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Desiccation tolerance of embryos of *Syagrus oleracea*, a cerrado native bitter palm heart

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There is great interest in seedling production of plant species with ornamental and food potential; however, there is great difficulty in propagating this species. A method of fruit drying was proposed with the goal of evaluating the desiccation tolerance of embryos of guariroba palm *Syagrus oleracea*. Tissue culture was used to test the effect of different fruit drying times (0, 4, 8 and 12 days) on embryo viability and *in vitro* germination. Desiccation tolerance of guariroba palm embryos was determined, and a methodology for their *in vitro* establishment was proposed. The seed water content was estimated from the fruit water content without extracting the seed. Drying at 37±2°C did not negatively affect embryo viability or germination. In addition, contamination of zygotic embryos decreased with increasing drying time. Fruit drying at 37±2°C for 8 days is recommended because it made the embryo extraction easier without embryo viability loss or contamination during the *in vitro* establishment of guariroba palm.

Key words: Desiccation, guariroba, propagation, ornamentation, physiological quality, Arecaceae.

INTRODUCTION

The guariroba palm (*Syagrus oleracea* (Mart.) Becc.), which belongs to the family Arecaceae, is commonly known as palmito-amargo (biter palm heart) and gueroba. The nut and palm heart of the guariroba palm are eaten fresh, and they are rich in peroxidase and polyphenoloxidase enzymes, which together with the phenols cause the darkening of the palm heart, giving it a characteristic taste much appreciated in cooking, especially in the central region of Brazil. Due to its beauty, this plant has become a popular ornamentation in

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urban and rural landscapes. The fruits of the guariroba palm are the basis of wildlife diets (Matteucci et al., 1995; Silva et al., 2001; Jaime et al., 2007).

To improve the process of seed extraction, some authors have recommended decreasing the seed water content in a forced air circulation oven at temperatures that result in the release of the seed from the fruit's endocarp, keeping the seed intact when the endocarp is broken (Ferreira and Gentil, 2006; Rubio Neto et al., 2012). However, the loss of seed water can decrease the seed viability and germination in some species, as reported for açaí seeds (*Euterpe oleracea* Mart.) (Nascimento et al., 2007) and red palm heart seeds (*Euterpe espiritosantensis* Fernandes) (Martins et al., 1999). For *S. oleracea*, there are no reports relating the loss of fruit and seed water content with germination, but there are reports on diaspores (endocarp+seed) that are collected with approximately 20% water content.

Because the drying process can make the embryos unviable, several quick tests to determine viability, such as the tetrazolium test and electrical conductivity, have been proposed for crops with high agricultural importance. These studies are scarce for plants of the family Arecaceae, but adaptations of the tetrazolium test have been performed using salt concentrations ranging from 0.075 to 1% and imbibition times in the tetrazolium solution for enzyme activation between 2 and 6 h, with preference for a 0.5% salt concentration and 4 h of imbibition at 30°C. Under these conditions, tetrazolium confers different colours to the embryos, allowing their classification into different vigour classes according to lesion proportion, quantity and localisation (Ferreira and Sader, 1987; Reis et al., 1999; Spera et al., 2001; Ribeiro et al., 2010).

Plants from the family Arecaceae often have difficulties in germination due to morphological characteristics of the seed and peculiarities of the germination process. It is common for some species to not germinate even under adequate germination conditions, which can result from dormancy mechanisms. For *S. oleracea*, the highest percentages of emergence have been obtained from seeds kept in vermiculite reaching 53.8% after 82 days of culture (Batista et al., 2011). The culture of embryos can therefore be advantageous because extracting the embryo from the seed removes all mechanical resistance and a large part of the germination inhibitors, thereby accelerating the process of seedling production (Hu and Ferreira, 1998; Lorenzi et al., 2004).

Embryo culture studies have focused on *in vitro* establishment, growth medium composition, embryo oxidation and maturation. Different disinfection times as well as alcohol and sodium hypochlorite concentrations have been recommended to minimise embryo contamination of species from the family Arecaceae (Sugii, 2011). Another important problem of embryo culture is the oxidation rate, which can be controlled by ascorbic acid in the case of *S. oleracea* (Melo et al., 2001).

