Full Length Research Paper

Effects of drying temperature on viability of macaw palm (*Acrocomia aculeata*) zygotic embryos

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In response to a growing interest in improving seedling production of oilseed species (like macaw palm), a fruit drying protocol for facilitating seed extraction was proposed. This enabled the production of macaw palm seedlings, but the temperature most suitable for seed extraction without losing its physiological quality is unknown. The goal of this study was to evaluate the effects of different drying temperatures on the physiological quality of macaw palm zygotic embryos to improve previously published drying methods. Fruits were dried in a forced-air drying oven at 57 or 37°C at different time periods (zero, two, four, six and eight days). Following each drying period, the fruits were removed from the drying oven, and the water content of the fruits and seeds were measured in addition to embryo viability and *in vitro* germination. Seed water content could be estimated based on fruit water content at both drying temperatures, eliminating the need to remove the seeds from the fruit. Drying at 57°C decreased the drying time by 50% compared to drying at 37°C; however, it was detrimental to embryo viability and germination. Therefore, drying of fruit at 37°C is recommended. Embryos dried at this temperature were still able to germinate after 16-day drying period, which corresponded to a decrease of 24.8% in the initial fruit water content.

Key words: Arecaceae, biodiesel, dehydration, physiological quality, propagation.

INTRODUCTION

Macaw palm [*Acrocomia aculeata* (Jacq.) Lood. Ex Mart.] is locally known as *macaúba, bocaiúva, coco-de-espinha, macaúva, marcová* and *mucajá*. This palm can reach 10 to 15 m in height and 3 to 4 m in crown diameter. The pinnate leaves are clustered at the stipe apex and are up to 5 m long. The yellow fruits are spherical or slightly flattened, globose and drupe shaped, with a diameter of 2 to 5 cm; have a cartaceous epicarp and a thin and fibrous

mesocarp. The bony endocarp is blackened and strongly attached to the mesocarp. There are generally between one and three seeds, which are oleaginous and edible. They are covered with a thin tegument layer, which, together with the endocarp, determines the high degree of seed dormancy in this species (Almeida et al., 1998; Silva et al., 2001; Lorenzi et al., 2004; Ramos et al., 2008).

Macaw palm is used for feed, ornaments, cosmetic oil and biofuels. Despite having many practical uses, macaw palm, like *tucumã* (*Astrocaryum aculeatum* Meyer) and wild areca palm [*Areca triandra* (Roxb.) ex Buch-Ham], is rarely cultivated. This is mostly due to its poor germination rates, as well as lack of studies that provide information about its germination. Palms are generally propagated through seeds, and palm seed germination is known to be slow and uneven because the seed's protective layer (endocarp) restricts water absorption and oxygen diffusion and exerts mechanical resistance to seedling emergence, resulting in a high degree of physical seed dormancy (Meerow, 1991; Ferreira and Gentil, 2006; Yang et al., 2007).

Due to the environmental and socio- economic impor-tance of macaw palm and the difficulty in propagating it by conventional methods, a seedling production protocol was recently proposed to enable efficient seedling production. With this, it can be used for family farming and will lead to increased profit. The protocol includes drying the fruits at room temperature to ease endocarp removal and seed extraction, followed by mechanical scarification of the tegument, immersion of the seeds in gibberellic acid and sowing of the seeds in a sterilised substrate (Motoike et al., 2007). This protocol can be time consuming, because it necessitates drying for five to seven days. An exploration of alternative protocols is warranted, given that seedling production of this species is still in its initial stage. Mechanical scarification of seeds followed by immersion in gibberellic acid was reported to increase seedling emergence by 50% after four weeks (Ribeiro et al., 2011).

To accelerate seed extraction and ensure the physical quality of the seeds, the water content of the seeds must be decreased to the point that they become detached from the endocarp; thus preventing damage to the seeds during extraction. However, the critical water content for seed survival is relatively high in this family; that is, there can be total loss of seed viability even at high water contents. For açaí palm (*Euterpe oleraceae* Mart.) fruits dehydrated in a drying oven at 30°C, a progressive decrease in seedling germination was observed in seeds with a water content below 15% (Nascimento et al., 2007).

Water content, drying method and storage time can interfere with seed germination in species of the family Arecaceae. This is because the drying process decreases seed moisture and consequently affects meta-bolic processes (Chien and Chen, 2008; Ribeiro et al.,

2012). For macaw palm, it was observed that drying of fruits for seven days at 37°C allowed for the extraction of physically intact seeds without a loss in their physiological quality. To reduce drying time is necessary for evaluating new drying temperatures. (Neto et al., 2012).

