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NOTE

Germination and emergence of *Astrocaryum murumuru* Mart. under stratification in different temperature regimes

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Jucimara Gonçalves dos Santos[1], Sidney Alberto do Nascimento Ferreira[2]*, Daniel Felipe de Oliveira Gentil[3]

ABSTRACT: *Astrocaryum murumuru* is a palm tree typical of Amazonian lowland areas (floodplains), whose seeds are exploited extractively for the manufacture of cosmetics. Germination of the species takes time, highlighting the need to develop techniques that shorten this process. Therefore, this research aimed to evaluate the germination and vigor of *A. murumuru* seeds, subjected to different thermal conditions of stratification. The experimental design adopted was completely randomized, with four treatments (different thermal conditions) and four replications. After drying the diaspores, extracting and soaking the seeds, these (with moisture content of 26.8%) were placed in double plastic bags containing vermiculite, moistened with water equivalent to 60% of their dry mass. They were then maintained under the following conditions: natural environment (27.8 ± 2.7 °C); oven with a temperature of 40 °C for 3 hours per day; oven with a temperature of 40 °C for 6 hours per day; and oven with a temperature of 40 °C for 9 hours a day. Stratification at a temperature of 40 °C/3 hours/day provides better germination performance and initial development of *A. murumuru* seedlings (51%), in addition to reducing remaining dormant (25%) and dead (24%) seeds.

Index terms: amazon palm tree, seed dormancy, temperature alternation.

RESUMO: *Astrocaryum murumuru* é uma palmeira típica de áreas de várzea amazônica, cujas sementes são exploradas extrativamente para a fabricação de cosméticos. A germinação da espécie é demorada, evidenciando a necessidade de desenvolvimento de técnicas que acelerem este processo. Desse modo, esta pesquisa objetivou avaliar a germinação e o vigor de sementes de *A. murumuru*, submetidas a diferentes condições térmicas de estratificação. O delineamento experimental adotado foi o inteiramente casualizado, com quatro tratamentos (diferentes condições térmicas) e quatro repetições. Após a secagem dos diásporos, extração e embebição das sementes, estas (com grau de umidade de 26,8%) foram acondicionadas em sacos plásticos duplos contendo vermiculita, umedecida com água o equivalente a 60% de sua massa seca. Em seguida, foram mantidas nas seguintes condições: ambiente natural (27,8 ± 2,7 °C); estufa com temperatura de 40 °C, por 3 horas, por dia; estufa com temperatura de 40 °C, por 6 horas, por dia; e estufa com temperatura de 40 °C, por 9 horas, por dia. A estratificação sob temperatura a 40 °C/3 horas/dia proporciona melhor desempenho germinativo e de desenvolvimento inicial de plântulas de *A. murumuru* (51%), além de redução das sementes remanescentes dormentes (25%) e mortas (24%).

Termos para indexação: palmeira amazônica, dormência de sementes, alternância de temperatura.

***Corresponding author**
sanf@inpa.gov.br

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[1]Instituto Nacional de Pesquisas da Amazônia, Coordenação de Tecnologia e Inovação, 69080-971, Manaus, AM, Brasil.

[2]Instituto Nacional de Pesquisas da Amazônia, Coordenação de Biodiversidade, 69080-971, Manaus, AM, Brasil.

[3]Universidade Federal do Amazonas, Faculdade de Ciências Agrárias, 69080-900, Manaus, AM, Brasil.

INTRODUCTION

The Amazon rainforest has a diversity of economically promising species, with possibilities to contribute to the development of industry and the regional bioeconomy. Among these, *Astrocaryum murumuru* Mart., popularly known as *murumuru*, stands out, a palm tree typical of periodically flooded areas (floodplains), whose seeds are exploited extractively due to the great demand by the cosmetics industries, also showing potential for use in food, emulsifier, soap, surfactant, and biodiesel industries (Vidal et al., 2021; Bezerra and Damasceno, 2022; Costa et al., 2024). Nonetheless, it is a species with little information about its management under cultivation conditions and even a lack of knowledge about its propagation, carried out only by seeds.