The goal of the study was to evaluate the effect of fruit drying on seed vigour and viability, enabling *in vitro* establishment of plantlets of guariroba palm. This study is warranted by the fact that *S. oleracea* has high social, environmental and economic importance, but its propagation using conventional methods is difficult. This difficulty can be overcome using *in vitro* embryo culture as highlighted by Melo et al. (2001).

MATERIALS AND METHODS

This study was performed at the Seed Laboratory (Laboratório de Sementes) and Plant Tissue Culture Laboratory (Laboratório de Cultura de Tecidos Vegetais) of the Goiás Federal Institute (Instituto Federal Goiano), Rio Verde Campus, Goiás (GO), Brazil. Ripe fruits of *S. oleracea* collected in October 2012 at the Gameleira farm in the Montes Claros de Goiás municipality, GO (16° 07' S and 51º 18' W; 592 m altitude) were used. Damaged fruits were discarded, and due to their high heterogeneity, fruits were classified into three classes according to their total mass (Figure 1A). The number of fruits per class followed the frequency distribution found at the field, that is, 30 small fruits (≤27.8 g), 35 medium fruits (28.0 to 34.9 g) and 15 large fruits (≥35.0 g). For this study, 80 fruits in total were used, from which 80 embryos were extracted.

To evaluate the effect of the drying temperature, whole fruits were dried in a force air circulation oven at 37±2°C for 0, 4, 8 or 12 days (Figure 1B). For all tested drying times, water contents of fruits and seeds were measured using an oven at 105°C until constant weight was reached.

For all tested times, a fruit lot was removed from the oven and broken using a 1.5 kg mallet and a concrete plate to evaluate the physiological quality of the embryos. Immediately following extraction, the embryos were covered in gauze and placed in running water for 3 h according to Melo et al. (2001). The embryos were then disinfected with 70% alcohol for 30 s, 20% commercial sodium hypochlorite for 20 min and rinsed three times in autoclaved distilled water (Figure 1C). Following inoculation in half-strength MS (Murashige and Skoog, 1962) growth medium, the embryos were kept in a growth chamber at 25±3°C in the absence of light for 15 days. Following this period, the embryos were kept under a 16 h light/8 h dark photoperiod at 25±3°C and 40-60 µmol m^{-2} s^{-1} active photosynthetic radiation supplied by fluorescent lights.

At the same time that embryos were extracted for *in vitro* germination, embryos were also removed for viability evaluation. The tetrazolium test was adapted from Ribeiro et al. (2010) using four replicates of 10 embryos for each drying time (Figure 1C).

The experimental design was completely randomised with four drying times and four replicates of 20 fruits and/or seeds. Analysis of variance was performed, and regression analysis was performed when necessary. The Pearson product-moment correlation coefficient was used to evaluate correlations at $p<0.05$.

RESULTS AND DISCUSSION

Regression analysis revealed pronounced fruit water loss, which increased exponentially with increasing drying time. The model fitted to the drying data was significant, and it showed that fruits exhibited 34.6% water content at the time of harvest and that a water content of 8.84% was reached after 12 days at 37°C (Figure 2A). Diaspores of