As the drying temperature increases, this can decrease the drying time in oven. The goal of this study is to test the effects of drying temperature and water content on the physiological quality of macaw palm embryos to improve the existing drying protocol.

MATERIALS AND METHODS

The study was performed at the Seed and Plant Tissue Culture Laboratory of the Federal Institute of Goiás (Instituto Federal Goiano), Rio Verde Campus, Goiás - Brazil, using ripe macaw palm fruits collected in January 2011 at the Gameleira farm in the municipality of Montes Claros de Goiás, GO (16° 07' S, 51° 18' W, 592 m altitude).

Fruit drying with varying drying time

Due to their high heterogeneity in size, healthy fruits were divided into three classes according to their total mass. The number of fruits per class corresponded to the size distribution observed in the field; that is, four small fruits (≤ 40.0 g), 14 medium fruits (40.1-55.0 g) and eight large fruits (≥ 55.1 g), making a total of 26 fruits. This was done to homogenize the replicates. The influence of size on seed germination is not known. A total of 40 embryos were removed from these fruits.

The fruits were dried whole in a forced-air drying oven (Tecnal TE 394) at 57 ± 2 or 37 ± 2°C for zero, two, four, six and eight days to test the effect of drying temperature. 57°C was selected as the temperature in order to minimize drying time based on previous studies.

Fruit drying with varying water contents

The effect of drying temperature on fruits with varying water content was tested. At the end of each drying period, the water content of fruits that were partially dried at 57°C was determined using the drying oven method. For this purpose, fruit fresh weight was measured, and the fruits were placed in a 105 ± 2°C drying oven until they reached a constant mass (Brasil, 2009). Subsequently, a second fruit lot dried at 37°C was removed from the oven when the fruits reached the same moisture content, as they dried at 57°C (Figure 1).

In vitro germination

For each drying time and water content tested, a separate fruit lot was removed from the drying oven. The fruits were opened using a 1.5 kg hammer and a concrete block; and the physiological quality of the embryos was evaluated according to Neto et al. (2012). The embryos were inoculated with half-strength MS culture medium (Murashige and Skoog, 1962) and kept in growth chambers at 25 ± 3°C in the dark for 15 days. Following this period, the embryos were subjected to a 16 h light/8 h dark photoperiod at 25 ± 3°C and 40-60 μmol.m^{-2}.s^{-1} photosynthetically active radiation supplied by fluorescent bulbs. A daily count of germinated seeds was
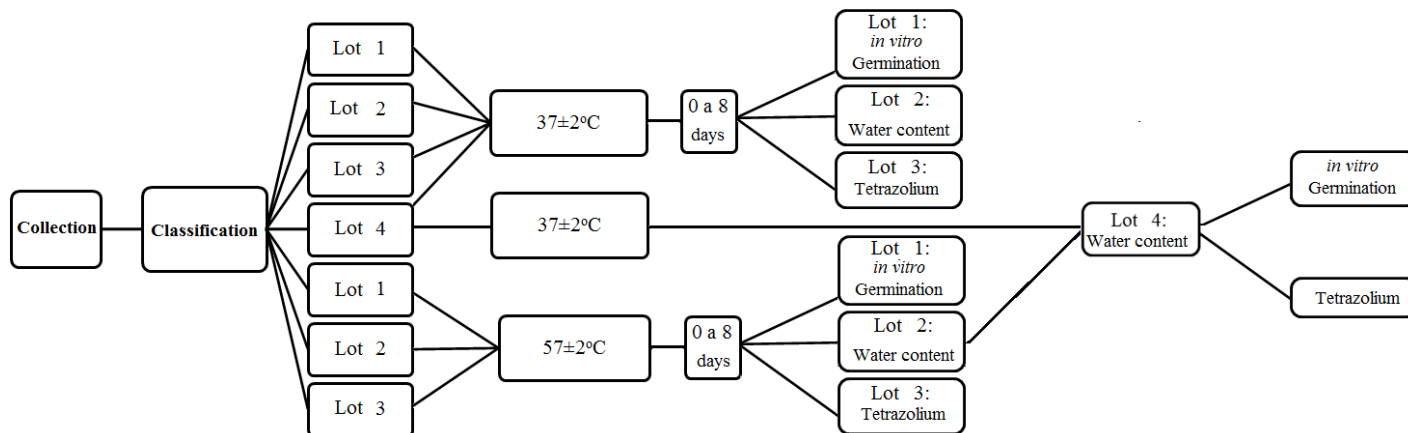
Figure 1. Diagram of procedures used for drying macaw palm fruits and testing *in vitro* germination and zygotic embryo vigour.
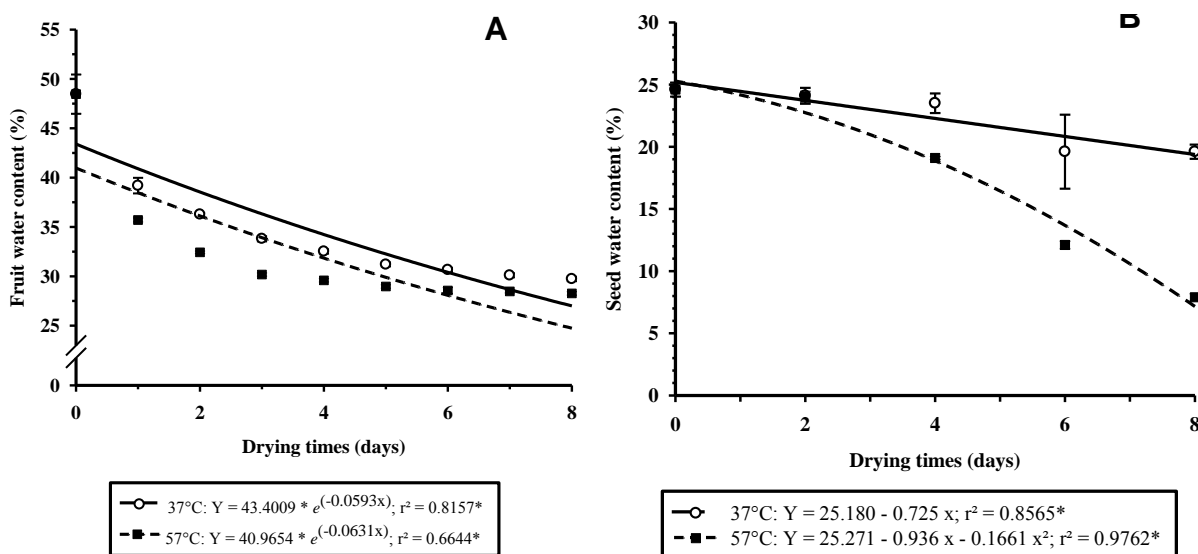