The germination of *A. murumuru* seeds is slow and irregular (Santos et al., 2022a) and can take up to a year to occur (Sousa et al., 2004). In a recent study, based on seeds devoid of endocarp, it was found that seedling emergence ranged from 12 to 73%, with an average emergence time between 64 and 106 days, whose variation was attributed, in part, to genetic variability and/or to the differentiated degree of dormancy among the progenies used (Santos et al., 2022a).

The slow and irregular germination of many palm trees is commonly caused by dormancy mechanisms of the seed itself. Dormancy in seeds of several species of the genus *Astrocaryum* (*A. aculeatum* G. Mey., *A. chambira* Burret, *A. jauari* Mart., *A. standleyanum* L.H. Bailey and *A. vulgare* Mart.) is considered to be of the morphophysiological type, in which the embryo is differentiated (has a plumular-radicular axis), but underdeveloped (needing to grow before radicle emergence), in addition to having a mechanism of physiological inhibition of germination (Baskin and Baskin, 2014). In general, the methods of overcoming dormancy in palm seeds consist of pre-germination treatments, which can be: scarification or elimination of the endocarp, immersion of the seeds in water to accelerate imbibition, stratification at low or high temperatures, use of gibberellic acid (GA_3) or potassium nitrate (KNO_3), among other chemicals (Broschat et al., 2014).

Temperature is a factor that significantly influences the germination of some palm species, and embryonic growth in seeds with morphological dormancy is strictly under control of temperature (Jaganathan, 2020). Thus, constant and high temperatures, between 30 and 35 °C, usually favor the germination of palm seeds, and often the alternation of temperature, from a minimum of 25-30 °C and a maximum of 30-40 °C, generates even more favorable results (Broschat et al., 2014).

Pre-germination heat treatment is a common practice in diaspores of *Elaeis guineensis* Jacq., and the use of a temperature of 39 ± 1 °C for a period of 60 days proved to be adequate for overcoming dormancy in different cultivars of the species (Green et al., 2013). In seeds of *Butia odorata* (Barb. Rod.) Noblic, dormancy reduction is mediated by a period of drying, followed by rehydration and exposure to high temperatures (Schlindwein et al., 2013). Although *B. odorata* is from a subtropical environment, this behavior is similar to that of *A. aculeatum*, which is from a humid tropical environment; to improve the germination of this species, its seeds (still coated by the endocarp) are subjected to drying, then to soaking in water (after extraction) and, finally, stratified under alternating temperatures (26/40 °C) (Ferreira et al., 2021). Hot and humid stratification (40 °C, for 6 hours daily) on seeds of the palm species *Bactris maraja* Mart. (Rodrigues et al., 2014) and *Phytelephas macrocarpa* Ruiz & Pavón (Ferreira and Gentil, 2017) also promoted superior germination and vigor results, when compared to other conditions and/or stratification temperatures tested. Mechanical scarification (removal of the germinal pore disc) followed by hot stratification (temperature at 40 °C, for 5 weeks) promoted an increase of emerged seedlings, as well as a reduction of emergence time in diaspores of *Butia yatay* (Mart.) Becc. (Santos et al., 2022b).

In view of the above, considering the importance of knowledge about the germination of Amazonian palm seeds, especially those exploited extractively, this study aimed to evaluate the germination and vigor of *A. murumuru* seeds, subjected to different thermal conditions of stratification.

MATERIAL AND METHODS

The experiment was conducted at the Seed Laboratory of the Biodiversity Coordination (COBIO), of the National Institute for Amazonian Research (INPA), Campus III, in Manaus, AM, Brazil. The seeds used came from a mixture of four racemes, of different plants, harvested at the beginning of the dispersal of ripe fruits, on Butija Island (04°05'12.8" S and 63°04'65.0" W), located in a floodplain area (Gleysol soil) in the municipality of Coari, AM, Brazil. The climate of the collection region is Af type (tropical, without dry season) according to Köppen's classification, with average annual temperature above 26 °C and average precipitation between 2,200 and 2,500 mm (Alvares et al., 2013).

