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Original Research Article

Germination Optimization of *Butia odorata* seeds

ABSTRACT

Aims: Based on the scarcity of research related to the germination of palm (*Butia odorata*) seeds, the present research aimed to optimize the germination of palm seeds, using different periods of immersion in gibberellic acid (GA3) solutions and water.
Study design: The experimental design was entirely randomized, with 30 replicates, and the experimental unit was composed of the doses of gibberellic acid and water, in a factorial scheme.
Place and Duration of Study: The experiment was carried out with plants from the Active Germplasm Bank (BAG) of the Federal University of Pelotas, in the agricultural experimental field, in the south of Brazil.
Methodology: The seeds were submitted to a cleaning and hygiene pre-treatment, pyrenes (kernels) were removed from 20 harvested bunches and stored in plastic boxes, kept in a protected place at a temperature of 20 ± 1C. The following treatments were performed: without immersion (control), seeds without operculum and immersed in GA_3 solution at 50 mg L^{-1} for 24 hours and 48 hours; seeds without operculum and immersed in GA_3 solution at 100 mg L^{-1} for 24 hours and 48 hours; seeds without operculum and immersed in distilled water for 24 hours and 48 hours
Results: Treatments H_2O/24h and H_2O/48h resulted in the highest percentages of emissions of cotyledon petiole and leaf primordia and the highest averages of germination velocity. Treatment H_2O/24h resulted in the shortest average germination time. H_2O/48h resulted in the lowest incidence of fungal and bacterial contamination. After the removal of the operculum, the GA_3 100/48h optimized the germination and anticipated the total germination of *Butia odorata* seeds.
Conclusion: The immersion of the palm seeds in water for 24 hours is the treatment that provided the best germination under the conditions established in this experiment.

Keywords: Arecaceae; gibberellic acid; seed propagation; plant hormone;

1. INTRODUCTION

According to Lorenzi et al. [1], the Arecaceae family is represented by more than 240 different genera, which can be subdivided into more than 2700 species. In Brazil, one of the most important genera is *Butia*, from which several species of economic importance are derived in each region where they occur. In Rio Grande do Sul, these species include *B. odorata*, *B. lallemantii*, *B. catarinensis* and *B. yatay*, which can be explored both in fruit production as well as in an ornamental character [2].

In order to meet the growing demand of the population and the industries for new essences and flavors, palm trees are an excellent source of alternative income for South-Rio Grande do Sul agriculture [3-4].

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According to Rodrigues et al. [5], since each species requires specific conditions for seed germination, research has been developed aiming at elucidating the ideal conditions for such, aiming at the generation of important information about the propagation of species.

Some of the factors that contribute to the low germination of palm seeds are the predation of the seeds by the larval stages of *Pachymerus aff. Nucleorum* (Bruchidae) and Curculionidae (in determination) [6], as well as the presence of a thick pyrene located on the outside of the seeds, hindering the entry of water and the expansion of the embryo, resulting in a delay occurrence of germination [7]. Under natural conditions, the germination period of the palm seeds may be longer than two years, depending on the environmental conditions.

With the help of some techniques, such as the use of phytoregulators, associated to temperatures up to 35 °C, besides the adoption of mechanical scarification methods, such as the removal of the protective film from the germinative pores (operculum), good results in germination rates can be obtained, with periods of up to 15 days [8].

For *B. odorata*, there is still no possibility of cultivation of meristematic tissues, or other forms of cloning of the matrix plant. The only way to propagate the species is through seeds, naturally derived from cross-pollination, resulting in the high genetic variability of the plants [9].

In order to increase the supply of raw material for the production of products based on native fruits, it is necessary to develop techniques that optimize the production of seedlings.

Based on this, and considering the scarcity of research related to the germination of palm seeds, the objective of this research was to optimize their germination, using different periods of immersion in solutions of gibberellic acid and water, and

2. MATERIAL AND METHODS

The seeds used in the experiment were collected from palms from the Germplasm Active Bank (BAG), from the Agricultural Center of Palma (CAP), at the Eliseu Maciel Faculty of Agronomy – of the Federal University of Pelotas (FAEM-UFPel), in the municipality of Capão do Leão, RS, whose geographic coordinates are latitude 31º52'00 "S and longitude 52º21'24" W, 13 m above sea level. The soil of the site is moderately deep, with average texture in the A horizon and clayey in the B. It is classified as Yellow Red Argisoil [10]. The climate is classified as "Cfa" [11], that is, temperate or humid subtropical with hot summers and average annual precipitation of 1582 mm, average annual temperature of 17.7 °C and average annual relative humidity of 78.8% [12].