Figure 2. Water content of macaw palm fruits (A) and seeds (B) dried in a forced-air drying oven over varying drying times at two drying temperatures (37 and 57°C). *Significant at *p*<0.05.

37°C: $Y = 43.4009 * e^{(-0.0593x)}$; $r^2 = 0.8157*$
57°C: $Y = 40.9654 * e^{(-0.0631x)}$; $r^2 = 0.6644*$

37°C: $Y = 25.180 - 0.725 x$; $r^2 = 0.8565*$
57°C: $Y = 25.271 - 0.936 x - 0.1661 x^2$; $r^2 = 0.9762*$

performed, and the germination rate index (GRI) (Maguire, 1962) and germination percentage were calculated. The height of the germinated seedlings at 60, 90 and 120 days was also measured.

Tetrazolium test and electrical conductivity

Electrical conductivity and embryo viability were measured using a second fruit lot that was removed from the drying oven at the same time with the fruit lot used for the *in vitro* germination test. Embryos were soaked in deionised water (50 mL) for 24 h at 25°C for the measurement of electrical conductivity. The tetrazolium test was performed according to Ribeiro et al. (2010).

A completely randomised experimental design was used, with a five (drying time and/or water content) x two (drying temperature) factorial scheme with four repetitions of 15 embryos each. An analysis of variance and a regression analysis were performed.

RESULTS AND DISCUSSION

Fruit drying with varying drying time

The results of the regression analysis showed that water loss occurred exponentially at both fruit drying temperatures. The models that fitted the dehydration data were significant and showed a greater decrease in water content at 57°C (Figure 2).

