such as mannitol,  $\text{KNO}_3$ , and polyethylene glycol (PEG), the latter being widely used, since it does not penetrate the cells, is not degraded, and

does not cause toxicity, due to its high molecular weight (Muscolo et al., 2013; Wang et al., 2018).

The germination of pequi tree seeds from the imbibition phase is still little known and PEG can regulate the osmotic potential of the solution. Therefore, this work aimed to evaluate the influence of different osmotic conditioning with PEG in the imbibition process and in the germination and vigor of pequi tree seeds, in addition to using the electrical conductivity test to evaluate the viability and vigor of seeds stored for 90 days and newly dispersed seeds.

## 2. Material and Methods

The experiment was carried out at the Plant Propagation Laboratory of the Institute of Agricultural Sciences of the Federal University of Minas Gerais (LPP-ICA/UFMG), Regional Campus of Montes Claros-MG, Brazil. The pequi fruits were collected from random matrices in the municipality of São João da Lagoa, north of Minas Gerais (16° 54' S and 44° 26' W), then they were pulped manually (removal of the internal mesocarp) using a sharp knife, without causing mechanical damage, and the pyrenes (seeds with endocarp) obtained were transported to the LPP-ICA/UFMG.

Part of the pyrenes were stored for 90 days, inside a wooden warehouse, packed in nylon bags and treated with phosphine to control insect attack. The seeds were extracted with the aid of an electric grinder to scarify the rigid endocarp, a manual bench vise to support it, an inverse pliers to open it, and tweezers to remove the seed. A careful selection was carried out eliminating seeds attacked by pests and fungi and with any physical damage caused by the seed extraction process, maintaining only those apparently intact.

The seeds that were obtained from recently dispersed pyrenes (new seeds) and the seeds stored for 90 days (old seeds), passed the membrane integrity test that evaluates the extravasation of solutes from the seed in the electrical conductivity of a solution water (Krzyzanowski et al., 2021). Five replications were carried out with 20 seeds immersed in 150 mL of distilled and deionized water, aerated (oxygenated) with an oxygen pump, and placed in a BOD-type chamber at a constant temperature of 30°C. The conductivity of the aqueous solution ( $\mu\text{S cm}^{-1} \text{g}^{-1}$ ) was measured at intervals of 6 to 48 hours with a Lutron model PCD-432 conductivity meter.

The seeds stored for 90 days were immersed in beakers containing polyethylene glycol solutions (PEG 6000) with the following osmotic potential levels: 0.0; -1.0; -2.0; -3.0; -4.0 MPa, plus the additional treatment with seeds without any imbibition, that is, dry seeds. The zero level of osmotic potential refers to the control (control), with only water. Distilled and deionized water

was used. To calculate the amount of PEG 6000 to be added to obtain each water tension, the equation 1 proposed by Michel and Kaufmann (1973) was used:

$$\begin{aligned} \Psi = & - (1.18 \times 10^{-2}) C (1.18 \times 10^{-4}) C^2 \\ & + (2.67 \times 10^{-4}) CT \\ & + (8.39 \times 10^{-7}) C^2T \end{aligned} \quad (1)$$

Where:  $\Psi$  = osmotic potential (Mpa); C = concentration (grams of PEG 6000/liter of water); and T = temperature ( $^{\circ}$ C). The design was completely randomized, with four replications of 25 seeds for germination and four replications of 10 seeds for monitoring water absorption at different imbibition times (0, 12, 24, 36, 48, and 60 hours). To determine the water content, the seeds were cut transversally into pieces of approximately three millimeters to facilitate drying in a Petri dish with the aid of a utility knife before being introduced into the oven for the drying process at  $105^{\circ}$ C for 24 hours (Brasil, 2009).

To avoid a high incidence of fungi during germination, the seeds were treated with the fungicide Vitavax-Thiram@ 200 SC [5,6-dihydro-2-methyl-1,4-oxathi-ine-3-carboxanilide (carboxina) + tetramethylthiuram disulfide (thiram)] with a concentration of 50% of the commercial product (Sousa et al., 2017a). Then they were sown in small, disinfected plastic trays containing vermiculite as substrate and moistened to 80% of their retention capacity.