The experiment was carried out at the Official Laboratory of Seed Analysis (LASO), at the Embrapa Temperate Weather Station (Embrapa-CPACT) - Experimental Low Earth Station

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(ETB), in the city of Capão do Leão, RS, Brazil. Where the seeds were extracted from pyrenes (kernels) from 20 bunches harvested in the production cycle and stored in plastic boxes, conditioned in a cool, ventilated place and protected from sun and rain, with a temperature of 20 ± 1ºC. For the extraction of the seeds, a manual bench clamp (Motomil® model TB-600P) was used in order to minimize damage by crushing seeds.

After the extraction process, the seeds were selected, discarding those that presented any type of visible mechanical damage. The seeds were then submitted to a disinfection procedure, applied sequentially, as follows: immersion of the seeds in 70% alcohol for one minute; triple wash with distilled water; immersion in sodium hypochlorite (concentration of 2.0-2.5%) for 25 minutes; triple wash with distilled water; treatment of the seeds with Vitavax-Thiram® (5,6-dihydro-2-methyl-1,4-oxathi-ine-3-carboxanilide, tetramethylthiuram disulfide and ethylene glycol) at the concentration of 300 mL 100 kg^{-1} of seed, diluted in distilled water in the proportion of 10 times the fungicide volume.

To facilitate the treatment of the seeds, small plastic bags were used, where the seeds were placed in contact with the fungicidal syrup and slightly rubbed manually to homogenize them with the syrup.

At the end of this process and superficial drying of the fungicide, the seeds were subjected to extraction of the operculum (a structure that composes the endocarp and protects the embryo), using a scalpel and magnifying glass, in order to avoid possible damages to the embryo. Afterwards, the seeds were submitted to the following treatments with distilled water and gibberellic acid from the pure gibberellin synthetic hormone (ProGibb® 400), which promotes the growth of the plant: control (intact seeds and without immersion), GA_3 50/24h (seeds without operculum + immersion in 50 mg L^{-1} gibberellic acid solution for 24 hours, GA_3 50/48h (seeds without operculum + immersion in 50 mg L^{-1} gibberellic acid solution for 48 hours, GA_3 100/24h (seeds without operculum + immersion in 100 mg L^{-1} solution of gibberellic acid for 24 hours), GA_3 100/48h (seeds without operculum + immersion in 100 mg L-1 of gibberellic acid solution for 48 hours, H_2O/24h (seeds without operculum + immersion in distilled water for 24 hours), H_2O/48h (seeds without operculum + immersion in distilled water for 48 hours).

Subsequently, the seeds of each treatment were sown manually in germ-boxes, on expanded vermiculite and kept in a germinator, under conditions of high humidity (over 90% ± 5%) and constant temperature of 25 ° ± 1 °C. All the material used in this experiment was previously sterilized to reduce the risk of contamination by fungi and bacteria.

The variables analyzed were: the emission of cotyledon petiole - ECP (the first structure emitted by the seed), emission of leaf primordia, ELP (a structure emitted from ECP),

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germination velocity index (GVI), obtained by the evaluation of cotyledon petiole emission of the seeds every two days and using the formula proposed by Maguire [13], average germination time (AGT) according to Fior et al. [14], percentage of seedlings contaminated by fungal colonies (CFC) and percentage of seeds contaminated by bacterial colonies (CBC).

The experiment consisted of 61 days of observation and the evaluations were performed at 48 hour intervals by persons trained by the Official Laboratory of Seed Analysis team prior to the installation of the experiment.

The experimental design was completely randomized, in a factorial scheme, composed of seven treatments, which were composed of four replicates of 30 seeds each, totaling 120 seeds analyzed in each of the treatments, totaling 840 seeds observed. Data were analyzed for normality by the Shapiro-Wilk test and for homoscedasticity by the Hartley test. Subsequently, they were submitted to analysis of variance ($p \leq 0.05$). In the case of significance, the effects of treatments on germination were analyzed by the Tukey test ($p \leq 0.05$), using the statistical program ASSISTAT 7.5.