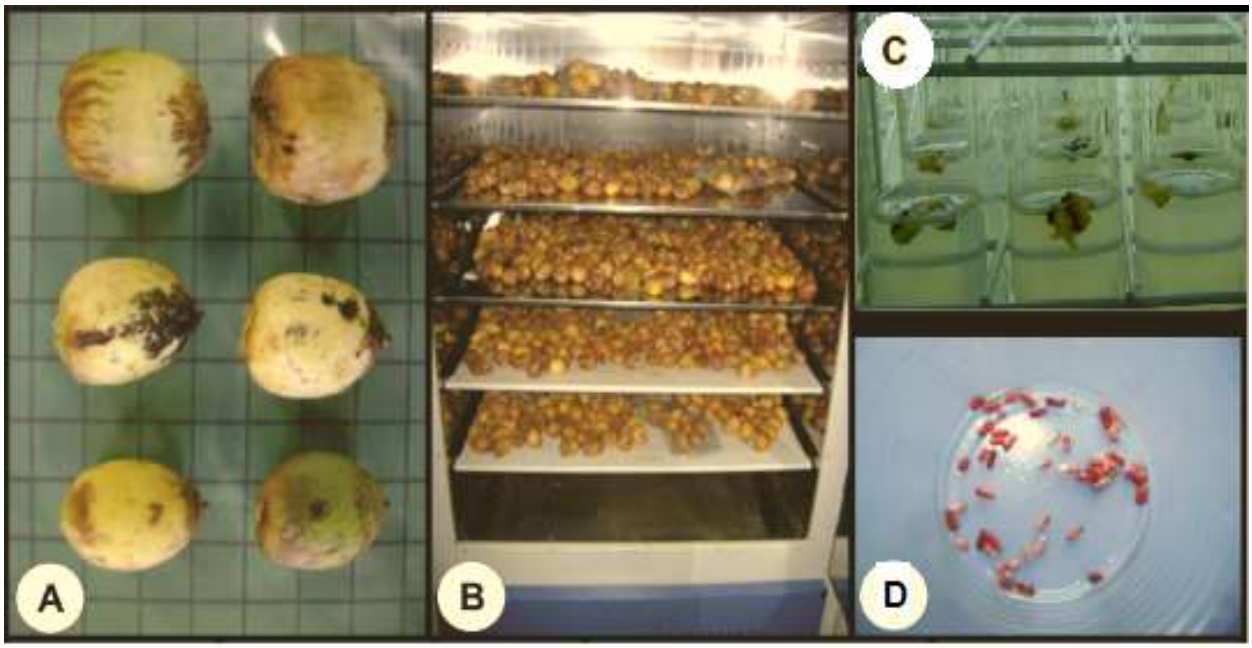

Figure 1. Method for *in vitro* establishment and tetrazolium test. A) Fruit classification according to their mass. B) Drying in a forced air circulation oven at 37°C. C) Embryo *in vitro* germination. D) Tetrazolium test following drying for 4 days.

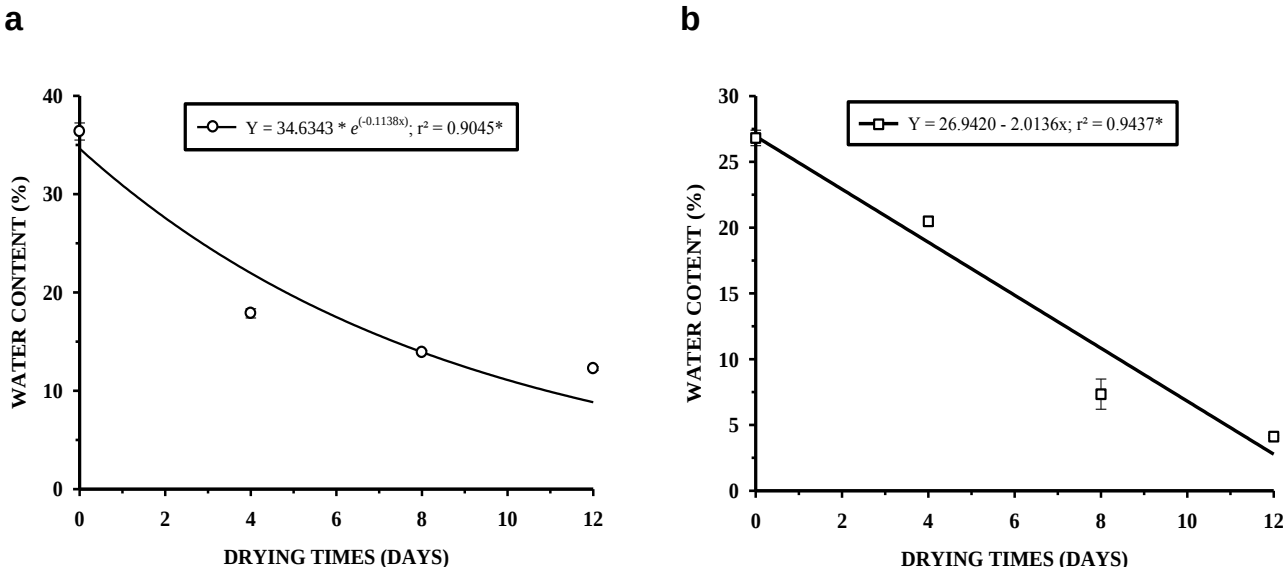

Figure 2. Water content of fruits (A) and seeds (B) of guariroba palm [*Syagrus oleracea* (Mart.) Becc.] dried in a forced air circulation oven at 37°C for different times. *Significant at $p<0.05$.

S. oleracea collected in 2009 have been shown to reach a 19.9% water content (Batista et al., 2011), indicating that the epicarp and mesocarp contain a large part of the whole fruit water content.