The fruit water content at harvest time was above 40%. Following eight days of drying, fruit water contents of 27 and 24.7% were observed for fruits that were dried at 37 and 57°C, respectively. Water loss was more pronounced in fruits dried at 57°C (Figure 2A), which is consistent with Neto et al. (2012).
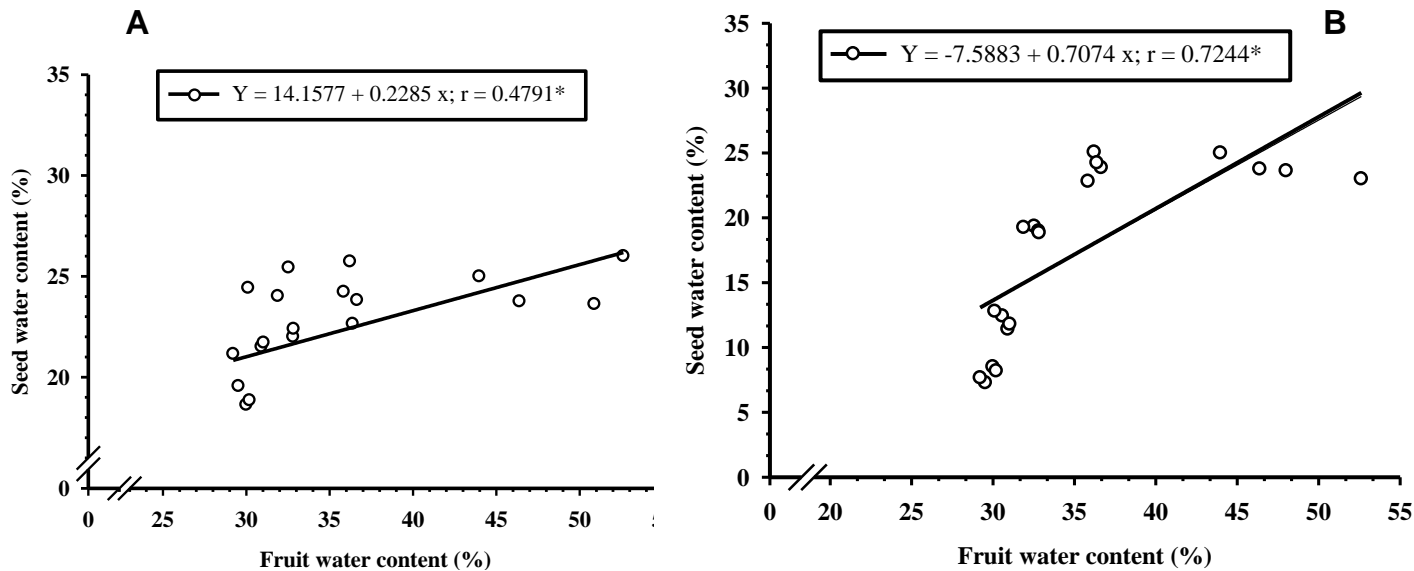
Figure 3. Relationship between water content of macaw palm (*A. aculeata*) fruits and seeds dehydrated in a drying oven for eight days at 37°C (A) and 57°C (B).* Significant at *p*<0.05.
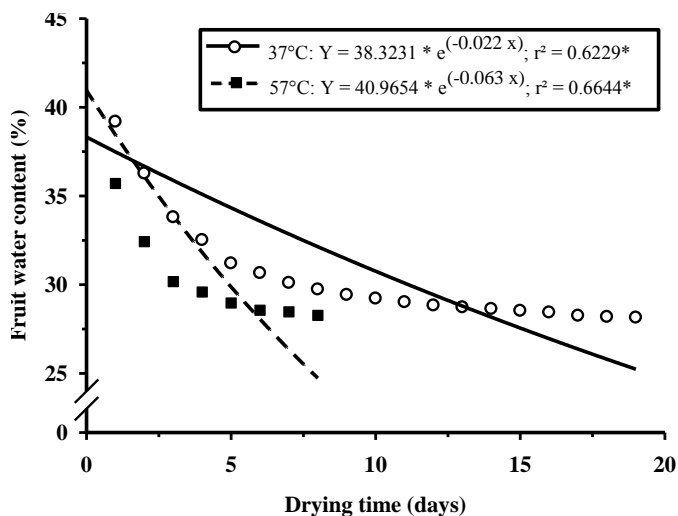

Figure 4. Water content of macaw palm fruits and seeds dried over varying drying times at two temperatures. *Significant at *p*<0.05.

The best explanatory models for seed water loss were different for the different drying temperatures. Drying at 37°C was best described by a linear model, and drying at 57°C was best described by a quadratic model. The same result was observed for fruit drying. Significant models were found for fruit drying, and there was greater water loss in fruits dried at 57°C. Seeds had an initial water content of 25%. Following eight days of drying, seed water contents of 19.4 and 7.15% were observed for fruits that were dried at 37 and 57°C, respectively (Figure 2).

