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Full Length Research Paper

In vitro germination and growth of babassu (*orbygnia phalerata* Mart.) embryos subjected to different drying temperatures

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The aim of this study was to evaluate the physiological quality of babassu (*Orbygnia phalerata* Mart.) embryos in relation to drying rate. The fruits were kept in a dry chamber with forced air circulation at 57 ± 2°C and 37 ± 2°C for 0, 6 and 11 days. An interaction between the drying time and temperature on water loss was not observed. Fruits dried at 37 ± 2°C failed to achieve the same water content values as those dried at 57 ± 2°C. Embryos dehydrated at 37 ± 2°C remained viable even after 11 days of drying, while embryos dehydrated at 57 ± 2°C were dead after 6 days of drying. Germination percentages above 67% were obtained for all times at drying 37°C, even for seeds with 9% water content, which highlights a possible orthodox behavior. Under the experimental conditions of the present study, drying embryos at 57 ± 2°C decreased the percentage and speed of germination as well as the initial growth of seedlings.

Key words: Arecaceae, desiccation tolerance, orthodox seeds.

INTRODUCTION

Babassu (*Orbygnia phalerata* Mart.) is a member of the Arecaceae family. This plant grows up to 20 m tall and the stem diameter can reach up to 40 cm. The babassu fruits are smooth, oblong ellipsoids and brown in color at maturity. The babassu bears fruit throughout the year with maximum production between August and January. The plant produces up to 6 bunches with an average yield of 2,400 kg ha^{-1} (Miranda et al., 2001; Lorenzi et al., 2004). The most important economic, social, and environmental use of babassu is in oil production. This plant is used on a commercial scale to produce fuel, soaps, cosmetics and food. The babassu residues (mesocarp and epicarp) are calorie-rich and thus have the potential to be used as biomass for energy production (Almeida et al., 2002; Albiero et al., 2007). This plant family primarily propagates through sexual reproduction. However, this process is slow, irregular and has a low efficiency because of the morphological seed charac-teristics and genetic heterogeneity (El-Kazzaz and El-Bahr, 2000). Therefore, several studies aimed at overcoming seed dormancy have been conducted on members of this family, including the peach-palm (*Bactris gasipaes* Kunth.) (Villalobos et al., 1992), inaja (*Maximiliana regia* Mart.) (Martins et al., 1996), dende (*Elaeis guinnensis* Jacq.) (Martine et al., 2009), tucuma (*Astrocharyum aculeatum* Mart.) (Ferreira and Gentil, 2006) and macauba palm (*Acrocomia aculeata* [Jacq.] Lodd. ex Mart.) (Rubio et al., 2011; Ribeiro et al., 2011).

Sexual propagation is a difficult method of reproduction in Arecaceae. Embryo culture has aided seedling production because this process can overcome certain germination inhibitors and shorten the plant reproductive

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Table 1. Water content of *O. phalerata* Mart. fruits and seeds that were subjected to different drying periods in forced-air dry chambers at 37 ± 2°C and 57 ± 2°C.

Drying (days)	Temperature 37 ± 2°C		Temperature of 57 ± 2°C	
	Seed (%)	Fruit (%)	Seed (%)	Fruit (%)
0	21.26^{Ay} (± 0.73)^{z}	23.88^{A} (± 0.98)	21.26^{A} (± 0.73)	23.88^{A} (± 0.98)
6	12.88^{B} (± 1.32)	20.80^{B} (± 0.29)	11.83^{B} (± 1.66)	19.38^{B} (± 0.27)
11	9.26^{B} (± 0.58)	20.38^{B} (± 0.32)	6.76^{C} (± 1.60)	18.98^{B} (± 0.28)

[y]Means that are followed by the same capital letter between the drying times do not differ according to Tukey's test at 5% probability. [z]Standard error of the mean.

cycle as well as contribute to the study of embryo hysiology (Bekheet et al., 2008; Hu and Ferreira, 1998). In palms, zygotic embryo culture has been used successfully, particularly in guariroba (*Syagrus oleraceae* Berg.), buriti (*Mauritia flexuosa* Mart.), babassu (*O. phalerata* Mart.), 'coquinho-azedo' (*Butia capitata* [Mart.] Becc.) and macauba (*A. aculeata* [Jacq.] ex Lodd.) (Melo et al., 2001; Spera et al., 2001; Ribeiro et al., 2011; Silva et al., 2011). These authors observed high germination rates after 2 months of *in vitro* cultivation of babassu (*O. phalerata* Mart.).

