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Desiccation sensitivity in seeds of Cerrado Pear (*Eugenia klotzschiana* O. Berg)

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Abstract: *Eugenia klotzschiana,* commonly known as pêra-do-cerrado, belongs to the Myrtaceae family. It is an unconventional food plant that is exploited in an extractive manner. The purpose of this study was to investigate the germination behavior of *E. klotzschiana* O. Berg seeds and their sensitivity to desiccation. The seeds were manually pulped from the fruits and stored in plastic containers. The initial water content was subsequently determined using the oven method at 105°C for 24 hours, yielding a value of 46.6%. Sub-samples were then separated to obtain the desired water contents of 40%, 32%, 22%, and 15% for treatments 2, 3, 4, and 5. The data underwent analysis of variance (ANOVA) and, if significant by the F test ($p<0.05$), the means were compared using the T test. Graphs with 95% confidence intervals were generated to illustrate the comparisons. The seedling development data was arranged in a box plot. The R software was used for these analyses. The data analysis revealed that *E. klotzschiana* O. Berg seeds do not have the potential to germinate with a water content lower than 22.50% moisture. Furthermore, the water content with the highest percentage, 46.6%, resulted in 100% germination.

Index terms: Germination, native fruit, water content.

Sensibilidade à dessecação em sementes de pêra-do-cerrado (*Eugenia klotzschiana* O. Berg)

Resumo – *Eugenia klotzschiana* (pêra-do-cerrado) pertence à família Myrtaceae. É caracterizada como planta alimentícia não convencional, sendo explorada de forma extrativista. Este trabalho foi desenvolvido para conhecer o comportamento germinativo das sementes de *E. klotzschiana* e ainda a sua sensibilidade à dessecação. Após a despolpa dos frutos, realizada manualmente, as sementes foram armazena-

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das em embalagens plásticas. O teor de água inicial foi determinado pelo método da estufa a 105°C por 24 horas, com valor de 46,6%. Separaram-se, em seguida, subamostras para obtenção dos demais teores de água desejados: 40%, 32%, 22% e 15%, sendo, respectivamente, os tratamentos 2, 3, 4 e 5. Os dados foram submetidos à análise de variância (ANOVA) e, quando significativas pelo teste F ($p<0,05$), as médias foram comparadas pelo teste T. Para ilustrar as comparações, foram gerados gráficos com intervalos de confiança de 95%. Foi realizada também a disposição dos dados do desenvolvimento das plântulas em *Boxplot*. Essas análises foram desenvolvidas utilizando-se o *software* R. A partir da análise dos dados, foi possível constatar que as sementes de *E. klotzschiana* O. Berg não apresentam potencial germinativo com teor de **água** inferior a 22,5% de umidade. Além disso, o maior teor de **água** de 46,6% proporcionou 100% de germinação.

Termos para Indexação: Germinação; frutífera nativa; teor de água.

The species *Eugenia klotzschiana* O. Berg is a fruit tree from the Myrtaceae family, commonly known as pêra-do-cerrado, pêra-do-campo or cabacinha-do-campo. The species is found in the Campo Limpo region of Goiás, Brazil, at altitudes above 749 m, in well-drained and permeable soils. The tree is classified as a PANCS, meaning it is an unconventional food plant, and it is still primarily harvested for its extracts (FONTES et al., 2018).

The population of Cerrado pear plants has decreased in recent years due to the expansion of the agricultural sector in the Cerrado biome. Therefore, given the risks that the decrease in the frequency of this species can bring to biodiversity, it is crucial to establish conservation strategies. Obtaining information on seedling production is one way to help conserve this species.

The fruit production period for *E. klotzschiana* is from October to January. The quantity found in the natural environment is low, making it difficult to re-establish the species naturally and obtain propagules to perpetuate it. However, plants grown in domestic orchards can produce up to 150 fruits per individual. Seedling production in the Myrtaceae family is primarily achieved through sexual reproduction (ANDRADE; FERREIRA, 2000). However, for several species, there are obstacles to this method, such as the short viability of seeds due to their low longevity during storage and sensitivity to water loss (Delgado & Barbedo, 2007; Amorim et al., 2020; Guardia et al., 2020).

To ensure successful seedling production, it is crucial to understand the germination behavior and water tolerance of the seeds. This is especially important for storage purposes or when there is a significant distance between the seed collection point and the seedling establishment site. The objective of this study was to evaluate the sensitivity of *E. klotzschiana* seeds to controlled drying.

The fruits were collected from three 8-year-old trees matrices in a domestic orchard located at the geographical coordinates (16° 39’ 47.9”S, 49° 11’ 59”W). The harvest occurred when the fruits turned dark yellow (Figure 1) and the flesh was slightly soft, as evidenced by gentle squeezing. The seeds were manually pulped and then stored for 25 days in transparent plastic containers with small holes to allow air circulation.