Fruit and seed water content were significantly and positively correlated (r=0.4791* and r=0.7244* for drying temperatures of 37 and 57°C, respectively). The ratio of water loss in fruits and seeds dehydrated at 37°C was 1:0.23% (Figure 3A), and the ratio in fruits and seeds dried at 57°C was 1:0.71% (Figure 3B). Therefore, drying occurred approximately three times faster at 57°C than at 37°C.

Seed water loss resulting from the drying of macaw palm fruits was previously described by Neto et al. (2012). These authors observed a drying ratio that was very similar to the ratio observed in this study (1:0.20%) when fruits were dehydrated at 37°C. A seed water loss ratio of 1:0.87% after drying at 37°C and a decrease in germination percentage after drying at 57°C were observed in babassu (*Orbignya phalerata* Mart.) seeds (Silva et al., 2012). These findings show the importance of determining the effects of fruit drying temperature and drying time on seed water content for each species. With this, the optimal seed water content can be estimated from fruit water content, thereby eliminating the need to remove the seeds and risk destroying them in the process.

Fruit drying with varying water contents

According to the fitted models, the same water content was reached after twice as much drying time at 37°C compared to 57°C. Therefore, the water content obtained after eight days of drying at 57°C (24.8%) was only reached after 16 days of drying at 37±2°C (Figure 4).

According to Rubio Neto et al. (2012), drying macaw palm fruit at 37°C for up to eight days makes the extraction of intact seeds easier and does not result in a loss of embryo vigour or seed viability. In this study, the
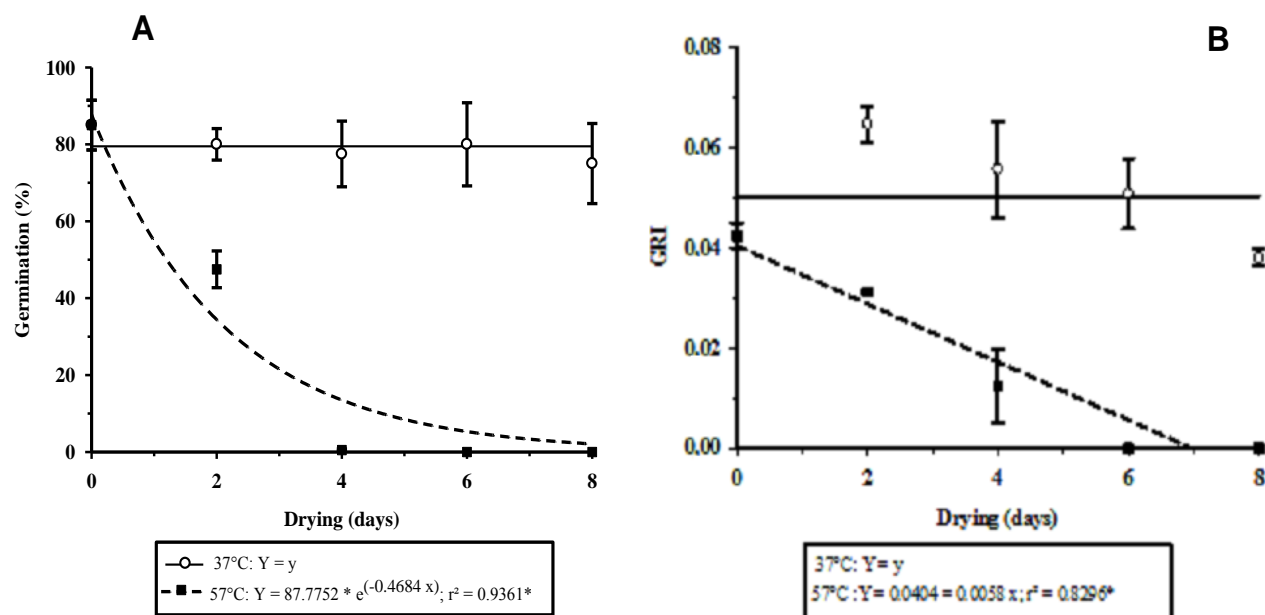
Figure 5. Germination percentage **(A)** and germination rate index (GRI) **(B)** for macaw palm zygotic embryos extracted from fruits dehydrated over varying drying times at two different temperatures (37 and 57°C).

germination of embryos decreased with increasing of temperature, although drying at 37°C did not affect the germination.