The seed water content was 26.9% at the time of harvest. The seed water content linearly decreased with increasing drying time at a rate of 2.01% per day, and it reached 2.79% after 12 days of drying. The exponential fruit water loss may have been due to the morphological characteristics of the fruits, which allow the easy exit of water from the epicarp and mesocarp. In contrast, the seed water loss was slower, which was most likely due to the presence of the rigid fruit endocarp that made the water loss linear with increasing drying time (Figure 2B).

Water loss at 37°C in a forced air circulation oven has been found to be efficient for the drying of macaúba palm

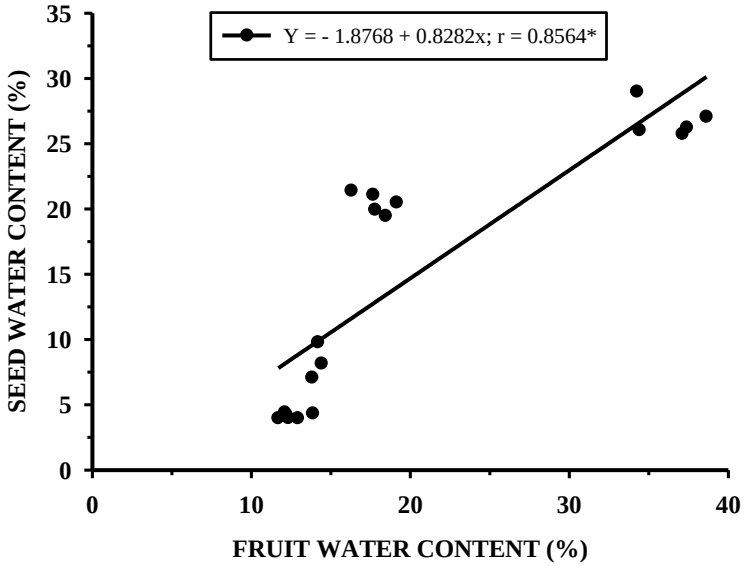

Figure 3. Correlation between water contents of fruits and seeds of guariroba palm [*Syagrus oleracea* (Mart.) Becc.] dried for 12 days in an oven at 37°C. *Significant at $p<0.05$.

[*Acrocomia aculeata* (Jacq.) Lodd. ex Mart.] and babassu palm (*Orbignya phalerata* Mart.) fruits, thereby reducing fruit water content and facilitating the extraction of intact seeds and zygotic embryos similarly to what has been observed for *S. oleracea* (Rubio Neto et al., 2012; Silva et al., 2012) .

A positive correlation was observed between the water content of fruits and seeds (r=0.8564*) during drying at 37±2°C. Therefore, the seed water content was correlated with the fruit water loss. In future studies, it will be possible to estimate seed water loss based on fruit water loss at 37°C without the need to break the fruits for seed extraction. The water loss of fruits and seeds dried at 37±2°C occurred at a 1:0.8282% ratio, thereby showing that the fruits lose water in a higher proportion than the seeds (Figure 3).

The importance of studying this effect for each species is reinforced by the fact that some species, such as palm heart (*Euterpe edulis* Mart.), açaí palm (*Euterpe oleracea* Mart.), red palm heart (*Euterpe espiritosantensis* Fernandes), bacaba (*Oenocarpus bacaba* Mart.) and Alexander palm (*Archontophoenix alexandrae* Wendl. and Drude), are considered recalcitrant because they lose germination capacity with increasing water loss. In contrast, other species, such as babassu palm (*Orbignya phalerata* Mart.), macaúba palm [*Acrocomia aculeata* (Jacq.) Lodd. ex Mart.] and carandá (*Copernicia alba* Morong.), are considered orthodox. In the present work, *S. oleracea* was observed to present orthodox characteristics because the decrease in seed water content did not affect embryo *in vitro* germination and viability. However, further studies should be performed to confirm these results, namely testing different times and storage environments (Martins et al., 1999; Reis et al., 1999; Nascimento et al., 2007; Ribeiro et al., 2010; José et al., 2012; Masetto et al., 2012; Silva et al., 2012).

At the beginning of the drying process, the *S. oleracea* embryos exhibited an intense red colour, thereby being classified as vigorous (Figure 4A and B). With the increase in drying time, the embryos became light pink at the haustorium and at some lesions at the petiole, thereby being classified as viable (class two of vigour) (Figure 4C and D).