In addition to tissue culture, the development of storage techniques and the tetrazolium test for viability has aided in the propagation and conservation of palm species. The tetrazolium test provides fast and definitive results for seed viability in the Arecaceae family, especially in *M. flexuosa*, macauba (*A. aculeata*, *B. capitata*, and pupunha (*Bactris gasipaes* Kunth.) (Ferreira and Sader, 1987; Spera et al., 2001; Fernandes et al., 2007; Ribeiro et al., 2010). There are peculiarities in the staining pattern of oleaginous seeds in which the diffusion of the solution or the dye can be impeded; in this case, specific staining patterns are required (Wood et al., 2005).

To elucidate the behavior of palm species during storage, certain factors should be considered, such as humidity, temperature and drying speed. Ferreira and Santos (1993) assessed the effects of drying time and drying rate on the germination of the peach-palm (*Bactris gasipaes* Kunth.) and observed that 2 days of fast drying on seeds, reduced the germination percentage, which demonstrates the deleterious effects increased drying times and rates cause. Panza et al. (2007), working with palmiteiro seeds (*Euterpe edulis* Mart.), observed high critical water contents, regardless of drying speed. A similar result was more easily observed with fast drying. Studies evaluating babassu fruit and seed water-loss behavior and its effect on seed germination were not found in the literature. Therefore, the aim of the present study was to evaluate the physiological quality of *O. phalerata* embryos that were dried subjected to different temperatures.

MATERIALS AND METHODS

This study was conducted in the seed laboratory and plant tissue culture laboratory at the Goias Federal Institute (Instituto Federal Goiano), Rio Verde Campus (GO). Mature babassu (*Orbygnia phalerata*) fruits were collected in December 2010 from plants grown in the Gameleira farm in the municipality of Montes Claros de Goiás - GO (16°07′ S – 51°18′ W, altitude of 592 m). To evaluate the effects of drying temperature, whole fruits were continuously maintained in a forced-air dry chamber at either 57 ± 2°C or 37 ± 2°C. The fruits remained under these conditions for 0, 6 and 11 days. The water content (on a wet weight basis) was determined according to the modified method of Brasil (2009) using an oven at 105 ± 2°C until a constant mass was reached.

The method of Silva et al. (2011) was used to assess the effect of dehydration on *in vitro* germination. The fruit samples were removed from the dry chamber at each time point and then broken using a mechanical press to obtain 40 embryos. The zygotic embryos were removed with a scalpel, covered with gauze, sterilized with 70% alcohol for 30 sec and 20% sodium hypochlorite (commercial bleach of 2.5% active chlorine) for 20 min and rinsed 3 times with sterile water. The embryos were inoculated in test tubes containing 20 ml of half-strength MS medium (Murashige and Skoog, 1962) that was supplemented with 30 gL^{-1} sucrose and 0.1 gL^{-1} myoinositol and solidified with 3.0 gL^{-1} agar. The pH of the culture medium was adjusted to 5.7 ± 0.1 prior to autoclaving at 121°C. After inoculation, the embryos were maintained in a growth chamber at 25 ± 3°C in the dark for 15 days, followed by a 16 h light photoperiod that was provided by fluorescent lamps as suggested by Silva et al. (2011).

A daily count was performed for 60 days to calculate the speed of germination index (SGI) and germination percentage (Maguire, 1962). A germinated embryo was characterized by a change in its initial color from white to yellow, followed by petiole and haustorium elongation. Additionally, the cotyledonary petiole length was assessed at 30, 60 and 90 days. At different drying times, the fruit samples were removed from the dry chambers to assess embryo viability using *in vitro* germination and the tetrazolium test, which was adapted from the method of Ribeiro et al. (2010). The experimental design was completely randomized, fully factorial (3 drying times × 2 drying temperatures) and used 4 replicates of 10 fruits and seeds for each variable combination. The F-test was applied and the treatment means were compared using Tukey's test (5%).

RESULTS AND DISCUSSION

The fruits were collected with initial water content above 20%. After 11 days of drying, the water content was reduced by 20.5% in the fruits dried at 57 ± 2°C and by 12.9% in those dried at 37 ± 2°C (Table 1). In the seeds, 62.8% of the initial water content was lost when the seeds were dried at *ca*. 57°C, while the seeds dried at *ca*.

