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Effect of drying and soaking fruits and seeds on germination of macaw palm (*Acrocomia aculeata* [Jacq.] Loddiges ex MART.)

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ABSTRACT. This study evaluated mechanisms of the dehydration and rehydration of fruits and seeds on the resulting embryo viability of the macaw palm, *Acrocomia aculeata* (Jacq.) Loddiges ex Mart. Dehydration was performed in a forced-air oven at $37 \pm 2^\circ\text{C}$ for 0, 3, 6, 9, 12 or 15 days, and after these periods, the embryo viability was evaluated by the tetrazolium test. The effect of hydration on the germination percentage and velocity index was evaluated by the imbibition of seeds with distilled water in a germinator at $30 \pm 2^\circ\text{C}$ for 0, 2, 4, 6, 8 or 10 days. The seeds were then treated with fungicide and maintained in a Mangelsdorf germinator at $30 \pm 2^\circ\text{C}$. Drying caused a progressive water loss in the fruits and facilitated the extraction of the seeds. Fruits subjected to drying for up to nine days had no loss of vigor; however, after dehydration for 15 days, a loss in seed viability was observed. Simple linear regression estimated the seed water content based on the fruit water content. Seed imbibition in distilled water favored an increase in dead by fungally contaminated seeds.

Keywords: germination, tetrazolium, drying tolerance.

Efeito da secagem e embebição de frutos e sementes na germinação de macaúba (*Acrocomia aculeata* [Jacq.] Loddiges ex MART.)

RESUMO. Objetivou-se com esse estudo elucidar os mecanismos de desidratação e hidratação de frutos e sementes na viabilidade de embriões de macaúba *Acrocomia aculeata* (Jacq.) Loddiges ex Mart. A desidratação foi realizada em estufa de circulação de ar, ajustada a $37 \pm 2^\circ\text{C}$ por 0, 3, 6, 9, 12 e 15 dias, após esses períodos, foi avaliada a viabilidade dos embriões pelo teste de tetrazólio. O efeito da hidratação na porcentagem e velocidade de germinação foi avaliado pela embebição das sementes em água destilada em germinador com temperatura a $30 \pm 2^\circ\text{C}$ por 0, 2, 4, 6, 8 e 10 dias. As sementes foram tratadas com fungicida e mantidas em germinador do tipo Mangelsdorf. A secagem promoveu perda progressiva no conteúdo de água dos frutos e facilitou a extração das sementes. Quando os frutos foram secos por nove dias não há perda de vigor, no entanto, depois de 15 dias de desidratação, foi observado perda na viabilidade das sementes. Com a regressão linear simples é possível estimar o conteúdo de água nas sementes baseando-se no teor de água dos frutos. A embebição das sementes em água destilada favoreceu e aumentou o número de sementes mortas pela contaminação fúngica.

Palavras-chave: germinação, tetrazólio, tolerância à dessecação.

Introduction

Macaw palm (*Acrocomia aculeata* [Jacq.] Loddiges ex Mart.), also known as bocaiúva, coco-de-espinha, macaúva, marcová or mucajá, can grow up to 10 to 15 meters and has petiolated 4-meter-long leaves clustered on the top of the trunk. The fruits of macaw palm are drupes that are approximately three cm in diameter and yellow when mature. The epicarp breaks easily when the fruit is mature, and the mesocarp is thin and fibrous; a very rigid endocarp is tightly adhered to the mesocarp. The nut is oily, edible and covered by a thin tegument

layer (ALMEIDA et al., 1998; LORENZI, 1996; SILVA et al., 2001).

The potential for producing combustible oil from their seeds has brought renewed interest to palm trees as crop plants, especially the macaw palm (*A. aculeata* Jacq. Loddiges ex Mart.), oil palm (*Elaeis guineensis* Jacq.), inajá palm (*Maximiliana regia* Mart.), tucumã (*Astrocaryum aculeatum* Meyer) and babassu (*Orbignya phalerata* Mart.). The macaw palm has been characterized as exceptionally promising due to the high oil content of the seeds and the fact that the fruits also produce edible meal, forage meal and a high-

energy endocarp (MARTINS et al., 2009; RAMOS et al., 2003).