Another factor to be considered about drying process is the quality of seed extraction because in this study, it was easy to remove physically intact seeds, with *in vitro* germination being maintained across varying drying times (Neto et al., 2012). Similarly, drying at 37°C did not affect germination percentage for embryos of babassu, which had a mean germination percentage of 93.3% following 12 days of drying (Silva et al., 2012).

In vitro germination

The embryo germination rate index decreased linearly with increasing drying time at a drying temperature of 57°C. At a drying temperature of 37°C, both the germination percentage and the germination rate remained stable over time, reaching 0.05 and 81.2%, respectively (Figure 5B). These germination results are considered satisfactory.

Drying at 57°C was more effective for fruit water loss and it drastically decreased embryo physiological quality, like fruit content. This is because germination percentage decreased linearly and germination ceased completely when fruit water content fell below 30%. Fruit drying at 37°C resulted in more superficial drying. A mathematical model that explained the behaviour of the data for drying at 37°C could not be identified. However, drying at 37°C resulted in an overall mean germination percentage of 70.6% (Figure 6A and B).

Tetrazolium test and electrical conductivity

Drying at 57°C, a high temperature, resulted in the absence of vigorous and viable embryos, in addition to a high mortality rate, for all drying times. These results show the deleterious effects of drying at this temperature. At 37°C, the percentage of viable embryos decreased with increased drying time (Figure 7).

An interaction between drying time and temperature was observed. The two factors are discussed here. The tetrazolium method proposed by Ribeiro et al. (2010) was used to classify the embryos by colour, vigor, viable, unviable or dead.

A linear decrease in the percentage of vigorous embryos was observed with increased fruit drying time at 37°C (Figure 8A). A mathematical model could not fit the data from the fruits dried at 57°C, and therefore, the mean values from these fruits were used to fit a model. The percentage of vigorous embryos was low for fruits dried at 57°C, regardless of drying time. Eight days of drying corresponded to an average of 18% vigorous embryos. The linear decrease in the percentage of vigorous embryos is not a concern, so long as the initially vigorous embryos remain viable. Viable embryos represent a class of vigour in which the capacity for *in vitro* germination is retained (Figure 7B). When fruits were dehydrated at 37°C, 39.2% of the embryos were viable.

The highest percentages of unviable and dead embryos, which do not germinate *in vitro*, were observed for drying at 57°C. No model was found that explained the behaviour of the data. However, the deleterious effect
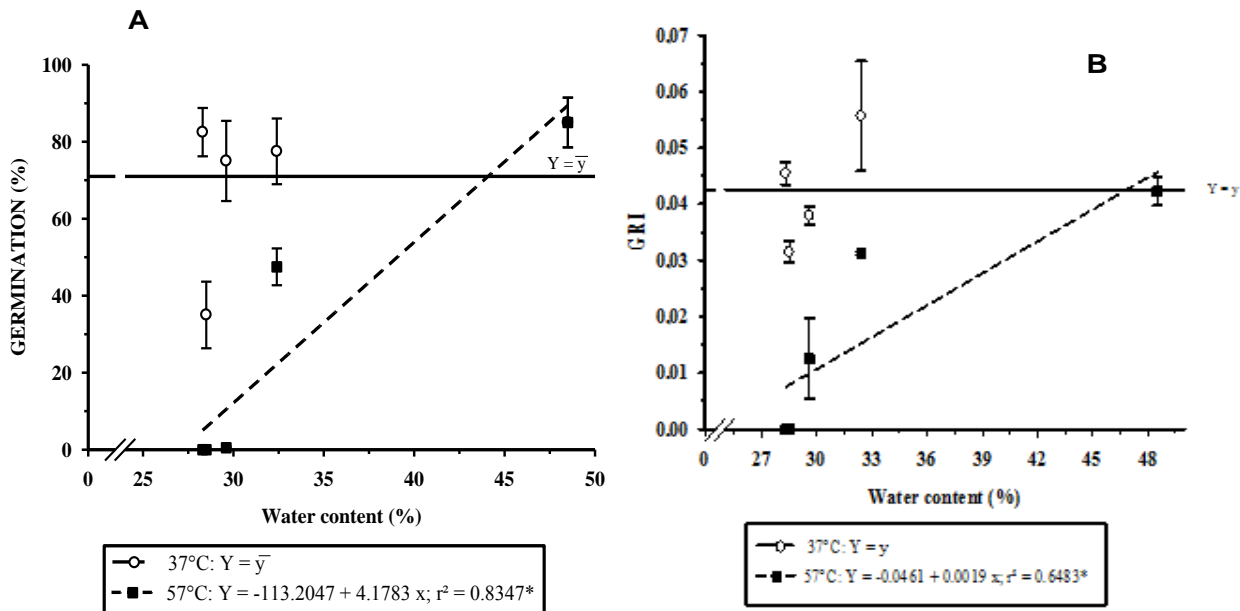
Figure 6. Germination percentage (A) and germination rate index (GRI) (B) for macaw palm embryos from seeds with different water contents.

