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ORIGINAL ARTICLE

Degree of seed desiccation sensitivity of the Amazonian palm *Oenocarpus bacaba* depends on the criterion for germination

Lydiane Lucia de Sousa BASTOS[1, 2], Geângelo Petene CALVI[2*], Manuel de Jesus Vieira LIMA JÚNIOR[3], Isolde Dorothea Kossmann FERRAZ[2]

[1] Universidade Federal de Viçosa (UFV), Departmento de Engenharia Florestal, 36570-900, Viçosa, Minas Gerais, Brazil

[2] Instituto Nacional de Pesquisas da Amazônia (INPA), Coordenação de Biodiversidade, CP 2223, Manaus, 69057-970, Manaus, Amazonas, Brazil

[3] Universidade Federal do Amazonas, Centro de Sementes Nativas do Amazonas, 69067-005, Manaus, Amazonas, Brazil

* Corresponding author: gpcalvi@inpa.gov.br; https://orcid.org/0000-0002-8631-2325

ABSTRACT

Across the seed-seedling transition, several germination criteria are used in studies of palm-seed germination. In *Oenocarpus bataua*, these criteria have differential tolerance to thermal stress. In this study, we evaluated the tolerance of germination criteria to seed desiccation of the congeneric *Oenocarpus bacaba*. We dried seeds to different moisture contents (MC) before scoring first cataphyll, second cataphyll, enclosed eophyll and expanded eophyll. Seeds without drying had 41.7% MC. Germination success reached close to 70% after 75 and 105 days, depending on the germination criterion. Safe MC was close to initial MC and all seeds were dead with MC < 26.7%. As the primordial organs of the cataphylls and the eophyll are already detectable in the palm-seed embryo, all were affected by drying. Critical MC, defined here as 50% loss of germination capacity, increased from 35.4% (first cataphyll) to 37.1% (expanded eophyll) and confirmed that, across the seed-seedling transition, more advanced germination stages had a higher sensitivity to desiccation. During germination and development, the criteria appear in sequence over several weeks. Consequently, the desiccation damage was only detectable when the last criterion was evaluated. To avoid an underestimation of damages, we suggest that seed-stress studies in palms should take into account an adequate period for seedling development, which, for *O. bacaba*, was 105 days until the expansion of the eophyll.

KEYWORDS: Arecaceae, drying, recalcitrant seeds, seedling, stress

O grau de sensibilidade ao dessecamento das sementes da palmeira amazônica *Oenocarpus bacaba* depende do critério de germinação

RESUMO

Ao longo da transição semente-plântula, vários critérios de germinação são usados em estudos de germinação de sementes de palmeiras. Em *Oenocarpus bataua*, esses critérios apresentam tolerância diferencial ao estresse térmico. Neste estudo, avaliamos a tolerância dos critérios de germinação ao dessecamento de sementes da congênere *Oenocarpus bacaba*. Dessecamos sementes a diferentes teores de água (TA) antes de avaliar primeiro catafilo, segundo catafilo, eofilo fechado e eofilo expandido. O TA de sementes sem dessecamento foi de 41,7%. O sucesso da germinação atingiu cerca de 70% após 75 e 105 dias, dependendo do critério de germinação. O TA de segurança foi próximo ao TA inicial e todas as sementes estavam mortas com TA < 26,7%. Como os órgãos primordiais dos catafilos e do eofilo são detectáveis no embrião da semente destas palmeiras, todos foram afetados pelo dessecamento. O TA crítico, definido aqui como 50% de perda da capacidade de germinação, aumentou de 35,4% (primeiro catafilo) para 37,1% (eofilo expandido) e confirmou que, ao longo da transição semente-plântula, estádios de germinação mais avançados tiveram maior sensibilidade ao dessecamento. Durante a germinação e o desenvolvimento, o aparecimento dos critérios ocorre em sequência ao longo de várias semanas. Consequentemente, o dano pelo dessecamento só foi detectado quando o último critério foi avaliado. Para evitar subestimação dos danos, sugerimos que estudos de estresse com sementes de palmeiras levem em consideração um período adequado para o desenvolvimento que, para *O. bacaba*, foi de 105 dias até a expansão do eofilo.