Then the trays were placed in a BOD germination chamber at a constant temperature of  $30^{\circ}$ C, in the presence of light. The mass of water consumed was measured using additional trays, which were replaced with deionized water weekly to rewet the substrate. The daily counts of seedling germination made it possible to determine the germination speed index (GSI) calculated with equation 2 (Maguire, 1962):

$$GSI = G1 / N1 + G2 / N2 + \dots + Gn / Nn \quad (2)$$

Where G1, G2, and Gn correspond to the number of seeds germinated on the first day, second day, and until the last day counted; and N1, N2, and Nn refer to the number of days from sowing to the first day, second day, until the last day counted. The criterion for germination was protrusion of the root and/or growth of the plumule to 3 mm (Sousa et al., 2017a). The final count was performed on the 40th day after setting up the germination test, computing the percentage of dead (deteriorated) seeds and the percentage of live (non-deteriorated) seeds at the end of that time. The dead, deteriorated seeds were considered as the inconsistent seeds, with bad odor, apparently in a deteriorating state, whereas the non-deteriorated ones were consistent, whitish, with no sign of deterioration (Brasil, 2009).

The data obtained were submitted to the Shapiro-Wilk normality test with the GraphPad Prism software

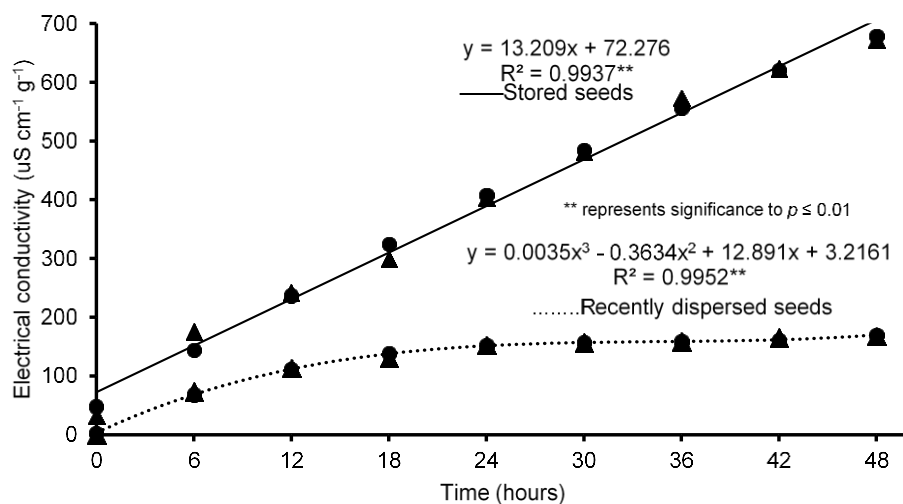
version 8.0.1 (San Diego, CA, USA). With all normal data, these were submitted to analysis of variance (ANOVA). The means of the electrical conductivity and water content of seeds were submitted to regression analysis, and the equations were adjusted by the TBC Software. The means of the osmotic conditioning data were compared by the Tukey test ( $p < 0.05$ ) using the Sisvar software version 5.6 (Lavras, MG, Brazil) and the results were presented as means with standard deviation (mean  $\pm$  SD).

### 3. Results and Discussion

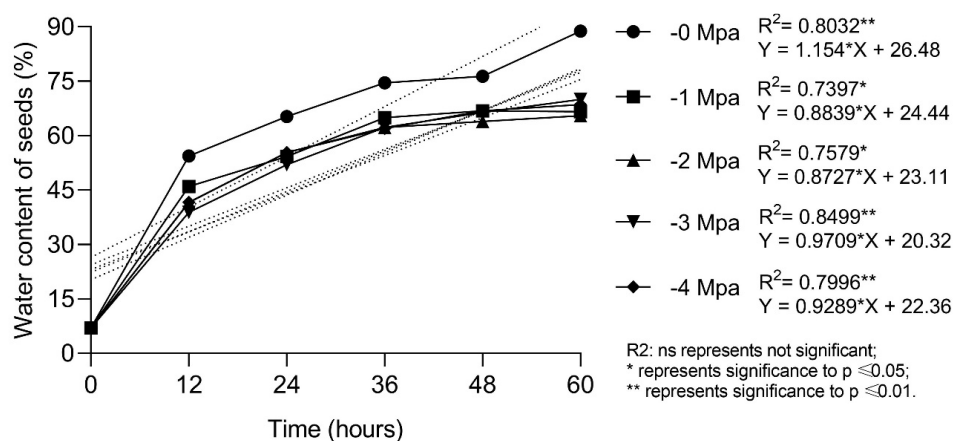
Freshly dispersed seeds showed increased electrical conductivity in the first 24 hours ( $153 \mu\text{S cm}^{-1} \text{g}^{-1}$ ) of immersion in water (distilled and deionized), remaining constant until the final time of 48 hours. Whereas the stored seeds showed a linear increase in electrical conductivity from the beginning to the end of time, reaching  $672 \mu\text{S cm}^{-1} \text{g}^{-1}$  at 48 hours, as shown in Figure 1. The initial water content of the newly dispersed seeds was 39%, whereas for the seeds stored for 90 days it was 7%.