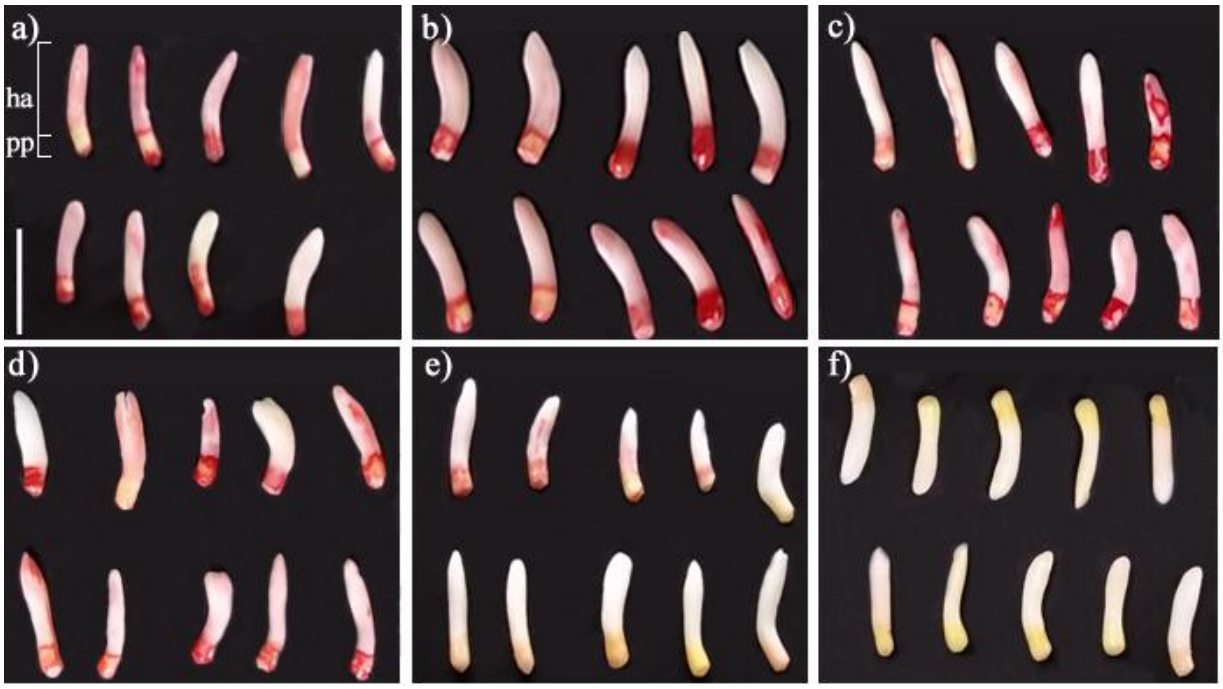

Figure 1. *O. phalerata* embryos subjected to the tetrazolium test. Bar = 2 cm. The haustorium (ha) and petiole proximal (pp) regions of the embryos are shown in the figure. Embryos obtained from fruits without drying (1a and 1d); embryos obtained from fruits dried at 37 ± 2°C for 6 (1b) and 11 days (1c) embryos obtained from fruits dried at 57 ± 2°C for 6 (1e) and 11 days (1f) are demonstrate.

37°C lost 57.2% of their initial water content. The seed water content varies among the species of this family at the time of harvest. For example, 46.5% water content (fresh wt. basis) was observed in bacaba (*Oenocarpus minor* Mart.) seeds and 21.8% water content was observed in pygmy date palm (*Phoenix roebelenii* O'Brien) seeds (Iossi et al., 2003; Silva et al., 2006). No interaction between drying time and temperature on water loss was observed; therefore, these two factors were treated separately. There was no significant difference in water content between the two temperatures tested. For the drying times tested, the fruits dehydrated at 37 ± 2°C initially lost a substantial amount of water. However, this rate of loss decreased and the water content remained stable for up to 11 days, eventually reaching 20.38%. Drying at 57 ± 2°C resulted in sustained water loss, and the fruits reached 18.98% water content.

The embryos were properly colored using the method of Ribeiro et al. (2010), which allowed for the identification of vigor. However, according to the method used, no embryo was classified as vigorous (vigor class 1). At most, the embryos were classified as viable (vigor class 2), even without drying. Although the embryos were colored with an intense red or pink color, uncolored areas were also observed in the proximal region of the petiole, which is characteristic of vigor class 2 (Figure 1).

The embryos obtained from the fruits that were dried at 37 ± 2°C for up to 6 days were classified as viable, according to tetrazolium test. The viability of these embryos was higher compared with the embryos obtained from the fruits that were dried at the same temperature for 11 days or from the fruits that were dried at 57 ± 2°C for 6 and 11 days. No dead embryos were observed at 37 ± 2°C for any of the time periods assessed. However, the embryos dried at 57 ± 2°C, regardless of the drying period, quickly lost their viability. At this temperature, 52.5 and 47.5% of the embryos were non-viable and dead, respectively, after 6 days of drying. These results suggest that drying temperature had a deleterious effect on the embryo viability (Table 2).