The seeds’ water content was measured using the oven method at 105°C for 24 hours, following the Rules for Seed Analysis (BRASIL, 2009). The results were expressed as a percentage of water on a wet basis (% b.u.). Two replicates of five seeds were used. After determining the initial water content of the seeds, a sub-sample was separated, consisting of treatment 1 with the highest water content (46.6%). The seeds were divided into

sub-samples of 20 seeds each and distributed on a screen in a single layer. They were then dried in an oven with forced air circulation at 30 ± 2ºC to obtain water contents of 40%, 32%, 22%, and 15%, which correspond to treatments 2, 3, 4, and 5, respectively.

Figure 1. Pear fruits used to obtain seeds.

Water loss was measured by monitoring the mass loss of sub-samples with a known initial mass. The samples were weighed at regular intervals until they reached the final mass determined by each treatment, using the equation described by Cromarty et al. (1985):

$$Mf = \frac{Mi\ (\ 100 - Ui)}{100 - Uf}$$

Mf = sample mass (g) after drying;
Mi = sample mass (g) before drying;
Ui = degree of moisture (%) before drying;
Uf = desired degree of moisture (%) after drying.

To confirm the different water contents obtained, the water content of the seeds was re-determined after reaching the mass predetermined by the formula in each treatment. This was done according to the methodology proposed by Brasil (2009), with two repetitions of five seeds each.

The seeds from each treatment were placed in aluminum containers and sealed to prevent moisture exchange with the outside environment. They were then stored in an oven with forced air circulation at 30 ± 2ºC for up to seven days to homogenize the water content of the seeds. The water content varied according to the time taken to obtain the different treatments. After this period, the following evaluations were conducted:

Germination test: was conducted with four replicates of five seeds each for every treatment. Sand moistened to 60% of its water retention capacity (BRASIL, 2009) with distilled water. Plastic containers with a depth of 5 cm, which were perforated, covered, and placed in a BOD at a temperature of 25 °C, were used. Counts of normal seedlings were taken 80 days after the test was set up, and the results were expressed as a percentage of normal seedlings.

Germination speed index (IVG): was carried out simultaneously with the germination test, and the seeds were evaluated at predetermined intervals until the last germination count, which was conducted 80 days after the germination test. Germination speed index (IVG) was calculated according to Maguire's (1962) method. The result was obtained using the formula:

$$IVG = \frac{E1}{N1} + \frac{E2}{N2} + \frac{En}{Nn}$$

Where E1, E2 and E*n* are the number of seedlings counted in the first, second, till the last count. N1, N2 and up to N*n* - number of days after the test was set up.

Seedling length: measured for all normal seedlings in the experiment at the end of the germination test using a graduated ruler. The length was analyzed from the base of the seedling to the apex of the terminal bud and expressed in centimeters (cm).

Root length: determined for all seedlings in the experiment by measuring from the root collar of the seedling to the furthest point of the largest root using a graduated ruler. The results were expressed in centimeters (cm).

Diameter of the collar: was measured for all seedlings using a digital caliper and expressed in millimeters (mm).

Number of secondary roots: was determined by counting them for each seedling. The results were then expressed as the number of secondary roots per plant.

Number of polyembryonic seeds: was determined by counting the seeds that have given rise to two or more developing seedlings.

The obtained data underwent analysis of variance (ANOVA), and if significant by the F test ($p<0.05$), the means were compared using the T-test. Graphs with 95% confidence intervals were generated to illustrate the comparisons. The seedling development data was presented on a Boxplot. These analyses were performed using R software.

The oven method at 105°C ± 3 used to obtain the water content of the seeds validated the pre-established values for monitored dehydration, confirming the efficiency of the method for obtaining the five different treatments (refer to Table 1). The drying period varies among species native to the Cerrado. However, species of the *Eugenia* genus have a slower desiccation time (DELGADO; BARBEDO, 2007; SILVA et al., 2016), which indicates intolerance to desiccation (BERJAK; PAMMENTER, 2000).