After being submitted to the osmotic potentials in the PEG solution, the water content varied between treatments (Figure 2). In the seeds treated with PEG, the entry of water into the seeds dropped. Regarding the treatment with water (zero potential), it led to higher water content in the seeds. The result of the analysis of variance (ANOVA) shows a statistical difference in osmotic potentials (MPa) in all parameters evaluated (Table 1). The seeds that were not immersed in the PEG solution, that is, seeds hydrated only with water and "dry" seeds, showed a higher percentage of germination with 40% and 37% respectively (Figure 3a), significantly differing from the other treatments, but not differing from each other.

The seeds that were not immersed in the PEG solution had a higher GSI (1.1298 in the treatment by soaking with water and 0.7284 in the dry seeds) and statistically differed compared with the treatments with PEG (Figure 3b), which presented 0.2030 in the potential of  $-2.0$  MPa, 0.1509 at  $-3.0$  MPa potential, 0.1144 at  $-1.0$  MPa potential and 0.0708 at  $-4.0$  MPa potential. The treatment with the highest PEG concentration, consequently lower osmotic potential ( $-4.0$  MPa) had the highest percentage of deteriorated seeds (Figure 3c). The dry seeds had a higher percentage of live seeds at the end of the germination process (Figure 3d), since they were consistent and whitish, also considered as live seeds, not deteriorated, but not germinated.



**Figure 1.** Electrical conductivity of pequi tree seeds extracted from recently dispersed pyrenes (new seeds) and from pyrenes stored for 90 days (old seeds) in a shed at room temperature



**Figure 2.** Percentage of water content evaluated with intervals of 12 hours, up to 60 hours, of pequi tree seeds stored for 90 days subjected to different osmotic potency of polyethylene glycol

**Table 1.** Results of analysis of variance (ANOVA) of germination percentage, germination speed index (GSI), percentage of dead and live pequi seeds (pyrenes stored for 90 days), at 40 days after sowing, submitted to different osmotic potentials (MPa) of polyethylene glycol and dry seeds without immersion, under germination chamber (BOD) conditions.

	Germination test (%)	GSI	Percentage of dead (%)	Percentage of alive (%)
MPa	0.000** <sup>1</sup>	0.000**	0.000**	0.000**
Mistake	7.166	0.006	35.222	5.555
MSD <sup>2</sup>	6.017	0.174	13.340	5.298

<sup>1</sup> ns represents not significant; \* represents significance to  $p \leq 0.05$  and \*\* represents significance to  $p \leq 0.01$ .

<sup>2</sup> Minimum significant difference.

Figure 4 shows graphically the electrical conductivity test, the water content in the seeds, and the parameters evaluated in germination. The increase in electrical conductivity in seeds stored for 90 days is related to seed quality, indicating changes in membrane integrity. Over time, endogenous changes occur, related to the presence of reactive oxygen species, since the seed has abundant lipid reserves, and is prone to oxidative processes (Sousa et al., 2017b). Thus,

metabolic alterations such as lipid and malonaldehyde peroxidation generate irreversible oxidative cell damage to proteins, carbohydrates, lipids, RNAs, DNA, in addition to deterioration and loss of seed vigor and membrane integrity (El-Maarouf-Bouteau et al., 2013; Barreto et al., 2014; Long et al., 2014; Waterworth et al., 2015). The presence of exudates in the solution was observed, which is probably related to the loss of integrity of the cell membranes of the seeds. The release