3. RESULTS AND DISCUSSION

The variable emission of cotyledon petiole (ECP = germination) showed significant differences between the treatments, where GA_3 100/48h and H_2O/24h resulted in the highest petiole emission averages (39% and 43%, respectively), while the rest of the treatments resulted in significantly lower averages (Control = 3%, GA_3 50/24h = 8%, GA_3 50/48h = 4%, GA_3 100/24h = 14% and H_2O/48h = 15%), due to the smaller cotyledon petiole emission percentage (Table 1).

In a study with *B. odorata* kernels, Schlindwein et al. [15] reported that the combination between periods of high humidity and temperature (90% moisture + 21 days at 40 °C + 30 °C) associated with pre-drying (24 hours), favors germination (70.0%), but does not significantly reduce the average germination time (39 days). Therefore, this pre-storage of the kernels in the period of 30 days in a chamber with 90% of humidity does not alter the germination.

In studies carried out with several species of palm trees, it was observed that each species presented specific requirements of temperature and germination period, besides the adoption of procedures that favor the imbibition of water by the seeds, for the promotion of germination.

Rodrigues et al. [5], on the other hand, affirm that in the case of the palm tree *Bactris maraja*, the removal of the endocarp is not necessary. If carried out, it can result in damage to

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germination and vigor of the seedlings of this species, suggesting that the seeds of the species do not present dormancy due to mechanical resistance.

During the development, it was observed that germination was stabilized from the seventh evaluation (40 days after sowing) in GA_3 100/48h, while in H_2O/24h, the germination stabilized from the tenth evaluation (47 days after sowing). Although there was no significant difference between the germination percentages after the seeds were submitted to these two treatments, it can be inferred that the gibberellic acid tends to anticipate the maximum germination at seven days (Figure 1). According to Murakami et al. [16], the use of 1g L^{-1} of GA_3 for 24 hours anticipated the germination of murici (*Byrsonima cydoniifolia*), which provided 5.33% germination on the ninth day after sowing.

Emission of leaf primordia (ELP) was also significantly higher in treatments GA_3 100/48h and H_2O /24h (34% and 42%, respectively) than the other treatments (control = 1%, GA_3 50/24h = 4%; GA_3 50/48h = 4%, GA_3 100/24h = 7% and H_2O/48h = 7%) (Table 1).