Drying time (days)

Figure 7. Macaw palm zygotic embryos subjected to the tetrazolium test (bar = 4 mm). The haustorial (ha), medial (pm) and proximal petiole (pp) regions of the embryo can be observed. a) and f) embryos obtained from fresh fruits; b) to e) embryos obtained from fruits dehydrated at 37°C for two, four, six and eight days; g) to j) embryos obtained from fruits dehydrated at 57°C.

of drying on the embryo viability was evident, reaching a mean value of 26%, independent of the drying time (Figure 8C).

Drying at 37°C minimised embryos' mortality, with a mean value of 0.3%. In contrast, drying at 57°C resulted in much faster drying but significantly increased embryos' mortality, with a mean value of 80%. These results show that drying at 37°C is preferable, as it allows for drying without the loss of embryos' physiological quality (Figure 8D).

An exponential decrease in germination percentage

with increased drying time was observed in seeds from fruits dried at 57°C, with germination reaching 2.85% after eight days. For seeds from fruits dried at 37°C, germination remained stable, with an overall mean germination percentage of 80% (Figure 6A). Drying at 57°C resulted in a more pronounced decrease in germination percentage and germination rate, showing that drying at a high temperature compromised the physiological quality of the embryos (Figure 8B).

For seeds dried at both temperatures, ions released from the embryo due to fissures in the embryonic membrane
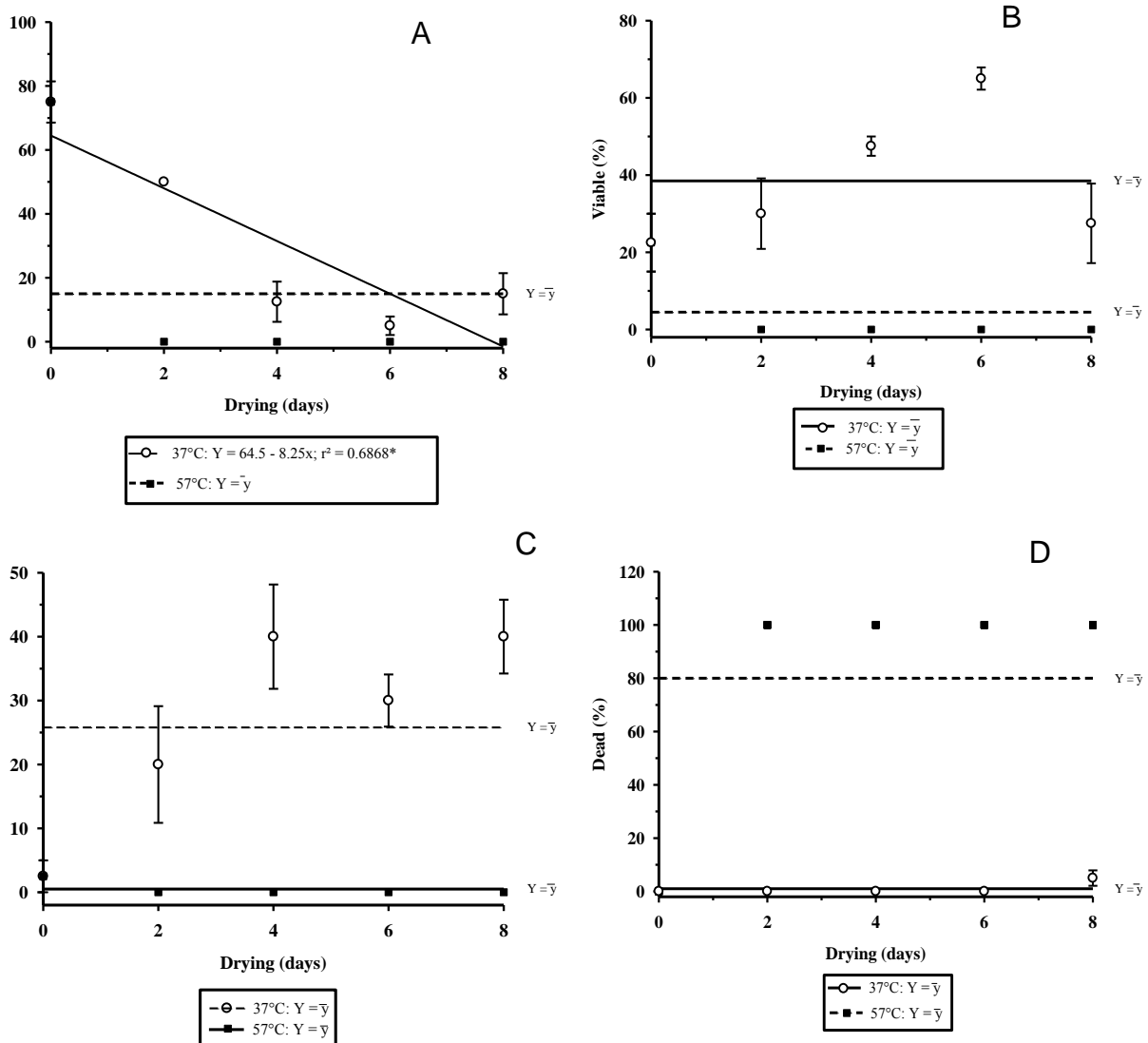
Figure 8. Percentage of vigorous (A), viable (B), unviable (C) and dead (D) macaw palm (*A. aculeata*) zygotic embryos extracted from fruits dehydrated over varying drying times at two different temperatures (37°C and 57°C).