After extraction and washing of the diaspores, they were allowed to dry under ambient conditions (27.9 ± 2.6 °C; 71.8 ± 15.9% relative humidity) for 30 days, at which time all the seeds had already detached from the endocarp. Next, the endocarp was broken to extract the seed, adopting procedures similar to those used in seeds of *A. aculeatum* (Ferreira et al., 2021) and *A. murumuru* (Santos et al., 2022a). Seeds that suffered some apparent mechanical damage during this process were eliminated.

The seeds were immersed in water for nine days, with daily water change, as has been done for *A. aculeatum* (Ferreira et al., 2021) and *A. murumuru* (Santos et al., 2022a). After soaking, the seeds (moisture content of 26.8%; obtained by the oven method, at 105 ± 3 °C, for 24 hours, Brasil (2009)) were packed in double plastic bags containing medium-texture vermiculite, in a volume equivalent to twice the volume of the seeds, moistened with water in an amount equivalent to 60% of their dry mass. Then, the closed packages containing the seeds were subjected to the following conditions: room temperature, in an airy room with a temperature of 27.8 ± 2.7 °C; oven with a temperature of 40 °C, for 3 hours, per day; oven with a temperature of 40 °C, for 6 hours, per day; and oven with a temperature of 40 °C, for 9 hours, per day. A timer was used to activate the ovens at temperature of 40 °C at 9:00 am and turn them off sequentially: one at 12:00 pm (after 3 hours), another at 3:00 pm (after 6 hours) and the third one at 6:00 pm (after 9 hours), depending on the duration of the 40 °C temperature of each treatment; during the rest of the time, in a 24-hour cycle, the internal temperature of the ovens decreased naturally, without any intervention, reaching a minimum average temperature of 26 °C.

The experimental design was completely randomized, with four treatments (different thermal conditions of stratification) and four replications, each containing 25 seeds. Periodically, every 15 days, for five months, the number of seedlings at the germinative button and first cataphyll stages (Figures 1A and 1B, respectively) were evaluated in each experimental unit (packaging of each replication), as described by Santos et al. (2022a). After each evaluation, and before closing the plastic bags again, the substrate was sprayed with water so as to maintain the initial moisture.

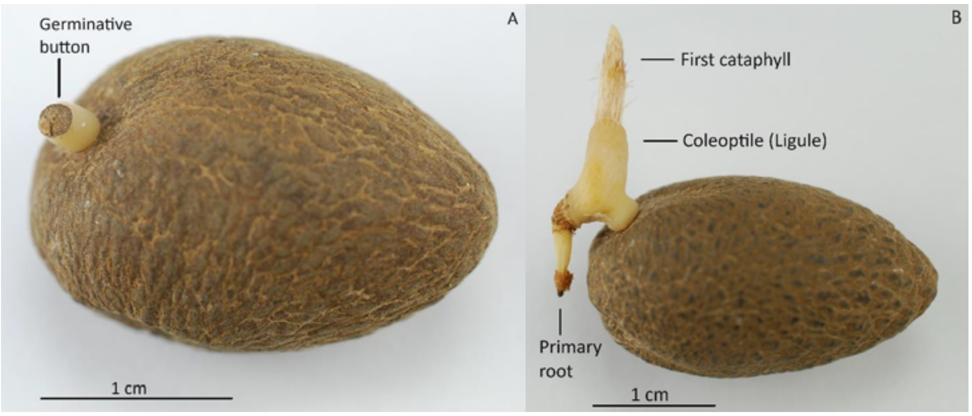

Figure 1. Germination (germinative button formation, A) and emergence (emergence of the first cataphyll, B) criteria adopted to evaluate the effect of stratification on *A. murumuru* seeds under different daily temperature regimes.

From the counting data, the percentages of germination (formation of the germinative button) and emergence (emergence of the first cataphyll), the germination and emergence speed index, and the mean germination and emergence times were calculated according to Ranal and Santana (2006). At the end of the experiment, by means of the cutting test (Brasil, 2009), seeds that did not germinate were classified as dormant (healthy with a firm and milky-white embryo) or dead (fully deteriorated or with only the rotten embryo).