Despite its manifold uses, the cultivation of macaw palm has been unattractive to growers, as is the case with tucumã (*A. aculeatum* Meyer) and wild areca palm (*Areca triandra* [Roxb.] ex Buch-Ham), mostly due to the difficulty in seed germination and the lack of studies in this area. Most palm trees do not propagate vegetatively, and the germination of the seeds is slow and heterogeneous, as the protective coat (endocarp) restricts water imbibition, oxygen diffusion and imposes mechanical resistance, resulting in seedling emergence problems that are characterized as physical dormancy (FERREIRA; GENTIL, 2006; MEEROW, 1991; YANG et al., 2007).

Treatments, such as imbibition in growth-regulating substances, scarification and depulping, have been successfully used in overcoming the seed dormancy of some related species, as demonstrated for the lady palm (*Rhapis excelsa* Thunberg Henry ex. Rehder), inajá palm (*M. regia* Mart.), wild areca palm (*A. tiandra* Roxb ex Buch-Ham.) and doum palm (*Hyphaene thebaica* Mart.). Varying the immersion period during water imbibition has also been used to optimize and speed the germination process in some palm trees, such as heart-of-palm (*Euterpe edulis* Mart.), *Thrinax parviflora* Swartz and tucumã (*A. aculeatum* Meyer), and was reported to require 2, 6 and 9 days, respectively (BOVI, 1990; FERREIRA; GENTIL, 2006; LUZ et al., 2008; MARTINS et al., 1996; MOUSSA et al., 1998; PÉREZ et al., 2008; PIVETTA et al., 2005; YANG et al., 2007).

It is an accepted fact among plant biologists that the positive effects of water immersion on seeds occur by favoring the first stage of the germination process, imbibition. The effects of desiccation on the germination percentage, as well as the ability of the seed to support storage molecules, classify seeds into different physiological groups, with the seeds of most palm trees being classified as recalcitrant, including heart-of-palm (*E. edulis* Mart.), açai (*Euterpe oleraceae* Mart.), areca palm (*Chrysalidocarpus lutescens* Wendl.) and peach palm (*Bactris gasipaes* Kunth) (ARAÚJO et al., 1994; BECWAR et al., 1982; BOVI et al., 2004; FERREIRA; SANTOS, 1993; MARTINS et al., 2006).

According to Nascimento and Silva (2005), the critical and lethal moisture levels are relatively high for palm trees in general. Indeed, the dehydration of açai (*E. oleraceae* Mart.) seeds in a forced-air oven at 30°C was shown to cause a progressive reduction in the germination percentage (from 43.4 to 30.3%) and seed vigor, and the germination process ceased completely when the seeds reached 15% moisture,

confirming the recalcitrant behavior of the seed of this species.

Additionally, the germination of red palmito (*Euterpe espirosantensis* Fernandes) seeds was found to be interrupted when they were dehydrated to 35.8% and stored in plastic bags at 15°C for up to 30 weeks, whereas seeds that were not desiccated (45% moisture) or were desiccated to only 40.1% moisture maintained germination ability for up to 52 weeks of storage (MARTINS et al., 2007).

Both the drying method and the rate of drying should be taken into consideration during desiccation to clarify in seed behavior classification. Accordingly, Ferreira and Santos (1993), in evaluating different drying periods and rates for peach palm (*B. gasipaes* Kunth), found an interaction between these factors, where slow drying (in a laboratory environment for 8 days) favored emergence and seed vigor, compared with seeds dried in silica gel, which ceased germination in only 4 days when they reached 19% moisture.

To date, no studies are available in the literature regarding the effects of moisture on the embryo viability and seed germination of the macaw palm. Therefore, the aim of this study was to evaluate the water content in macaw palm fruits and seeds subjected to different periods of drying and imbibition.

Material and methods

The study was performed at the Seeds Laboratory of the Instituto Federal Goiano, Campus Rio Verde, Goiás State, Brazil, with ripe macaw palm (*Acrocomia aculeata* [Jacq.] Loddiges ex Mart.) fruits that were collected in the months of August and December, in the counties of Rio Verde and Indiara (Goiás State, Brazil), respectively.

Trial 1. The drying curve

Fruits were removed from the clusters, and fruits that were damaged or weighed less than 25 g were discarded. Due to the high heterogeneity among the fruits, they were classified into three classes, according to their total weight. The number of fruits per class followed the distribution frequency observed in the field: 8 small fruits (less than 30 g), 8 medium fruits (30-35 g) and 4 large fruits (more than 35 g), for a total of 20 fruits per repetition.