Few studies concerning seed water loss behavior in the Arecaceae family have been reported, which hinders the discussion of the present study. Unlike the water loss, an interaction between drying time and temperature on viability was observed; therefore, these two factors were analyzed together. No reduction in the germination percentage or speed of *in vitro* germination was observed when the embryos were dehydrated at 37 ± 2°C for up to 11 days. In contrast, dehydration at 57 ± 2°C reduced these values, which indicates that this temperature exerts a deleterious effect. Germination rates above 67% were obtained for all times at drying 37°C. This result suggests a possible orthodox behavior, which has been verified by the work of Silva et al. (2011) with the same species. Similar to what was observed for the germination percentages; the embryos that were dried at 57 ± 2°C suffered a reduction in vigor, which was evident from their

Table 2. Mean percentage of viable, vigorous, nonviable, and dead *O. phalerata* embryos that were dried in a forced-air dry chamber for different time periods and at different temperatures estimated by triphenyl tetrazolium chloride test.

Drying (Days)	Drying temperature							
	37 ± 2°C				57 ± 2°C			
	Vigorous	Viable	Non-viable	Dead	Vigorous	Viable	Non-viable	Dead
0	0.0^{Aay}	52.7^{Aa}	47.2^{Ba}	0.0^{Aa}	0.0^{Aa}	52.7^{Aa}	47.2^{Aa}	0.0^{Ba}
6	0.0^{Aa}	57.5^{Aa}	42.5^{Ba}	0.0^{Aa}	0.0^{Aa}	0.0^{Bb}	52.5^{Aa}	47.5^{Ab}
11	0.0^{Aa}	10.0^{Ba}	90.0^{Aa}	0.0^{Aa}	0.0^{Aa}	0.0^{Ba}	42.5^{Ab}	55.0^{Ab}

[y]Means that are followed by the same uppercase letter between the drying times and the same lowercase letter between the drying temperatures do not differ according to Tukey's test at 5% probability.

Table 3. The germination percentages and speed of germination indices (SGIs) of *O. phalerata* embryos that were extracted from fruits subjected to different drying times and temperatures in a forced-air and inoculated *in vitro*.

Drying (Days)	Drying temperature			
	37 ± 2°C		57 ± 2°C	
	Germination (%)	SGI	Germination (%)	SGI
0	67.10^{Aay} (± 9.22)[z]	0.56^{Aa} (± 0.05)	67.10^{Aa} (± 9.22)	0.56^{Aa} (± 0.05)
6	67.64^{Aa} (± 12.9)	0.40^{Aa} (± 0.03)	11.11^{Bb} (± 6.41)	0.04^{Bb} (± 0.01)
11	86.88^{Aa} (± 2.37)	0.42^{Aa} (± 0.03)	2.50^{Bb} (± 2.50)	0.06^{Bb} (± 0.02)

[y]Means that are followed by the same uppercase letter between the drying times and the same lowercase letter between the drying temperatures do not differ according to Tukey's test at 5% probability. [z]Standard error of the mean.

speed of germination indices (SGIs) (Table 3). Ferreira and Santos (1993) also observed an interaction between different drying temperatures and times on peach-palm (*Bactris gasipaes* Kunth) seeds and noted that slow drying for up to 8 days does not affect seed germination and vigor. However, when submitted to fast drying in silica gel, germination was interrupted, which suggests a possible recalcitrant behavior for this species.

Some differences between the results of the viability test (tetrazolium) and the *in vitro* germination assay were observed after 6 days of drying. The tetrazolium test indicated that dehydration for 11 days at 37 ± 2°C rendered the embryos non-viable, which was not observed during embryo culture. In contrast, the *in vitro* germination test at 57 ± 2°C confirmed the results that were obtained by the viability test. In a study of the zygotic embryos of *A. aculeata*, the *in vitro* germination test corroborated the tetrazolium test results (Ribeiro et al., 2010). The method of Ribeiro et al. (2010) was effective for testing macauba (*A. aculeata*) embryo viability. However, some modifications of the method were necessary for the use of this test on the babassu embryos (*O. phalerata* Mart.). Although the babassu embryos were classified as vigor class 2, we observed that the embryos were highly viable in the *in vitro* germination assay and reached a germination rate of 86.8%. Additionally, insufficient soaking time or the high oil content of the embryo may hinder salt diffusion (Wood et al., 2005).