The modification of the tetrazolium test proposed by Ribeiro et al. (2010) resulted in adequate colouring of *S. oleracea* embryos, enabling the identification of their vigour. With the increase in drying time, a decrease in class one (vigorous) embryos and an increase in class two (viable) embryos (which also germinated *in vitro*) were observed, thereby confirming the results of the vigour tests.

Tetrazolium salt concentrations between 0.075 and 1% in addition to imbibition times varying between 2 and 6 h have been tested for the family Arecaceae. The tetrazolium test has been evaluated for several different species. According to Ferreira and Sader (1987), the efficiency of the tetrazolium test should be confirmed by correlation with germination tests. The concentration of and imbibition time in tetrazolium solution most often used is 0.5% and 4 h, respectively, for enzyme activation at 30°C (Ferreira and Sader, 1987; Reis et al., 1999; Spera et al., 2001; Ribeiro et al., 2010; Rubio Neto et al., 2012).

The number of viable embryos increased quadratically,

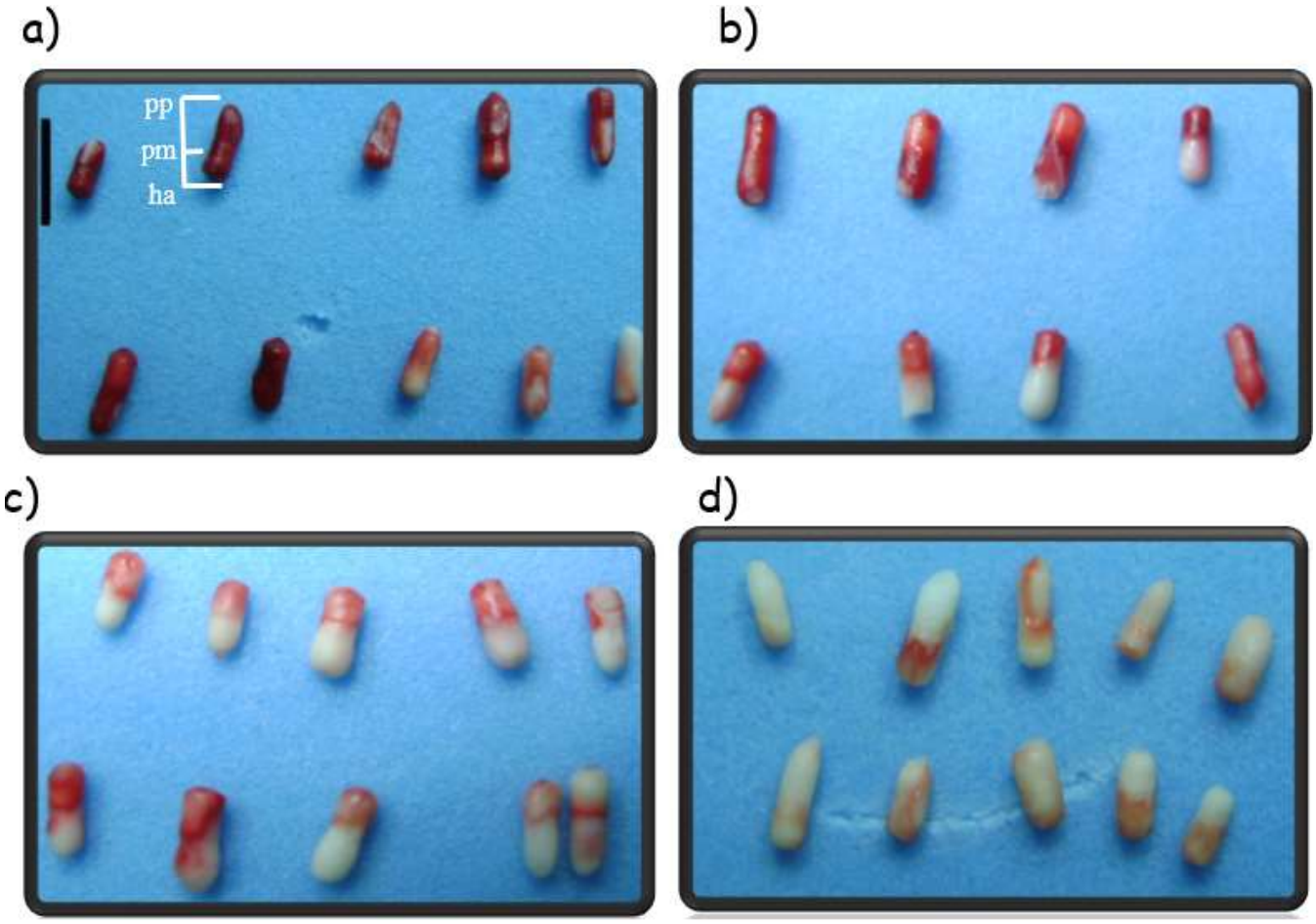

Figure 4. Zygotic embryos of guariroba palm [*Syagrus oleracea* (Mart.) Becc.] in the tetrazolium test (Bar = 1 cm). ha, Haustorium; pm, median; pp, proximal petiole regions. **a)** Embryos obtained from fruits without drying. **(b, c and d)** Embryos obtained from fruits dried at 37±2°C for 4, 8 and 12 days, respectively.

with increasing drying time reaching its maximum (75.2%) following 7.5 days of drying (Figure 5A and B). The percentages of unviable and dead embryos were low at all drying times, with averages of 12.0 and 3.5%, respectively (Figure 5C and D). However, the average percentage of vigorous embryos (vigour class one) linearly decreased with increasing drying time, with values decreasing from 67.2% at the time of harvest to 0% following 12 days of drying. This trend was accompanied by an increase in the average percentage of viable embryos (vigour class two), which also germinate *in vitro.*