The initial condition of the fruits was evaluated in a lot of 80 whole fruits and seeds by determining the water content of both, with the moist weight as the basis, using an oven-drying method at 105 ± 2°C, until reaching a constant mass. To accelerate the drying process, the whole

fruits were enveloped by a metal galvanized screen and dried in a forced-air oven at $37 \pm 2^\circ\text{C}$ for 0, 3, 6, 9, 12 or 15 days to obtain each target moisture content both for the fruit and the seed inside the fruit. The seeds were removed by breaking the fruits of a lot with a 1.5-kg hammer on a concrete block and their moisture content was determined. The effect of dehydration on the embryos was evaluated by the tetrazolium test (0.075%) in four repetitions with ten embryos from the seeds used for the moisture determination, according to the method described by França Neto et al. (1998).

The experimental design was completely randomized, with six drying periods and four repetitions of 20 fruits and/or seeds. The analysis of variance test was conducted, and the averages were compared by Tukey's test at a 5% probability ($p < 0.05$); simple linear regression was used to compare the moisture contents of fruits and seeds, using the Sigma Plot®11.0 software.

Trial 2. The imbibition curve

Subsequent to manual harvest, whole fruits were broken to remove the seeds and determine the water content by the above-described oven-drying method at $105 \pm 2^\circ\text{C}$, using four repetitions of 20 seeds. The remaining fruits were dried in a forced-air oven at 35°C for six days to facilitate seed extraction, after which, the seed moisture content was determined.

As described above, the endocarp was broken with the aid of a 1.5-kg hammer and a concrete block. The evaluation of the extraction process was performed as proposed by Ferreira and Gentil (2006), which consisted of a determination of the percentage of physically intact seeds, those with visible mechanical damage, those completely broken, and those that remained adhered to the endocarp.

Seeds that were physically whole and those with mechanical damage were separated and similarly used in all of the repetitions of the experiment. The effect of imbibition was conducted in beakers containing distilled water that were kept in a Mangelsdorf-type germination chamber adjusted to $30 \pm 2^\circ\text{C}$ for 0, 2, 4, 6, 8 or 10 days with daily replacement of the imbibition water.

An imbibition curve was determined using four samples of 20 seeds kept under the same conditions. During the first 12 hours, sample weighing was performed every 2 hours. Subsequently, until the third day, the samples were weighed every 12 hours; from the third until the tenth imbibition day, the samples were weighed every 24 hours. Before

weighing, the seeds were blotted with four paper towels for 1 minute.

After the pre-established times, four samples of 20 seeds were placed on germitest paper® that was previously moistened with distilled water at 2.5 times the dry substrate weight and placed in the germination chamber at $30 \pm 2^\circ\text{C}$.

Before sowing, the seeds were treated with fungicide (active ingredient, carboxin+thiram at $200+200 \text{ g L}^{-1}$) at a dose of 500 mL per 100 kg seeds; 500 mL of distilled water per 100 kg seeds was used as a control.

The experimental design was completely randomized, with four repetitions of 20 seeds. Analysis of variance was performed, and the averages were compared by Tukey's test at a 5% probability.

Results and discussion

Trial 1. The drying curve

As depicted in Figure 1A, there was a drastic drop in the water content of the fruit immediately after the beginning of the drying process, with an average reduction from 46.92 to 32.51% and 21.69% moisture after 6 and 15 days of drying, respectively; a total loss of 53.77% was observed from the beginning until the end of drying. The initial seed moisture content was lower than that of the fruits, and the dehydration process was slow during drying. After 15 days, the seeds reached 15.09% moisture, losing 29.45% of their initial water content (21.39%) (Figure 1B).

The high determination coefficients of the regression analyses were 95.15 and 89.16% for fruits and seeds, respectively, and suggested a good fit of the model to the water content data, indicating that the drying time was closely related to the water content. By the comparison of the angle coefficients, the fruits lost water faster than the seeds, which can be explained by the fact that the drying process in a forced-air oven at 37°C initially removes surface water from the fruits, and subsequently from the internal tissues and organs, such as the seeds.