Data analysis began with the distribution of temporal frequency (fortnightly) of germination and emergence, and calculation of asymmetry using the Excel® program – version 2409. Prior to the analysis of the variables, data normality was checked using the Shapiro-Wilk test, and, if abnormalities were found, transformations were made aiming at normalization: the values of germination, emergence, dead seeds and dormant seeds were transformed into arcsine $\sqrt{x/100}$; those of mean germination and emergence times were transformed into $\sqrt{x}$; and the values of germination speed and emergence speed indices were not transformed. Then, the homogeneity of the variance was checked using the Bartlett test. Once the assumptions of randomness, normality and homogeneity were met, analyses of variance were performed, followed by comparisons of the means using Tukey test at 5% probability level. Shapiro-Wilk tests, transformations, Bartlett tests, analyses of variance and comparisons of means were performed using the Assistat 7.7 beta program (Silva and Azevedo, 2016). The results were presented and discussed in their original form, without transformation.

RESULTS AND DISCUSSION

The temporal distributions of germination (germinative button) and emergence (first cataphyll) showed distinct behaviors (Figures 2A and 2B, respectively, and Table 1). In the stratifications at 27.8 ± 2.7 °C and 40 °C/3 hours/day, germination showed reduced and negative asymmetries, while, under these same conditions, emergence had reduced and positive asymmetries; under 40 °C/9 hours/day, the result was reversed: germination with positive asymmetry coefficient and negative emergence coefficient, both with reduced values. In the stratification at 40 °C/6 hours/day, high degrees of positive asymmetry were observed for germination (1.274) and emergence (1.508), resulting from the longer periods to complete these events (105 days for germination and 135 days for emergence).

Regardless of the stratification condition, germination began at fifteen days of conditioning, while emergence began at 45 days. For the best performing treatments (40 °C/3 hours/day and 40 °C/6 hours/day), the maximum germination and emergence values were reached at 30 and 60 days, respectively. It is worth noting that, at a temperature of 40 °C/3 hours/day, germination and emergence occurred between 15 and 45 days and between 45 and 90 days, respectively, while at the temperature of 40 °C/6 hours/day, they occurred between 15 and 105 days and between 45 and 135 days, respectively. The results found are in accordance with those reported by Santos et al. (2022a), who studied six progenies of *A. murumuru* and showed approximate periods of formation of these structures of 15 to 100 days for the germinative button and 30 to 140 days for the first cataphyll.

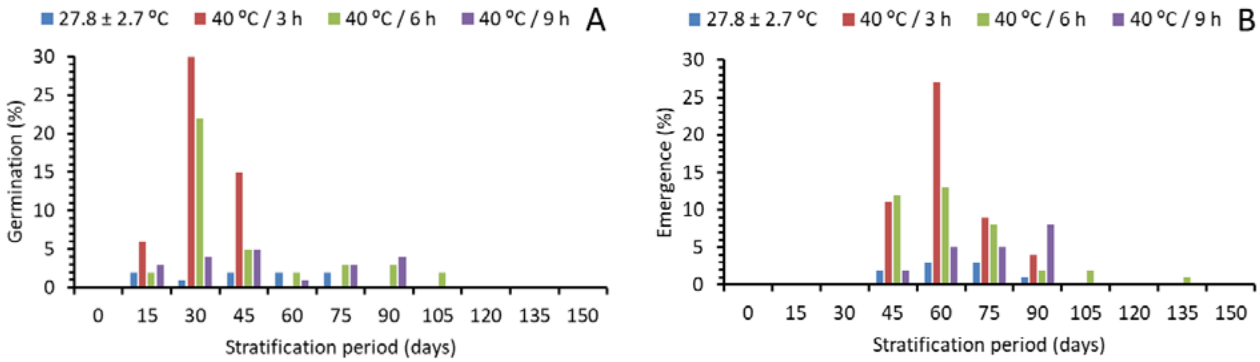

Figure 2. Fortnightly germination (A) and emergence (B) in *A. murumuru* seeds subjected to stratification in different daily temperature regimes for five months.