PALAVRAS-CHAVE: Arecaceae, dessecamento, sementes recalcitrantes, plântulas, estresse

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INTRODUCTION

The global distribution of palms (family Arecaceae) is mainly restricted to tropical and sub-tropical climates, and only a few species are found in warm-temperate regions (Dransfield *et al.* 2008). Of the 124 palm species studied so far, a similar percentage of desiccation-tolerant (29%) and desiccation-sensitive seeds (33%) has been identified (Royal Botanic Gardens Kew 2020). Palms are among the most useful plants, thus knowledge on seed storage behavior is of great importance for the development of handling and storage protocols.

In palms, several germination criteria during initial development were used for germination assessment: the germination button (Beckman-Calvacante *et al.* 2012), primary root development (Iossi *et al.* 2003; Silva *et al.* 2006; José *et al.* 2012), first and/or second cataphyll (Bastos *et al.* 2017) or expanded eophyll (Nazario and Ferreira 2012). Brazilian seed testing rules (Brasil 2009) indicate a seedling with the expanded eophyll as suitable for germination assessment and evaluation of a normal seedling.

Four development stages during the seed-seedling transition of *Oenocarpus bataua* Mart. were found to have differential tolerance to thermal stress (Bastos *et al.* 2017). Thus we aimed to assess whether stages of development in palm seeds also differ in tolerance to desiccation. We choose the congeneric *Oenocarpus bacaba* Mart. as our study subject. The seeds of this species are known to be sensitive to desiccation, however, only the germination button and radicle growth had been assessed (José *et al.* 2012).

Oenocarpus bacaba is one of the most useful plants for regional communities in Amazonia. A milk-like beverage is produced with the fruit pulp, and the oily supernatant is used for medicinal, cosmetic and culinary purposes (Tropical Plants Database 2020). This palm occurs in the Amazon Basin in *terra firme* forests to an altitude of about 700 m (Flora do Brasil 2020). The palm has a single, straight stalk and can reach 7-20 m in hight and 15-25 cm in diameter. The branched inflorescences are pendulous like a ponytail and can reach 80-150 cm. Ripe fruits are dark purple ellipsoid drupes (1.3-1.5 cm) with a single seed (Ferreira 2005). In drupes, the seed is enclosed in a hard endocarp, which is botanically a pyrene and is the handling and seeding unit, hereafter referred to as the "seed".

The present study aimed to evaluate the tolerance of four seedling development stages used as emergence criteria to different levels of desiccation stress in *O. bacaba* seeds.

MATERIAL AND METHODS

Ripe, dark purple fruits of *O. bacaba* were collected from seven palm trees in Autazes, Amazonas state, Brazil (3°37'22.8"S; 59°28'44.3"W) in September (lot 1) and October (lot 2). Fruits were transported in polyethylene bags to the Seed Laboratory of Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus, Amazonas, Brazil. For seed extraction, fruits were submerged in water at 32 ± 4 ºC for 24 hours before pulp removal using a pulper machine. The remaining pulp was removed by rubbing the seeds against a metal sieve under running tap water. Drying for two hours in the shade at 32 °C reduced excess water. For each lot (lot 1 and lot 2), initial seed moisture content (MC) was determined with four replicates of five seeds (approximately 10 g per replicate), cut into quarters and dried at 105 °C for 24 hours, according to Brasil (2009) and expressed on fresh weight (FW) basis. The remaining seeds were kept for a maximum of 30 hours at 15 °C in closed polyethylene bags to prevent desiccation.