It was possible to establish a relationship between the two plant tissues analyzed (fruits and seeds), obtaining a linear fit with $R^2 = 0.6402$ (Figure 2), and the interference of fruit moisture on seed moisture was noteworthy. It was observed that after the removal of one half of the fruit water content, the seeds began to lose water substantially. This could be related to the ease of removing the water available in the mucilaginous mesocarp of the fruit, forming a moisture gradient between the fruit and the seed.

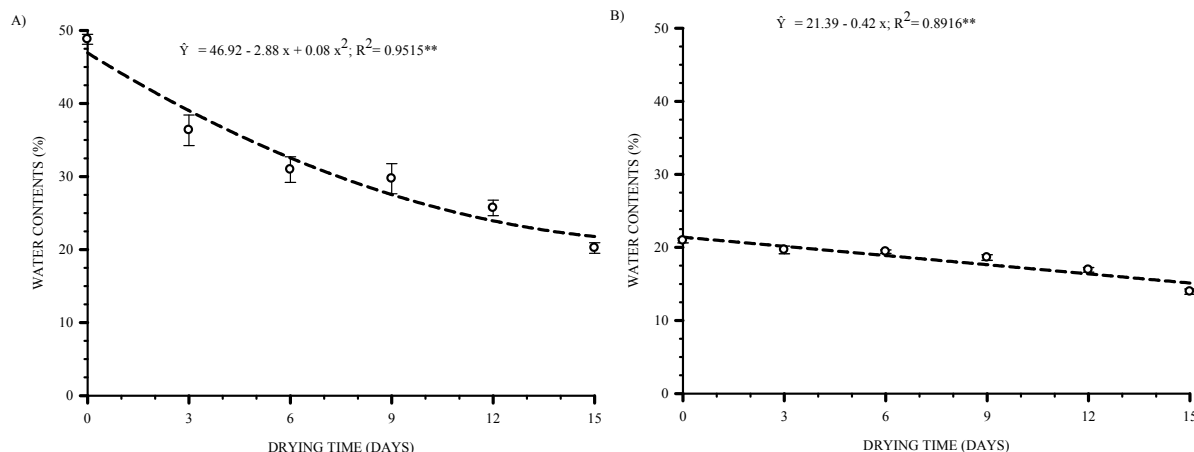


Figure 1. Water loss in the fruits (A) and seeds (B) of macaw palm (*Acrocomia aculeata* [Jacq.] Loddiges ex Mart.) subjected to different drying periods in a forced-air oven at 37°C. **Significant at 5% probability ($p < 0.05$). Rio Verde, Goiás State, 2010.

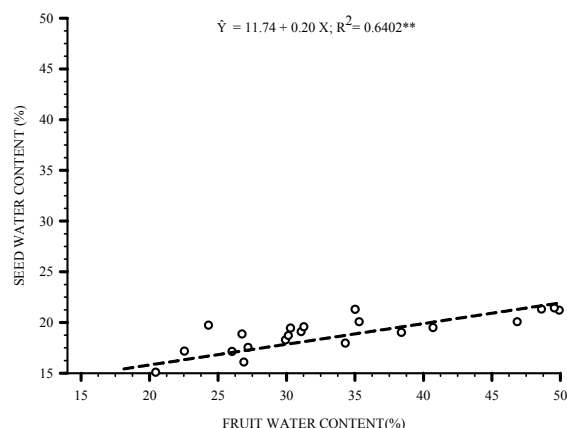


Figure 2. The relationship between the water contents of macaw palm (*Acrocomia aculeata* [Jacq.] Loddiges ex Mart.) fruit and seed subjected to different drying periods in a forced-air oven at 37°C. **Significant at 5% probability. Rio Verde, Goiás State, 2010.

The imbibition of macaw palm embryos in water for 16 hours at 30°C and the subsequent reaction with tetrazolium solution at 0.075% in darkness allowed an adequate coloration of the embryos, which enabled the classification of vigor. Because they were completely damaged during the opening of the seed, we were unable to remove the embryos at initiation time point. The embryos of seeds that were imbibed for other lengths of time were classified as vigor class 2 (high vigor) for 3 and 6 days of drying and vigor class 3 (medium vigor) for all of the other periods evaluated. The major distinguishing factor between the vigor classes was in relation to the size of the lesion (creamy white) caused to the embryo: most of them were dark red, indicating live tissue (Figure 3).