Table 1. Asymmetries regarding the temporal distribution of germination (germinative button) and emergence (first cataphyll) related to *A. murumuru* seeds subjected to stratification in different daily temperature regimes, for five months.

Event	Stratification conditions			
	27.8 ± 2.7 °C	40 ˚C/3 h/d	40 ˚C/6 h/d	40 ˚C/9 h/d
Germination	-0.235	-0.134	1.274	0.213
Emergence	0.107	0.613	1.508	-0.498

Table 2. Summary of the analysis of variance plus Shapiro-Wilk and Bartlett tests referring to the following variables: germination (G), emergence (E), germination speed index (GSI), emergence speed index (ESI), mean germination time (MGT), mean emergence time (MET), and dormant (DOS) and dead (DES) remaining seeds referring to *A. murumuru* seeds subjected to stratification in different daily temperature regimes, for five months.

Source of variation	Degree of freedom	Mean square							
		G	E	GSI	ESI	MGT	MET	DOS	DES
Treatment	3	650.5**	640.3**	0.00017**	0.426**	1.651^{ns}	0.377^{ns}	521.5**	189.5*
Residual	12	24.5	23.7	0.00001	0.018	0.667	0.284	27.7	38.0
CV (%)	-	15.6	15.4	37.3	28.2	12.3	6.5	15.6	16.3
^{a}W	-	0.937 (0.318)	0.945 (0.411)	0.946 (0.429)	0.938 (0.323)	0.970 (0.840)	0.921 (0.176)	0.945 (0.416)	0.936 (0.307)
$^{b}X^2$	-	1.342	1.770	1.715	0.485	2.428	4.415	7.492	3.392

* and ** - significant at 5% and 1% probability levels by the F test, respectively; ^{ns} – not significant at 5% probability level by the F test; ^{a} – Shapiro-Wilk test (W) plus p-value (0.05) in parentheses, attesting that the variables have a normal distribution; ^{b} – Bartlett's test statistics (X^2) whose critical significance values are 7.815 (5%) and 11.345 (1%), not rejecting the null hypothesis (the variances are homogeneous).

The variables evaluated showed significant differences between the treatments, except for the mean germination (MGT) and emergence (MET) times (Table 2). The highest percentages of germination and emergence (both 51%) were obtained with stratification at 40 °C/3 hours/day, not differing significantly from the values obtained at 40 °C/6 hours/day (Table 3). The period of 9 hours per day at a temperature of 40 °C seems to have been excessive, as it led to lower germination and emergence percentages (both 20%), although still higher than the values found in the control treatment (ambient temperature of 27.9 ± 2.6 °C), which resulted in only 9% of germination and emergence. In many cases, effective treatments to overcome dormancy in seeds simulate the natural conditions of occurrence of the species (Baskin and Baskin, 2014).

The germination (GSI) and emergence (ESI) speed indices showed similar behaviors to those of the germination and emergence variables, even following the same hierarchical order of performance between the treatments (Table 3). In *B. maraja* (Rodrigues et al., 2014), *P. macrocarpa* (Ferreira and Gentil, 2017) and *A. aculeatum* (Ferreira et al., 2021), seed stratification at a temperature of 40 °C/6 hours/day promoted higher germination speed indices.

The mean germination (MGT) and emergence (MET) times did not differ significantly from each other, and on average were 45.1 and 67.6 days, respectively (Table 3). Despite these results, there is a trend towards lower values of MGT and MET in the stratification at 40 °C/3 hours/day (33 and 62 days, respectively). In stratification under different thermal conditions, *Phytelephas macrocarpa* seeds showed no significant differences between the mean germination times; however, the trend of favorable results was also evident under the temperature of 40 °C/6 hours/day (Ferreira and Gentil, 2017).

Table 3. Germination (G), emergence (E), germination speed index (GSI), emergence speed index (ESI), mean germination time (MGT), mean emergence time (MET), and dormant (DOS) and dead (DES) remaining seeds, referring to *A. murumuru* seeds subjected to stratification in different daily temperature regimes, for five months.