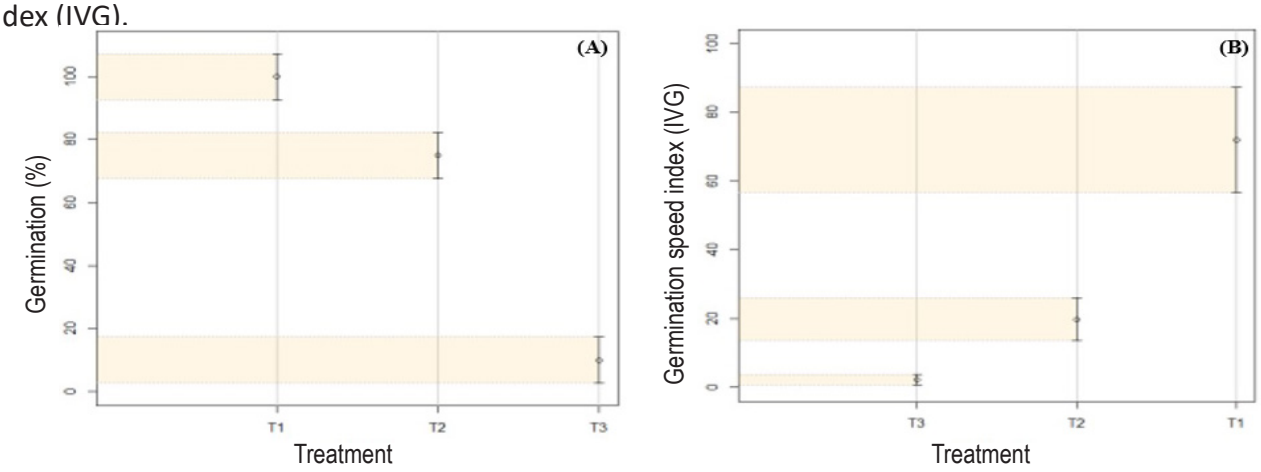
Table 1. Desired water content (desired TA, %) and water content after drying (obtained TA, %) and drying time (hours) of *E. klotzschiana* seeds.

Treatment	Desired TA	Obtained TA	Drying time
	%		hours
1	46.00	46.60	0
2	40.00	39.90	24hr 44min
3	32.00	31.70	41hr 30min
4	22.00	22.50	± 68 hr
5	15.00	14.90	± 174 hr

No germination occurred in treatments 4 (22.5% humidity) and 5 (14.9% humidity), indicating that these water levels are critical for the species, causing loss of viability and consequent death of the seeds. Studies have shown that species of the *Eugenia* genus reduce or lose their germination potential when subjected to desiccation (DELGADO & BARBEDO, 2007).

Treatment 1, with a humidity level of 46.6%, outperformed treatments 2 (39.90% humidity) and 3 (31.7% humidity), resulting in 100% germination and higher IVG. This indicates that the seeds are sensitive to desiccation, which may pose challenges for long-term storage (Figure 2). Similar behavior has been observed in other species of the same genus, including *Eugenia cerasiflora*, *Eugenia involucrata*, *Eugenia pyriformis*, *Eugenia umbeliflora*, *Eugenia uniflora*, and *Eugenia brasiliensis* (DELGADO; BARBEDO 2007; LAMARCA; BARBEDO 2014; LAMARCA et al., 2020).

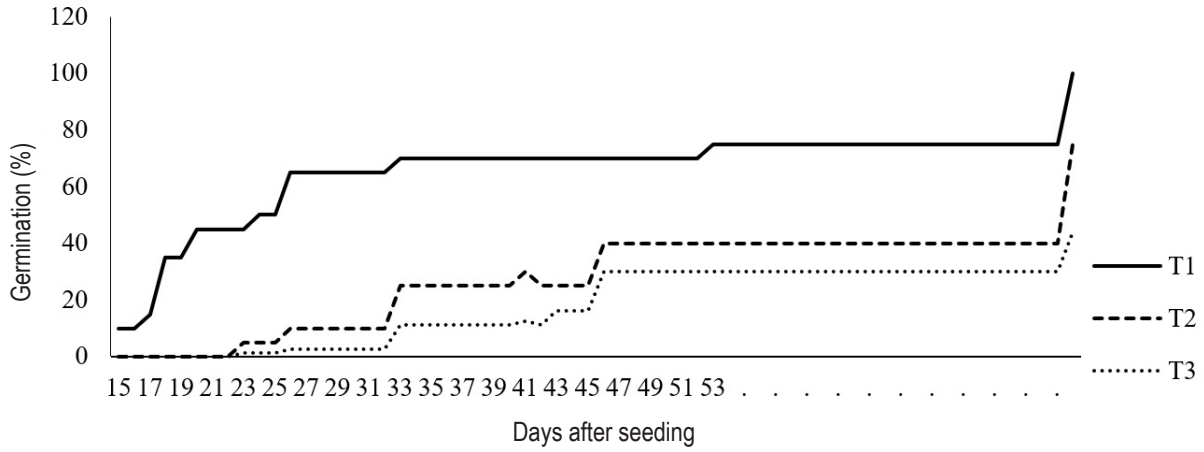
Figure 2. Physiological quality of *E. klotzschiana* seeds (A) Germination; (B) Germination speed index (IVG).