According to the results of the water content measurements and the tetrazolium test, it was

observed that drying for up to six days was favorable for seed removal without the loss of embryo viability or a significant reduction in the seed water content.

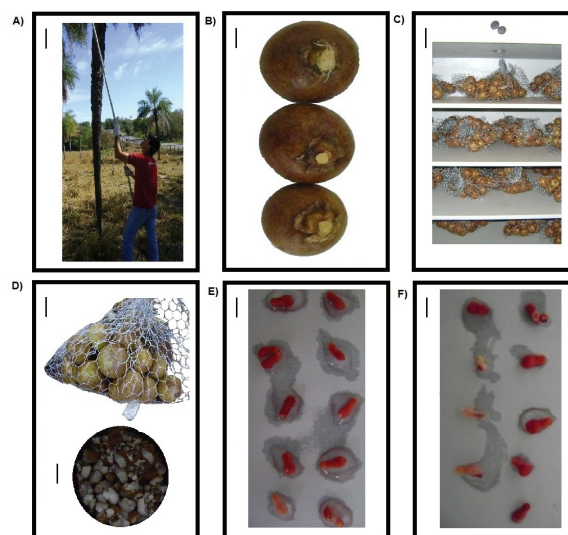


Figure 3. The effect of dehydration on macaw palm (*Acrocomia aculeata* [Jacq.] Loddiges ex Mart.) fruit subjected to different drying times, as evaluated by the tetrazolium test. A) Harvest. Bar = 0.5 m; B) Fruit classification: small (< 30 g), medium (30-35 g) and large (> 35 g). Bar = 1 cm; C) Drying in a forced-air oven at 37°C for up to 15 days. Bar = 30 cm; D) Determination of fruit moisture (above) and seed moisture (below). Bar = 5 cm and 1 cm; E) Embryos of high vigor, obtained after drying for up to 9 days. Bar = 3 mm; F) Embryos of medium vigor, obtained after drying for 12 to 15 days. Bar = 3 mm. Rio Verde, Goiás State, 2010.

Similar results have been reported for peach palm (*B. gasipaes* Kunth), by Bovi et al. (2004), who considered the drying process slow because it required 15 days for drying until the initial seed water content (20.94%) had been reduced to approximately 14%. According to Ferreira and Santos (1993), the drying velocity is a determinant

factor for seed germination. These authors found an increase in the emergence and seedling vigor of greater peach palm (*B. gasipaes* Kunth) when the seeds were dried for up to eight days in a laboratory environment (slow drying), in comparison to drying in desiccators containing silica gel (fast drying). It is important to note that drying promotes low seed moisture, thus, facilitating prolonged storage. However, it is of the utmost importance that the process is conducted in controlled-temperature ovens to prevent a loss in the germination ability and seed vigor (CHIN, 1988).

In addition, the use of the tetrazolium test to determine embryo viability has been performed in many ways in the Arecaceae family, including varying the salt concentration from 0.1 to 1% and the imbibition time from 2 to 6 hours; thus, an adjustment of the test for each species is required (LIN, 1988; SPERA et al., 2001).

Trial 2. Imbibition curve

After breaking the endocarp, 60.83% of the seeds were apparently undamaged (physically whole), whereas 12.63% of the seeds showed some type of visible damage, 10.88% of the seeds were broken and 15.65% adhered to the endocarp. The seed extraction yield was 494 seeds per man-hour. According to Ferreira and Gentil (2006), the removal of the rigid endocarp of palm seeds always presents risks of damage to the endosperm and embryo; therefore, this process should be optimized for each species.

As shown in Figure 4, the water absorption by the seeds was rapid on the first day and remained stable until the 10th day of imbibition. After drying, seed moisture was 19.84%, and, after 10 days, the water content reached 29.95%. The water acquisition was fast, probably due to the absence of the endocarp.