The present study demonstrated that drying for up to 12 days in a forced air circulation oven does not negatively affect germination and plantlet initial growth (Figure 6). Using *S. oleracea* diaspores (seed + endocarp), however, Matteucci et al. (1995) obtained higher germination percentages in seeds of depulped freshly harvested fruits than in fruits dried for 30 days and not depulped. Thus, it can be concluded that different results can be obtained for the same species depending on the form of drying because the water removal can damage cell walls and result in loss of vigour.

The average germination percentage remained stable with the increase in drying time, with an average of 71.1% (Figure 7). In contrast, contamination by micro-organisms was high in zygotic embryos extracted from fruits that were not dried at 37°C. This contamination decreased with increasing drying time in an oven over 7.5 days of drying, reaching an average of 3.33% contamination, which is considered satisfactory for *in vitro* establishment.

A positive correlation was observed between *in vitro* contamination and seed water content. As the seed water content decreased, the average percentage of *in vitro* contamination also decreased in a 1:0.82 ratio, which indicated that drying at this temperature improves seed and embryo extraction but also results in lower *in vitro* embryo contamination (Figure 8). Similar results have been observed for macaw palm [*Acrocomia aculeata* (Jacq.) Lood. ex Mart.] (Rubio Neto et al., 2012){Rubio