In addition to the control (no drying), seeds were dried to ten levels of MC: 40, 35, 30, 25, 20, 15, 12.5, 10, 7.5 and 5%. To determine whether seeds have reached the desired moisture content, seed moisture content was monitored by weighing before and during desiccation. The following formula was applied according to Hong and Ellis (1999):

$$\text{Desired seed weight} = \frac{(100 - \text{initial MC})}{(100 - \text{desired MC})} * \text{Initial seed weight}$$

For each treatment, three replicates with 25 seeds of lot 1 and three of lot 2 were put in nylon nets and maintained above a fan at ambient conditions of 27 ± 3 °C, 50-60% relative humidity (RH). Weight was monitored every two hours during the first 12 hours, and then at 24-hour intervals. Additional desiccation above silica gel was required to achieve MC < 15%. Once the seeds reached the desired weight, 20 seeds of each replicate were sown and five seeds were dried to confirm MC at 105 °C for 24 hours, as described above.

The germination trial was conducted in a nursery at 30 ± 5 °C, 86 ± 3% RH. Seeds were sown in vermiculite (medium grain, BrasilMinerios®) in perforated plastic trays (25 × 30 × 5 cm), initially moistened (3 g water to 1 g vermiculite) and irrigated periodically. Three times a week, four germination criteria were scored: first cataphyll, second cataphyll, enclosed eophyll and expanded eophyll, based on Bastos *et. al.* (2017).

After 105 days, final germination was expressed as the percentage of germinated seeds relative to total seeds sown, and mean germination time (MGT) was calculated for each germination criterion according to Ranal and Santana (2006). Germination results were evaluated for each desiccation degree. Moisture content was considered as safe when no significant loss of germinability was detected after desiccation, critical when initial germination was reduced by 50% and lethal when germinability was completely lost, according to Sacandé *et al.* (2004).

Data were compared between lot 1 and lot 2 using Hartley's maximum F > 7.0 (Ranal and Santana 2006) which indicated homogeneity between the residual variances. Hence, the data of both lots were pooled and the effects of desiccation on germination and MGT were analyzed by regression analyses of the pooled data. Calculation of MGT can only be done if germination occurs, thus treatments which had reached lethal MC were excluded. Germination was analyzed by sigmoidal models and MGT by linear models. For all mathematical models, the significance levels were tested by *F* test using SigmaPlot.

RESULTS

Without any desiccation (control), *O. bacaba* seeds had MC of 41.7%. In the control, the emergence of the first cataphyll started 20 days after sowing and reached 70% of emergence after 75 days. Seedlings with fully expanded eophyll were observed 46 days after germination and reached 67.5% at day 101 (Figure 1).

The regression of final germination on MC was expressed by sigmoid models (Figure 2; Table 1). After only two hours of drying, MC was reduced from 41.1% to 38.7%, which had significant consequences for final germination. The resulting regressions equations (Table 1) indicate that, in the range between 34.5 and 38.5% MC, a reduction of only 0.5% MC, will result in a reduction of at least 4% germination, independent of the criteria (see also Figure 2). Thus *O. bacaba* seeds hardly tolerate any desiccation, as the safe MC was close to the initial MC. All seeds were dead at MC < 26.7%, which indicates that lethal MC had been reached or exceeded.

The critical MC was 35.4% for the first cataphyll, 35.8% for the second cataphyll, 36.3% for the enclosed eophyll, and 37.1% for the expanded eophyll (Figure 2), showing that, with progress in seedling development, the scored criteria became more sensitive to desiccation. Abnormal seedlings, such as atrophied eophylls and/or absence of root development, were noted when MC fell below 36.5%. MGT increased linearly with drying in a similar impact on the enclosed and expanded eophyll, and a more pronounced impact on the first and second cataphyll was observed (Figure 3; Table 1).