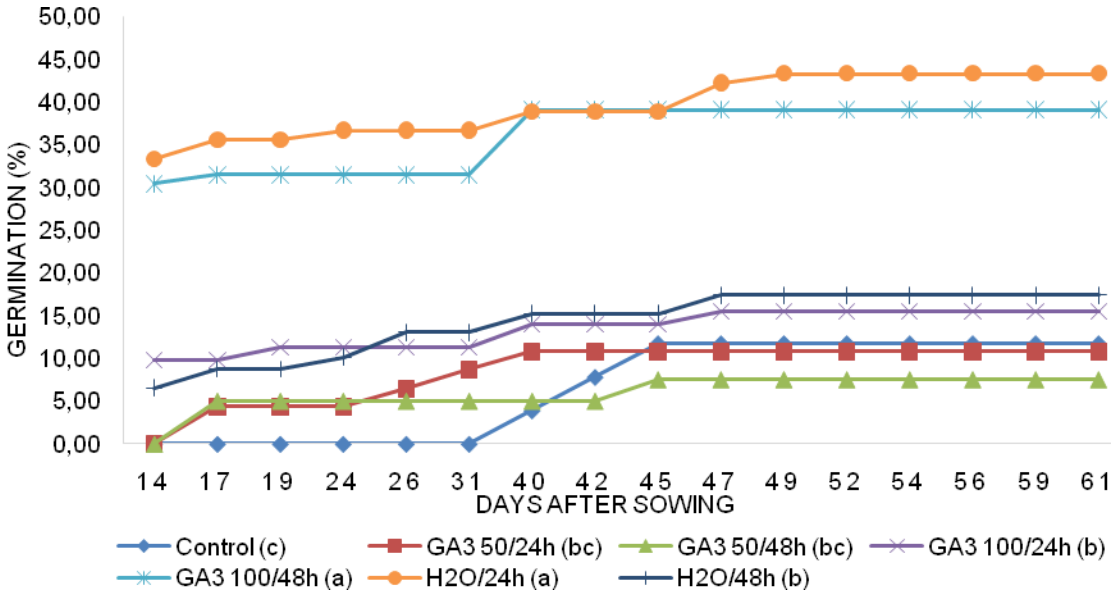

Figure 1. Germination behavior of *Butia odorata* seeds according to the different evaluation days (14-61 days after sowing), under the influence of the control (T1), GA_3 50 mg L^{-1} for 24 hours (T2), GA_3 50 mg L^{-1} for 48 hours (T3), GA_3 100 mg L^{-1} for 24 hours (T4), GA_3 100 mg L^{-1} for 48 hours (T5), H_2O for 24 hours (T6) e H_2O for 48 hours (T7) of *B. odorata* seeds. LASO-CPACT/FAEM-UFPel, Capão do Leão - RS, 2016.

According to Kerbauy [17], leaf primordia emission is of fundamental importance for the seedlings, when they become active photosynthetically, guaranteeing a greater oxygen supply to the embryo. In addition, these authors state that the presence of newly germinated green

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embryos, a fact that occurred in this experiment, may be related to the embryo need for oxygen, caused by the low supply of oxygen at the site where germination is in progress.

The extraction of the seeds from the interior of the pyrenes and removal of the operculum seem to have greatly favored the absorption of water by the seeds. Fior et al. [14] suggest that the opening of the embryo cavity by the removal of the operculum is the treatment that allows obtaining a higher percentage of seedlings of emerged palms, besides the shortest average time for emergence. The authors report the occurrence of a high percentage of germination in treatments composed of almonds without operculum (72.0%) and whole diaspores kept submerged in distilled water for 18 hours (67.2%) at the end of 440 days of evaluation.

Fior et al. [18] obtained germination greater than 80% in a period of 13 days, in *Butia odorata* seeds submitted to the total opening of the embryo cavity, as well as the isolation of embryos. On the contrary, in the present study, a percentage of less than 30% emission of cotyledon petiole was observed in a similar period (14 days after sowing).

For the variable germination velocity index (GVI), significant differences were observed between treatment averages, with GA_3 100/48h and H_2O/24h resulting in the highest indexes (0.61 for both), compared to other treatments (control = 0.02, GA_3 50/24h = 0.07, GA_3 50/48h = 0.03, GA_3 100/24h = 0.18 and H_2O/48h = 0.16).

According to Kerbauy [17] the speed of germination may be associated to the availability of water in the substrate, as well as to the maintenance of the optimum temperature for favoring seed germination. In addition, the granulometry of the substrate used in relation to the size of the seed may influence the germination. If unsuitable, the contact surface of the seed with the wet substrate particles may be reduced. However, temporary water restriction may favor an increase in the speed of development of the primary root.

According to the data on the average germination time (AGT), presented in Table 1, there was a significant difference between the means of the treatments, where the control obtained the highest mean (control = 76 days), while GA_3 100/48h had the lowest mean (18 days). The means of treatments GA_3 50/24H (34 days), GA_3 50/48H (35 days), GA_3 100/24h (24 days) and H_2O/24h (21 days) did not differ from each other. H_2O/48h presented an intermediate mean (45 days).

The use of gibberellic acid at the maximum dose (100 mg L^{-1} for 48 hours) may have contributed to a lowest germination time in treatment GA_3 100/48h.

The bacterial contamination level of the seeds (CCB) showed significant differences between treatments, with treatment H_2O/24h presenting the lowest percentage of contamination

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(3%), H_2O/48h presented the highest percentage (47%) and the rest of the treatments did not present significant differences from each other (Control = 6%, GA_3 50/24h = 9%, GA_3 50/48h = 10%, GA_3 100/24h = 12%, GA_3 100/48h = 12%).

As for fungal contamination (CFC), significant differences were observed between the means of the treatments. Treatment H_2O/24h resulted in the lowest percentage (81%), while, for the other treatments, 100% of the seeds presented fungus contamination (Table 1).

Up until the 14th day after the experiment was installed, bacterial contamination was predominant, with the possibility of visual detection of colony formation, which presented gelatinous appearance and characteristic odor. From the results presented in Table 1, it was observed that treatment H_2O/24h (removal of the operculum followed by immersion in water for 24 hours) was the only one in which bacterial contamination (CCB) remained at low levels (3%) throughout the evaluation period.