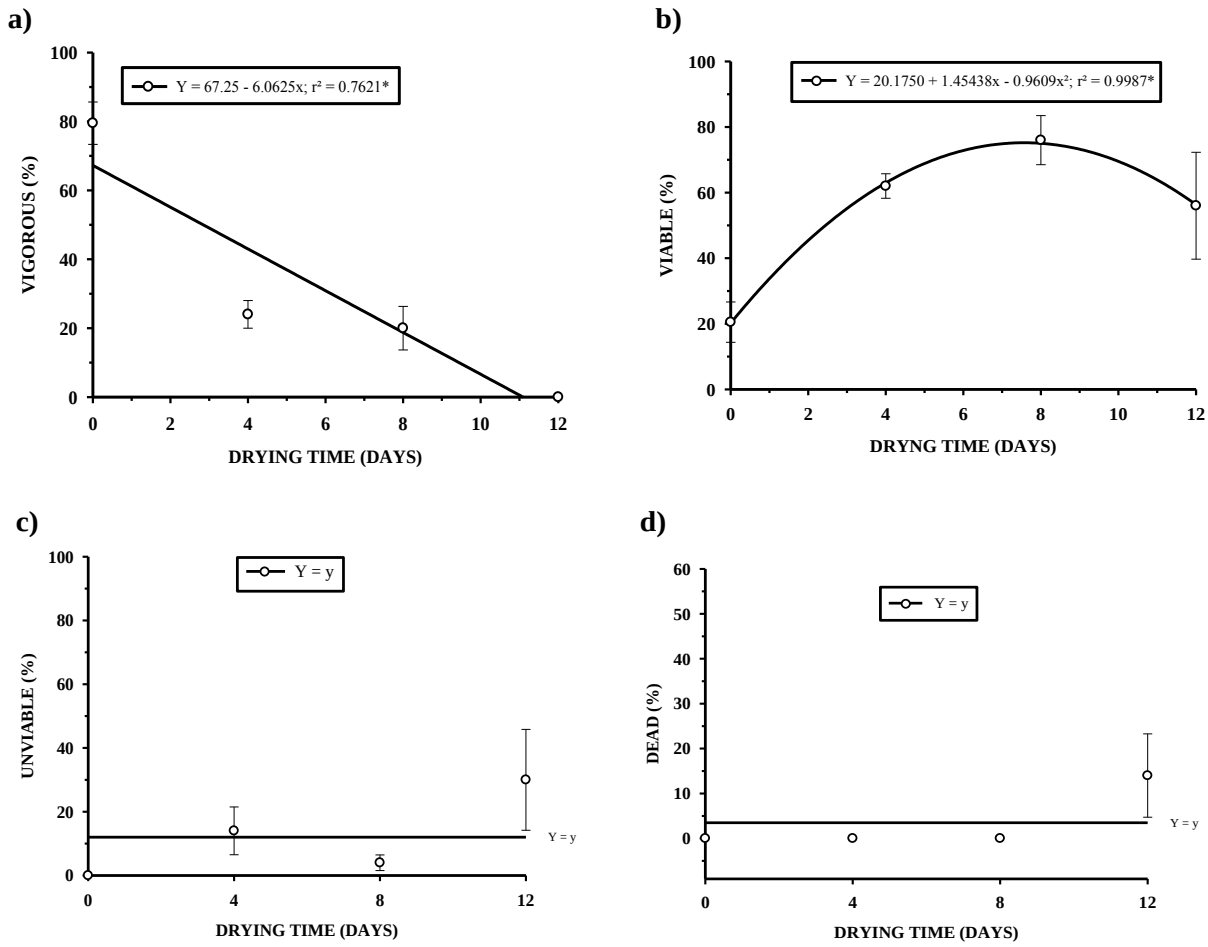

Figure 5. Percentage of vigorous (A), viable (B), unviable (C) and dead (D) zygotic embryos of guariroba palm [*Syagrus oleracea* (Mart.) Becc.] dried for 12 days in an oven at 37°C. *Significant at $p<0.05$.

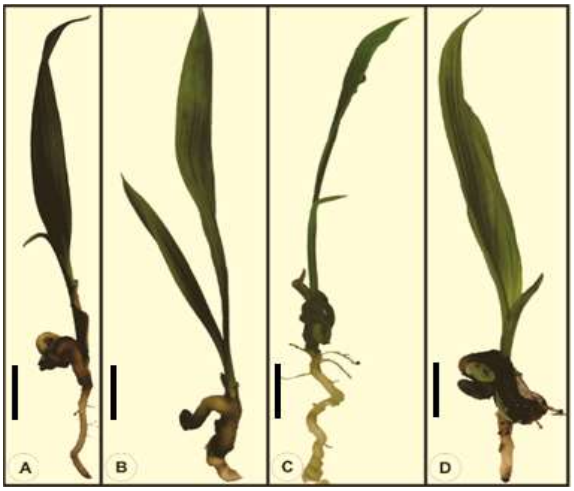

Figure 6. Plantlets of guariroba palm [*Syagrus oleracea* (Mart.) Becc.] grown for 90 days obtained from zygotic embryos extracted from fruits dried superficially A) without drying, B) with 4 days of drying, C) with 8 days of drying and D) with 12 days of drying at 37°C. Bar = 2 cm.