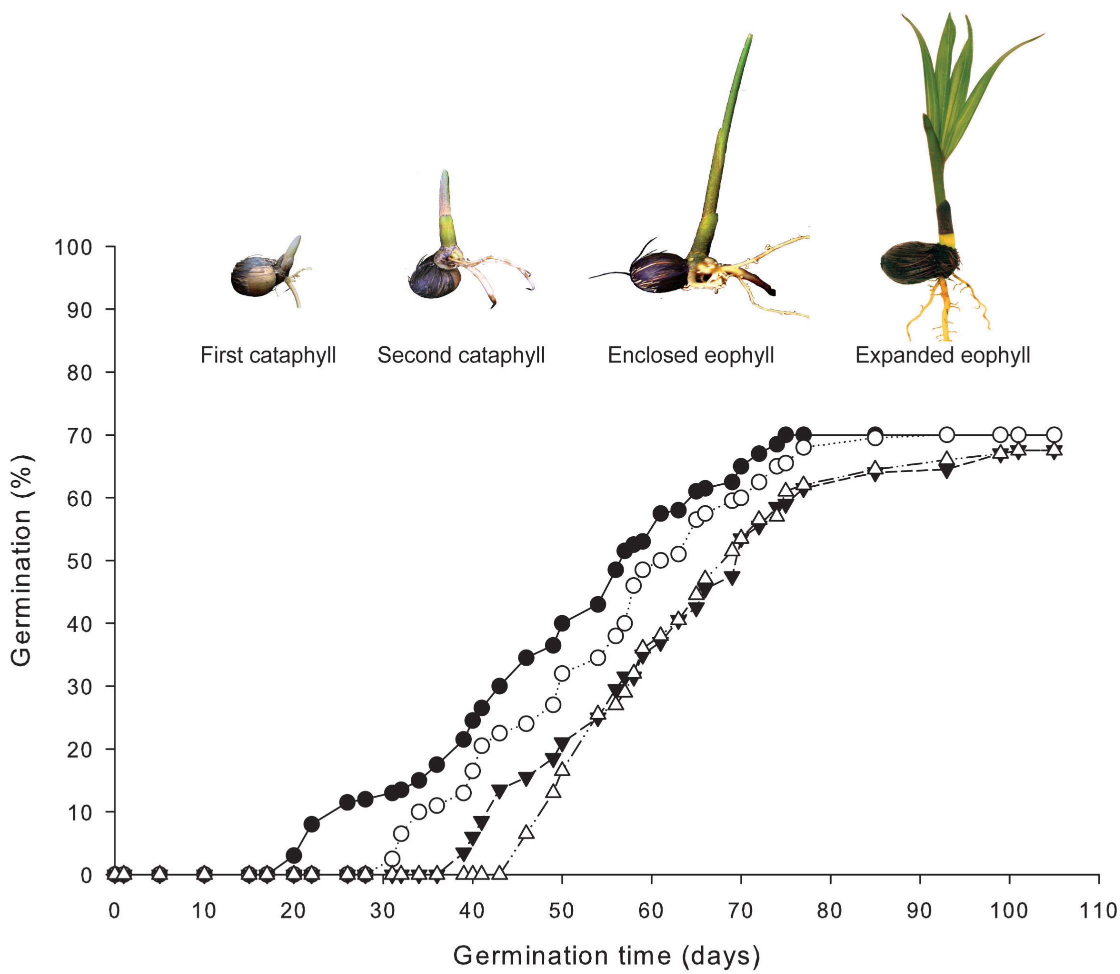

Figure 1. Germination progress of *Oenocarpus bacaba* seeds without drying (control), scoring first cataphyll (●), second cataphyll (○), enclosed eophyll (▼) and expanded eophyll (Δ). Data points represent means of six replicates of 25 seeds. This figure is in color in the electronic version.