Condition	G (%)	E (%)	GSI (% day^{-1})	ESI (% day^{-1})	MGT (days)	MET (days)	DOS (%)	DES (%)
27.8 ± 2.7 ˚C	9 b	9 b	0.067 c	0.145 b	51.6 a	69.4 a	57 a	34 ab
40 ˚C / 3 h	51 a	51 a	1.733 a	0.859 a	33.0 a	62.0 a	25 bc	24 b
40 ˚C / 6 h	39 a	38 a	1.103 ab	0.639 a	45.1 a	65.1 a	15 c	46 a
40 ˚C / 9 h	20 b	20 b	0.545 bc	0.283 b	50.5 a	73.8 a	32 b	48 a

Means followed by the same letter vertically do not differ significantly by Tukey test at 5% probability level.

The highest percentage of dormant remaining seeds (57%) was found in the ambient condition (27.8 ± 2.7 °C), where germination and emergence were significantly lower (both 9%), indicating a trend of conservation of viability under this condition (Table 3). Rodrigues et al. (2014), using constant temperatures between 20 and 35 °C, obtained low germination rates in *B. maraja*, with almost all seeds remaining dormant. *A. aculeatum* seeds also showed high levels of dormancy and/or conservation when stratification temperatures were constant, ranging between 25 and 35 °C (Ferreira et al., 2021), similar to what occurred with *P. macrocarpa* seeds (Ferreira and Gentil, 2017).

The lowest value of dead remaining seeds (24%) was obtained in the stratification at 40 °C/3 hours/day and did not differ significantly from that obtained with the stratification under ambient conditions (34%), which did not differ from those obtained with the stratifications at 40 °C/6 hours/day (46%) and 40 °C/9 hours/day (48%) (Table 3). Under stratification, seeds of *B. maraja* (Rodrigues et al., 2014), *P. macrocarpa* (Ferreira and Gentil, 2017) and *A. aculeatum* (Ferreira et al., 2021) showed the highest mortality rates at a continuous temperature of 40 °C, and the lowest values at a temperature of 40 °C/6 hours/day.

Despite the favoring promoted by hot and humid stratification, there was low germination and emergence in *A. murumuru*, with a maximum effectiveness of 51% (40 °C/3 hours/day). This may be related to the origin and/or genetic variability of the seeds, since they came from a mixture of progenies, which, individually, showed distinct physical and physiological characteristics (Santos et al., 2022a).

The high mortality of the seeds, under all thermal conditions of stratification (Table 3), may be associated with mechanical damage (crushing) suffered during seed extraction (endocarp breakage) and that was not noticed, in order to discard these seeds prior to stratification. Santos et al. (2022a) found, as a function of progenies, variations in the percentages of seeds lost during extraction and seeds that died after sowing, which showed a correlation of 0.76. Thus, a better selection of the extracted seeds and/or improvement of the processing process are suggested. In the industrial extraction of fat from seeds, the quality of the product may be compromised due to drying, as smaller seeds dry faster, allowing earlier detachment from the endocarp, while larger ones take longer to detach from the endocarp (Bezerra and Damasceno, 2022).

In the present study, a high number of remaining seeds in a dormancy state was also observed, even in the stratification at a temperature of 40 °C/3 hours/day, for which the best germination/emergence results were obtained, with a dormancy value of 25%. Apparently, in addition to the probable existence of morphological dormancy, there is still the possibility of physiological dormancy occurring at different levels. According to Baskin and Baskin (2022), in tropical forests such as the Amazon, this type of dormancy can be overcome by a long period of hot and humid stratification. Thus, there is a need for a better understanding of the type(s) and cause(s) of dormancy, as well as the continuity of studies on overcoming this phenomenon in *A. murumuru* seeds.

CONCLUSIONS

Stratification at a temperature of 40 °C/3 hours/day promotes better performance in terms of germination (formation of the germinative button) and emergence (emergence of the first cataphyll) of *Astrocaryum murumuru* (51%), in addition to a reduction in dormant (25%) and dead (24%) remaining seeds.

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