According to the results discussed here, we suggest that the vigor classes (vigorous, viable and non-viable) should be reclassified as viable and non-viable when the method of Ribeiro et al. (2010) is applied to babassu (*O. phalerata* Mart.). Viable embryos are those that stain intensely red or pink but also include those embryos that lack dye in up to 30% of the areas of the haustorium and petiole proximal regions. Embryos that lack dye in more than 30% of the haustorium and petiole proximal regions are non-viable. Martins et al. (2009) observed that the drying time had a deleterious effect on the germination of palmiteiro (*Euterpe edulis* Mart.) seeds. We observed that drying babassu (*O. phalerata* Mart.) fruits at 57 ± 2°C reduced the physiological quality of the embryos and decreased the viability, germination percentage and SGI. These findings suggest that dehydration at higher temperatures allows for the faster release of water from the embryo tissues, thus damaging their membrane structures.

Under the experimental conditions used in the present study, we observed that dehydration at 57 ± 2°C reduced the germination percentage and speed. A similar result was observed for palmiteiro (*Euterpe edulis* Mart.) seeds, in which fast drying in silica gel promoted the deterioration of apical meristem cells (Panza et al., 2007). The germination process, average time and vigor were impaired when assai (*Euterpe oleraceae*) seeds were

Table 4. Length (cm) of *O. phalerata* seedlings cultured *in vitro* for 30, 60 and 90 days from the zygotic embryos obtained from fruits subjected to different times and temperatures.

Drying (Days)	Drying temperature					
	37 ± 2°C			57 ± 2°C		
	Cultivation time (days)					
	30	60	90	30	60	90
0	1.34^{Aay} (±0.13)^{z}	1.99^{Aba} (±0.12)	3.24^{Ba} (±0.40)	1.34^{Aa} (± 0.13)	1.99^{Aa} (± 0.12)	3.24^{Aa} (± 0.40)
6	1.58^{Aa} (± 0.10)	2.58^{Aa} (± 0.20)	5.17^{Aa} (± 0.12)	1.12^{Ab} (± 0.12)	1.69^{Ab} (± 0.12)	2.44^{Ab} (± 0.25)
11	1.35^{Aa} (± 0.05)	1.86^{Ba} (± 0.13)	2.76^{Ba} (± 0.14)	0.25^{Bb} (± 0.25)	0.27^{Bb} (± 0.28)	0.62^{Bb} (± 0.62)

[y]Means that are followed by the same uppercase letter between the drying times and the same lowercase letter between the drying temperatures do not differ according to Tukey's test at 5% probability. [z]Standard error of the mean.

slowly dehydrated until they reached 30.3% water content (Nascimento et al., 2007). These observations suggest that the drying process should be adjusted for each species. The *in vitro* growth rate at 30, 60, and 90 days after inoculation showed that drying *O. phalerata* fruits at 57 ± 2°C decreased the embryo germination percentage and speed of germination, and slowed the initial seedling growth rate compared with those dried at 37 ± 2°C (Table 4).

Slow initial growth has also been observed in royal palm (*Archontophoenix alexandrae* Wendl.and Drude) seedlings when the fruits were dried in a dry chamber at 28.52 ± 2.5°C (Martins et al., 2003). Those authors suggested that the damage caused to the membrane, as demonstrated by an electrical conductivity test, influenced this result. During the first 30 days of *in vitro* culture, the drying time was not observed to affect the length of seedlings that originated from the embryos dried at 37 ± 2°C.

However, at 60 and 90 days of culture, we observed that the largest seedlings were derived from the embryos that had not been subjected to dehydration or were dehydrated for only up to 6 days. The embryos that were dried at 57 ± 2°C resulted in smaller seedlings. There was no difference in seedling length between the embryos not dehydrated (control) and those that were dehydrated for 6 days.

Conclusion

With the proposed changes to the tetrazolium test interpretation for *O. phalerata*, it is now possible to determine the exact number of viable, non-viable and dead embryos.

Unlike drying at 57 ± 2°C, drying at 37 ± 2°C for up to 11 days does not decrease *in vitro* embryo germination. Our results demonstrate an orthodox behavior for this species because the embryo germination capacity was not affected, even when seeds were dried to below 10% of their water content. The embryos that were not dehydrated or those that were dehydrated at 37 ± 2°C for up to 6 days resulted in seedlings that initially grew faster *in vitro.*

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