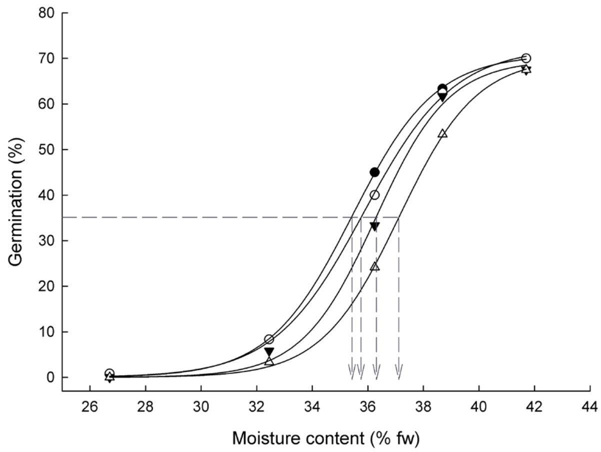

Figure 2. Final germination of *Oenocarpus bacaba* seeds after seed desiccation to different moisture contents, scoring first cataphyll (●), second cataphyll (○), enclosed eophyll (▼) and expanded eophyll (Δ). Vertical lines indicate the critical MC (50% of initial germination capacity lost) for each criterion. Data points represent means of six replicates of 25 seeds.

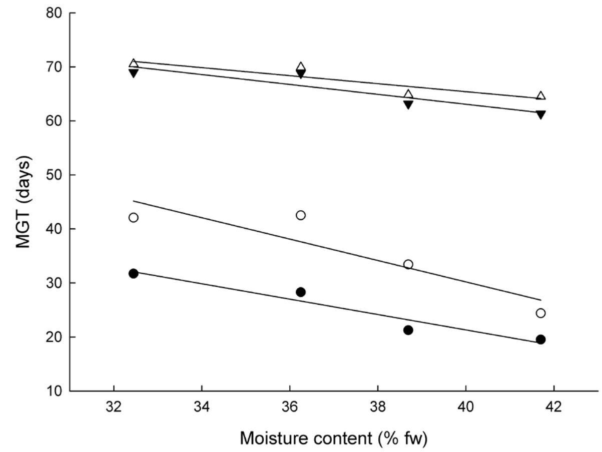

Figure 3. Mean germination time (MGT) of *Oenocarpus bacaba* seeds after seed desiccation to different seed moisture contents, scoring first cataphyll (●), second cataphyll (○), enclosed eophyll (▼) and expanded eophyll (Δ). Data points represent means of six replicates of 25 seeds.

Table 1. Mathematical models to predict germination (G) and mean germination time (MGT) based on seed moisture content (MC) for four germination criteria of *Oenocarpus bacaba* seeds. Dataset from Figures 2 and 3.

Criterion		Germination	Mean germination time
First cataphyll	Equation	$G = \frac{70.8955}{1 + \exp\left(-\frac{(MC - 35.4377)}{1.4992}\right)}$	$MGT = 78.2776 - 1.4240 * MC$
	Adj R²	99.99% **	93.13% **
	SE	0.5317	1.8528
Second cataphyll	Equation	$G = \frac{72.3539}{1 + \exp\left(-\frac{(MC - 35.8550)}{1.6175}\right)}$	$MGT = 109.4390 - 1.9810 * MC$
	Adj R²	99.95% ***	81.92% *
	SE	0.9447	4.4586
Enclosed eophyll	Equation	$G = \frac{69.6521}{1 + \exp\left(-\frac{(MC - 36.2740)}{1.3250}\right)}$	$MGT = 95.0638 - 0.7413 * MC$
	Adj R²	99.74% **	81.20% *
	SE	2.2284	1.7090
Expanded eophyll	Equation	$G = \frac{70.1901}{1 + \exp\left(-\frac{(MC - 37.1166)}{1.3966}\right)}$	$MGT = 99.7419 - 0.9165 * MC$
	Adj R²	99.97% **	83.53% *
	SE	0.7528	1.9496

Adj R^2 = Adjusted R^2; SE = Standard error; Asterisks indicate the significance level of the model according to the F test: * = P < 0.05; ** = P < 0.01; *** = P < 0.001