For this treatment (H_2O/24h), even though the percentage observed for the fungal contamination (TFC) was high, it was also the lowest (81%), in relation to the other treatments, remaining at low levels until the 17th day after sowing. It was observed that the treatment of the *B. odorata* seeds with Vitamax-Thiram® presented satisfactory results regarding the control of the proliferation of microorganisms in the first 15 days after the installation of the experiment, maintaining the level of fungal contamination stable. After this period, the percentage of contamination increased significantly until reaching the maximum level presented in Table 1.

Table 1. Emission of cotyledon petiole (ECP in %), emission of leaf primordia (ELP in %), germination speed index (IVG), seeds with fungal contamination (CFC in %), seeds with bacterial contamination (CBC in %) and average germination time (AGT in days) of seeds extracted from palm trees (*Butia odorata*) fruits, under the influence of the control, GA_3 50 mg L^{-1} for 24 hours, GA_3 50 mg L^{-1} for 48 hours, GA_3 100 mg L^{-1} for 24 hours, GA_3 100 mg L^{-1} for 48 hours, H_2O for 24 hours and H_2O for 48 hours. LASO-CPACT / FAEM-UFPel, Capão do Leão - RS, 2016.

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Treatments	ECP	ELP	GVI	CFC	CBC	AGT
Control	3 ±6.00 c*	1 ±4.23b*	0,02 ±1.38 b*	100 ±0,99 a*	6 ±4.23 bc*	76 ±3.65 a*
GA_3 50/24h	8 ±5.24bc	4 ± 3.25 b	0.07 ±1.73 b	100 ±0.79 a	9 ±5.87 bc	34 ±3.79 bc
GA_3 50/48h	5 ±6.25bc	4 ±1.98 b	0.03 ±2.67 b	100 ±0.71a	10 ±5.46 bc	35 ±2.99 bc
GA_3 100/24h	14 ±5.61b	7 ±1.78 b	0.18 ±2.14 b	100 ±0.66 a	12 ±3.13 b	24 ±2.54 bc
GA_3 100/48h	39 ±4.16a	34 ±1,39 a	0.61 ±2.73 a	100 ±0.60 a	12 ±3.44 b	18 ±2.39 c
H_2O /24h	43 ±2.94a	42 ±0.98 a	0.61 ±1.57 a	81 ±0.36 b	3 ±2.78 c	21 ±3.21 bc
H_2O /48h	15 ±2.76b	7 ±0.77 b	0.16 ±1.60 b	100 ±0.45 a	47 ±3.22 a	45 ±3.50 b
M.G.	18.14	14.14	0.24	97.29	14.14	36.14
CV (%)	28.2	34.4	36.4	6.6	26.7	29.21

**The mean ± standard deviation of replicates*, and means followed by the same letter in the column do not differ from each other by the Tukey test (p≤0.05).

The main characteristics of the seed germination of *B. odorata* are long periods of occurrence, as well as high contamination by fungi and bacteria. According to the results obtained in the present study, the disinfection of the seeds, associated to the treatment with fungicide, was extremely necessary to avoid germination suppression by phytopathogenic organisms.

During seed germination it was observed that the immersion of the seeds in gibberellic acid solution at concentrations of 50 and 100 mg L^{-1} did not provide significant improvements in the germination performance of the seeds, relative to the treatment H_2O/24h (removal of the operculum, followed by immersion in water for 24 hours). Although the maximum concentration of GA_3, together with the maximum immersion period, resulted in a significantly higher percentage of germination than the other treatments (GA_3 100/48h = 39%), this did not differ from immersion in water for 24 hours.

In addition, treatments GA_3 100/48h and H_2O/24h resulted in similar seed performance, which was higher than observed in the other treatments, suggesting that the germination speed was not altered by GA_3 (in relation to immersion in distilled water) and that the addition of this phytohormone is dispensable as a promoter of *B. odorata* seed germination.

4. CONCLUSION

The immersion of *B. odorata* seeds, after the removal of the operculum, in 100 mg L^{-1} of gibberellic acid for 48 hours anticipates the total germination, while the immersion in distilled

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water for 24 hours provides the lowest percentage of fungal and bacterial contamination. Both methods optimizes the germination of *B. odorata* seeds.

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