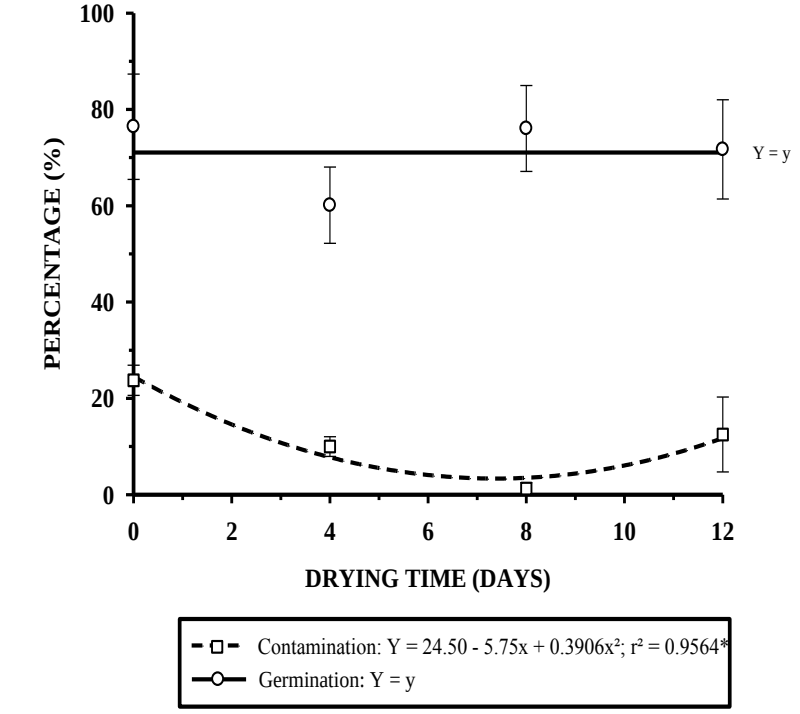

Figure 7. Germination and contamination by microorganisms (%) in zygotic embryos of guariroba palm [*Syagrus oleracea* (Mart.) Becc.] extracted from fruits dried for different times at 37±2°C.

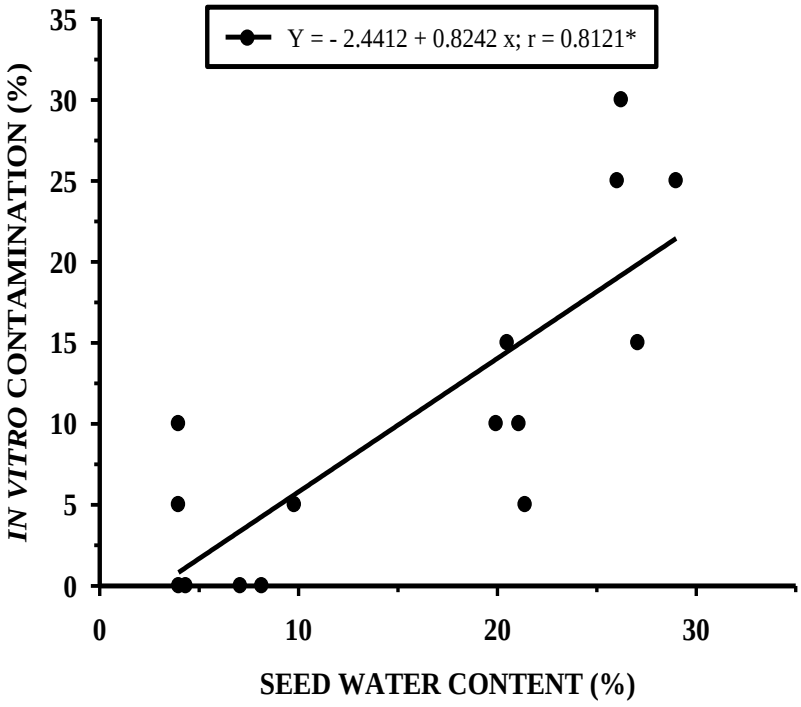

Figure 8. Correlation between *in vitro* contamination and water content of seeds of guariroba palm [*Syagrus oleracea* (Mart.) Becc.] extracted from fruits dried at 37±2°C for different times.

Neto, 2012 #50}.

Conclusions

Drying at 37±2°C for 8 days was effective for the extraction of *S. oleracea* embryos without being detrimental to embryo viability and *in vitro* germination, thereby indicating a possible orthodox behaviour of this species. *In vitro* establishment of *S. oleracea* via zygotic embryo culture can be optimised by drying the fruits at 37°C, as there was a decrease in contamination without decreasing embryo vigour. It was possible to estimate the seed water content without seed extraction by determining the fruit water content. The tetrazolium test, as modified for this species, in association with the germination test allows the evaluation of *S. oleracea* embryo viability.

Conflict of interests

The authors did not declare any conflict of interest.

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