DISCUSSION

In a previous study on seed desiccation of *O. bacaba* that scored the protrusion of the germinative bud and the growth of the primary root (> 2 mm), the control (MC = 39.9%) had the highest germination percentage and all seeds were dead after drying to a MC of 26.6% (José *et al.* 2012). These results are similar to ours in that best germination performance was achieved without desiccation (MC = 41.7%), and lethal MC was close to 26.7%. Our study confirmed that *O. bacaba* seeds do not tolerate any desiccation, as all four criteria had a significant reduction in germination after only two hours of drying. Vigour is very sensitive to stress and can be measured, for example, by germination time (Onwimol *et al.* 2016). Stress imposed by drying may reduce vigour and, consequently, increase MGT, as observed in our study. Thus, the safe MC for *O. bacaba* is close to the initial MC and producers must sow the seeds immediately after fruit processing or store them for only a short period without any drying.

Fresh seeds of *O. bataua* had 38.0% MC and seem to be less sensitive to water loss, with a critical MC close to 27% (estimated for 50% germination loss) and lethal MC below 24.3% (Nazário and Ferreira 2012). Both *O. bacaba* and *O. bataua* produce fruits at the same time and are often harvested and processed together and in the same manner. However, the greater sensitivity to desiccation of *O. bacaba* suggests that its seeds should be handled separately, to avoid loss in germinability.

In this study, the critical MC gradually increased when more developed germination stages were assessed. This was unexpected, as these structures develop gradually over more than three months during germination in moist substrate. However, an anatomical study of *Oenocarpus minor* Mart. revealed that the first and second cataphyll and the eophyll are clearly visible in the seed, formed by undifferentiated cells, covered by an external and internal protoderm (Oliveira *et al.* 2010). The embryonic axis and the cataphylls and eophyll are also well-defined in *Euterpe edulis* Mart. (Iossi *et al.* 2016) and *Euterpe oleracea* Mart. (Aguiar and Mendonça 2002). *Oenocarpus* and *Euterpe* are close relatives of the same tribe Euterpeinae of the subfamily Arecoideae (Henderson 1999). Thus, desiccation may affect all these structures in the seed, but any desiccation damage will only be detectable when the eophyll is expanded.

A similar increase in sensitivity to stress conditions was observed in the more advanced stages of development of the congeneric *O. bataua* when comparing the four germination criteria across the seed-seedling transition for temperature tolerance (Bastos *et al.* 2017). The latter authors discussed their results in terms of temperature preference between the development of early structures and reserve mobilization at a later stage. In any case, both results on temperature tolerance and on desiccation sensitivity suggest that more advanced seedling development stages are more sensitive to stress conditions.

It is important to mention that, for seed quality analysis, the germination results need to be conclusive in the shortest time possible, to allow producers to price their seeds according to quality. Scoring early germination stages will reduce the duration of the germination tests, which is especially important for desiccation-sensitive palm seeds. For example, in *O. bataua*, by scoring the first cataphyll instead of the expanded eophyll, the germination test can be reduced from three to one month (Bastos *et al.* 2017). So there is an economic driver for the choice of early germination-scoring criteria. However, our study revealed that the anticipation of the germination assessment can mask the results of normal seedling development, especially for desiccation-sensitive seeds.

CONCLUSIONS

The four stages of seedling development in *Oenocarpus bacaba* were affected in different degrees by desiccation and more advanced stages of development were more sensitive to desiccation. As the primordial leaves of all four germination criteria are already visible in the seed embryo, all of them can be damaged by desiccation of the seed. The consequences of this damage only will be detectable when the embryonic organs become visible during germination and early seedling development. We suggest that seed-stress studies with palms should take into account an adequate species-specific period for seedling development to avoid an underestimation of damages, which was of at least 100 days for *O. bacaba*.

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