The seeds' initial water content of 46.6%, determined after 25 days, was similar to that of recently collected and processed seeds of the *Eugenia pyriformis* Cambess. species of 45% (SCALON et al., 2012). The seed conditioning method likely prevented moisture loss during storage. Silva et al. (2016) found an initial water content of 36% for *Eugenia dysenterica* DC seeds stored for 15 days in a cold room.

Germination started in treatment 1 (46.6% humidity) 15 days after sowing. Treatments 2 (39.9% humidity) and 3 (31.7% humidity) showed seed germination on the 23rd day, indicating a delay in the germination process after the water content was reduced (Figure 3). At 35 days after the experiment was established, it became evident that germination had stabilized for treatment 1 (46.6% moisture) at a rate of 70%. However, treatments 2 (39.9% moisture) and 3 (31.7% moisture) only recorded 25% and 11.5% germination, respectively. It is noteworthy that even after 75 days, the seeds remained viable enough to germinate. Treatments 1 (46.6% humidity), 2 (39.9% humidity), and 3 (31.7% humidity) reached maximum germination rates of 100%, 75%, and 43.75%, respectively. This observation of germination capacity after the 75-day period demonstrates the slower germination of seeds with reduced water content.

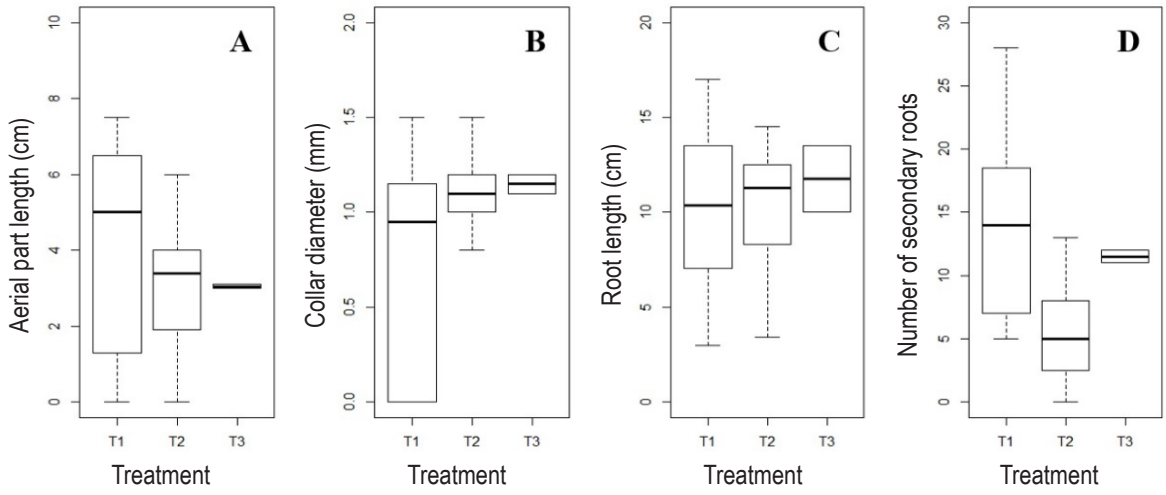
Figure 3. Germination behavior of *E. klotzschiana* seeds with moisture contents of 46.6%, 39.9% and 31.7% over 76 days.



Polyembryony was observed in 24.3% of the pêra-do-cerrado seedlings. This phenomenon is defined as the occurrence of more than one embryo in the same seed (COSTA et al., 2004). It is a common characteristic in species of the Mytaceae family, which provides an advantageous strategy from an ecological perspective, as it increases the possibility of greater reproductive success for these species (GOMES et al., 2016). However, one disadvantage of this phenomenon is the potential for competition among developing embryos and seedlings (MENDES-RODRIGUES, 2010).

There was less variation in the data for all assessed parameters related to seedling development in treatment 3 (31.7% humidity), which is justified by the smaller number of data points computed in this treatment (Figure 4). The length of the aerial part in treatment 1 (46.6% humidity) had a median of 5 cm, while treatments 2 (39.9% humidity) and 3 (31.7% humidity) had medians of 3.5 and 3.0 cm, respectively. In all treatments, the collar diameter data was approximately 1.0 mm. Treatment 1 (46.6% humidity), treatment 2 (39.9% humidity), and treatment 3 (31.7% humidity) had median root lengths close to 10 cm.

Figure 4. Development of *E. klotzschiana* seedlings: (A) Aerial part length; (B) Collar diameter; (C) Root length; (D) Number of secondary roots.



The study revealed that *E. klotzschiana* seeds cannot germinate with a water content below 22.50%. However, the highest water content (46.6%) resulted in 100% germination and superior IVG. Moreover, treatment 1 (46.6% humidity), treatment 2 (39.9% humidity), and treatment 3 (31.7% humidity) exhibited a similar development pattern, particularly in terms of collar diameter and root length.

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