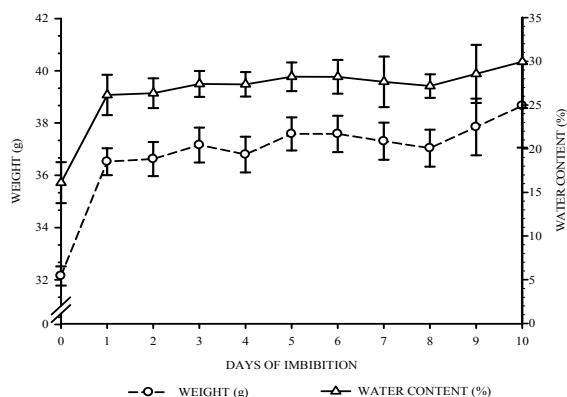


Figure 4. The imbibition curve and water contents of macaw palm (*Acrocomia aculeata* [Jacq.] Loddiges ex Mart.) seeds. Bar = Standard error of the mean. Rio Verde, Goiás State, 2010.

No statistically significant differences were observed for any of the characteristics evaluated; however, there was a decreasing trend for both the germination percentage and velocity as the imbibition time in distilled water increased (Table 1).

Table 1. Germination percentage and the index of germination velocity for *Acrocomia aculeata* (Jacq.) Loddiges ex Mart. The seeds were subjected to different imbibition periods. Rio Verde, Goiás State, 2010.

Days of imbibition	Germination (%)	IVG
0	10.0 ¹ ± 2.04 ²	0.068 ¹ ± 0.01 ²
2	6.2 ± 2.39	0.047 ± 0.01
4	7.5 ± 2.50	0.049 ± 0.03
6	7.5 ± 1.15	0.062 ± 0.04
8	6.2 ± 3.15	0.042 ± 0.03
10	2.5 ± 2.50	0.038 ± 0.04

¹Averages in the column do not differ by Tukey's test at 5% probability. ²± the Standard Error of the Mean.

The percentage of dead seeds increased as the imbibition time increased (Figure 5), which compromised the germination percentage because of the growth of microorganisms that consumed almost all of the reserves in the seed. Seed death after imbibition also could have been favored by the extraction method adopted, as any damage to the integument allows deterioration by microorganisms.

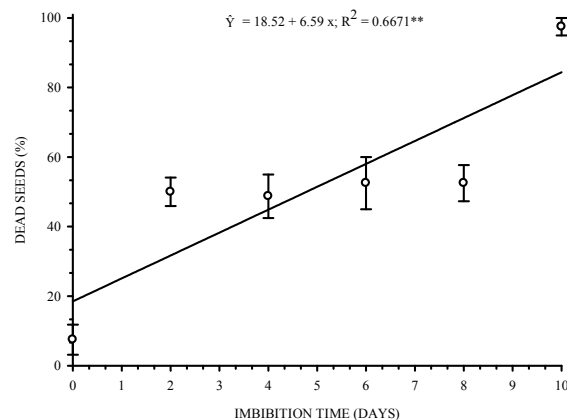


Figure 5. The percentage of *Acrocomia aculeata* (Jacq.) Loddiges ex Mart. seed death after the seeds were subjected to different imbibition times. **Significant at 5% probability. Rio Verde, Goiás State, 2010.

Similar results have been obtained by Ledo et al. (2002) in peach palm (*B. gasipaes* Kunth). These authors have reported that the imbibition of the diaspores in water (28°C) for 48 hours or the removal of the endocarp did not increase the germination percentage (41 and 37%, respectively), and the results were not different from the control diaspores, which reached 46% germination. In addition, the seeds of *Pritchardia remota* (Kuntze) Beck. subjected to 336 hours of imbibition in water (23°C) showed no radicle protrusion in any of the embryos (PÉREZ et al., 2008).

In contrast, Bovi (1990) observed a greater emergence percentage (85%) in heart-of-palm (*Euterpe edulis* Mart.) seeds after subjecting them to imbibition for 2 to 4 days. Although beneficial, imbibition for 2 days did not increase the emergence velocity. According to Ferreira and Gentil (2006), imbibition of tucumã (*A. aculeatum* Meyer) seeds in water (24°C) for up to nine days promoted a positive effect on germination and in the index of germination velocity. An increase from 58 to 70% germination was observed; however, on average, 104 days were required for this process.

Conclusion

The water loss in macaw palm fruits was more rapid immediately after the beginning of the drying process. Seed dehydration was slow for all of the drying periods in the forced-air oven at 37°C. It was possible to estimate the seed water content based on the fruit water content using simple linear regression. Fruit drying for up to six days eased the extraction of the seeds yet did not affect vigor. However, after dehydration for 15 days, a loss in viability was observed.

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