caused by the drying process resulted in an exponential increase in electrical conductivity on the second day of drying. There was no interaction between drying time and temperature for electrical conductivity, and there were no differences in electrical conductivity for different drying times or temperatures. Beginning on the second day of drying, electrical conductivity generally remained stable (Figure 9). This result suggests that further studies are required to improve the methodology for determining electrical conductivity. It is likely that the membranes of dehydrated embryos are damaged when they are subjected to soaking in distilled and deionised water for 24 h (Brasil, 2009). Therefore, soaking the embryos for a shorter time period than the time period suggested in the methodology proposed by Brasil (2009) may be more appropriate.

The tetrazolium test applied according to Ribeiro et al. (2010) resulted in staining of the embryos that was sufficient for the quantification of embryos' vigour. This test has helped researchers to know quickly and accurately the viability of embryos; thus, it was found that increasing the temperature result in decreased viability.

The present study demonstrated that it is possible to estimate the water content of macaw palm seeds based on fruit water content for both drying temperatures tested (37 and 57°C). In contrast to drying at 37°C, drying at 57°C resulted in faster drying of the fruits but hindered embryos' viability and germination. It shows that if the goal is to extract seed to produce seedlings, 37°C is recommended, but if the goal is to extract oil, drying of fruits at 57°C is recommended. This is one of the major findings of this study.
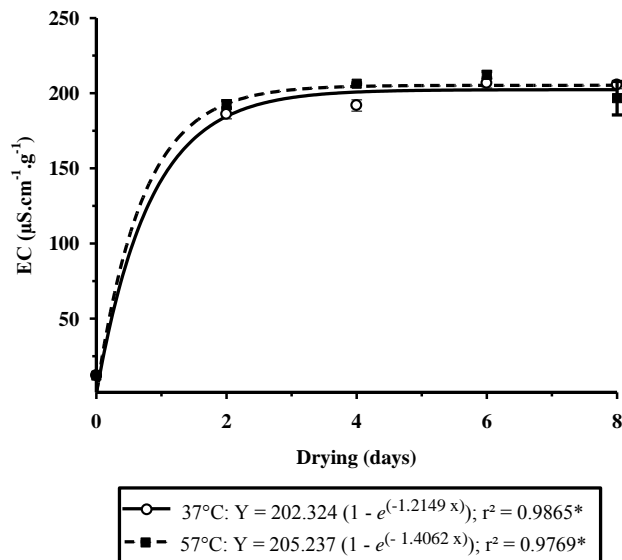
$$37°C: Y = 202.324 \, (1 - e^{(-1.2149 \, x)}); \; r^2 = 0.9865*$$
$$57°C: Y = 205.237 \, (1 - e^{(-1.4062 \, x)}); \; r^2 = 0.9769*$$

Figure 9. Electrical conductivity (EC) of macaw palm zygotic embryos extracted from fruits dehydrated over varying drying times at two different temperatures (37 and 57°C).

Conflict of interests

The author(s) have not declared any conflict of interests.

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