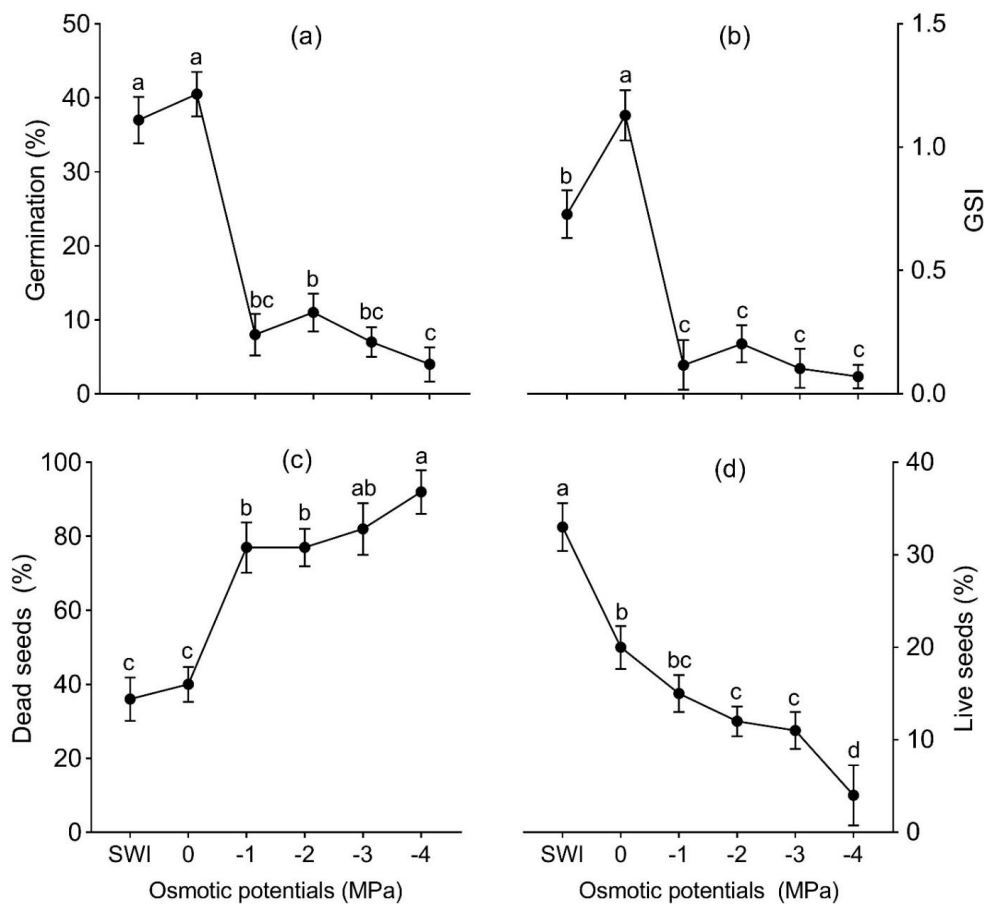
of exudates is also an irreversible process, which makes it impossible to restructure the membrane systems, since the structure of the membranes is disorganized, reducing seed vigor (Bewley et al., 2013; Finch-Savage, 2013), which allowed the extravasation of solutes to the external environment.

Barbosa et al. (2012) rated the electrical conductivity in peanut seeds (*Arachis hypogaea* L.) with different percentages of initial water content and observed that seeds with lower moisture contents, although stored in a controlled environment and considered to have good physiological quality according to the test of vigor, are particularly sensitive to stress and damage during the soaking process. Also, according to these authors, this is related to the structural disorganization of cell membranes and the great loss of electrolytes during the initial period of imbibition.

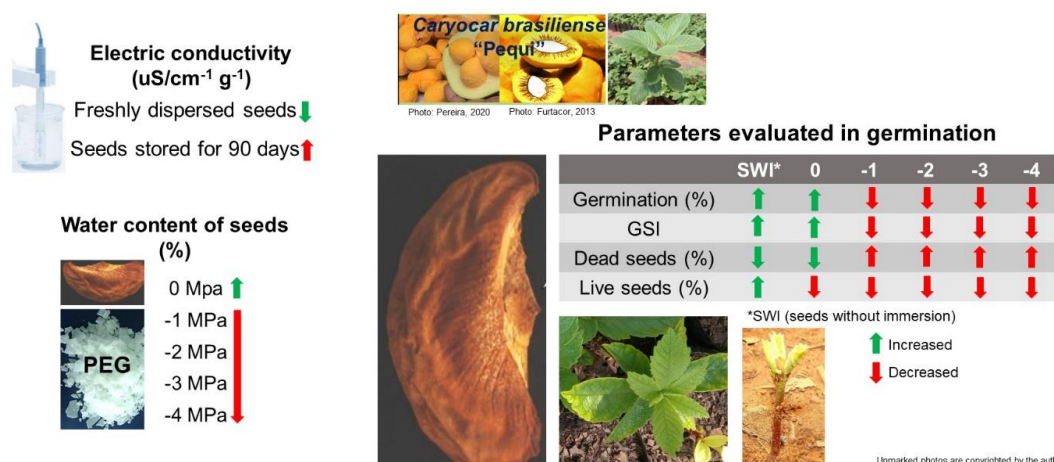
Although isolated seeds were used, taken from the endocarp, in which they control the flow of water and mechanically restrict the potential growth of the embryo (Bewley et al., 2013; Baskin and Baskin, 2014), they still showed the dormancy phenomenon (Dombroski et al., 2010; Baskin and Baskin, 2014), not exceeding 50%

germination in seeds stored for 100 days (Sousa et al., 2017a). Even those stored showed no significant increase, nor did they differ from those recently dispersed, since storage contributes to overcoming, albeit partially, dormancy in pequi tree seeds (Sousa et al., 2017a).

In this work, storage for 90 days may have contributed to increase the percentage of germination of seeds (40%) without osmotic conditioning, as well as reducing their viability, due to the high number of deteriorated seeds (80%) with osmotic conditioning. Note that the responses within the same species are different, since different genotypes occur, due to genetic variability and, consequently, seeds show different levels of dormancy (Baskin and Baskin, 2014; Finch-Savage, 2013). Other authors, such as Patané et al. (2016), using PEG 6000 in the soaking of sweet sorghum seeds [*Sorghum bicolor* (L.) Moench], observed that the decrease in the water potential of the soaking solution progressively inhibited and delayed germination. Similarly, other authors found a reduction in germination percentage, germination speed, and even in the initial development of seedlings (Luan et al., 2014; Muscolo et al., 2013; Wang et al., 2018).



**Figure 3.** Percentage of germination (a), germination speed index (GSI) (b), percentage of dead seeds (c) and live seeds (d) of pequi (pyrenes stored for 90 days), at 40 days after sowing, submitted to different osmotic potentials (MPa) of polyethylene glycol and dry seeds without immersion (SWI), under germination chamber (BOD) conditions.



**Figure 4.** Graphical abstract of the tests carried out with pequi tree seeds and main results of this work.

Note that each species has typical characteristics of density and chemical composition, factors that interfere with germination due to the osmotic potential, which depends on the initial quality of the seeds, the type of salt, and the concentration (Muscolo et al., 2013). The reduction in germinability and GSI and increase in seed mortality may be associated with reduced viability and vigor in stored seeds (Waterworth et al., 2015; Finch-Savage, 2013). Seed vigor defines their ability to germinate and establish seedlings quickly, affecting germinability, germination speed index, and seed deterioration in each environment (Finch-Savage and Bassel, 2016), thus the seeds had low physiological quality at the time they received the conditioner, which resulted in a high percentage (%) of deterioration. Added to the fact that the dormancy was not completely overcome.

The PEG reduces the osmotic and water potential of the concomitant solution, reduces water absorption by seeds (Wang et al., 2018), and can prevent the rapid entry of water into the cell tissue, and consequent damage by rapid imbibition to the embryo. However, the reduction of the osmotic potential negatively affects the mobilization of stored reserves, the structural organization of proteins, the activity of phytohormones, in addition to increasing oxidative stress, due to the toxic effect of ions in the solution (Ibrahim, 2016). Sunflower (*Helianthus annuus* L.) and *Crotalaria spectabilis* Roth seeds under water stress (Carneiro et al., 2011; Nóbrega et al., 2022) showed a reduction in germination and vigor with osmotic potentials below  $-0.20$  MPa; however, the use of solutions with potential above  $-0.20$  MPa did not affect germination, which remained around 90%.

Another activity probably affected was enzymatic activity, an essential part in the germination process, mainly of amylases, which are commonly reduced by water stress (Muscolo et al., 2013; Finch-Savage and Bassel, 2016). Amylase enzymes mobilize reserves and provide energy for embryonic growth (Muscolo et al.,

2013). Low water and osmotic potentials negatively affected the stored seeds, since they may have their vigor reduced with storage. More detailed studies are essential and will allow more precise conclusions.

#### 4. Conclusions

The electrical conductivity test shows that pequi tree seeds stored for 90 days have higher electrical conductivity and consequently reduced viability and vigor compared with newly dispersed seeds. Polyethylene glycol solutions (PEG 6000) at osmotic potentials from  $-1.0$  to  $-4.0$  MPa reduce the water content level of the seeds but negatively affect germinability and increase the mortality of pequi tree seeds. The minimum germinability limit of pequi tree seeds is possibly above  $-1.0$  MPa of osmotic potentials close to zero, with the need for studies with osmotic conditioning of higher potentials.

#### Authors' Contribution

Vander Rocha Lacerda contributed to setting up the experiment, reviewing the data, writing the manuscript, analyzing data, and designing the graphs. Levi Fraga Pagehú and Armando Pego Gonçalves contributed to setting up the experiment, making evaluations, and collecting and tabulating data. Paulo Sérgio Nascimento Lopes contributed by donating inputs and correcting the manuscript. Delacyr da Silva Brandão Junior contributed to setting up the experiment, writing the manuscript, and analyzing data.

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