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Physiological potential of stored *Schinopsis brasiliensis* Engler diaspores[1]

Potencial fisiológico de diásporos armazenados de *Schinopsis brasiliensis* Engler

Monalisa Alves Diniz da Silva[2]*, Vanessa Renata de Sousa Barboza[3], Joyce Naiara da Silva[4], Edilma Pereira Gonçalves[5], Jeandson Silva Viana[5]

ABSTRACT - Storage of diaspores makes it possible to provide seedlings for reforestation programs or restoration of degraded areas, especially for perennial species that do not regularly produce diaspores or when germination is difficult and slow. The objective of the present study was to verify the physiological potential of *Schinopsis brasiliensis* diaspores stored in two environments for 12 months, with subsequent manual scarification of the endocarp. The experimental design was entirely randomized, using a 2 × 7 × 2 factorial scheme, with two environments (natural and refrigerator), seven periods (0, 2, 4, 6, 8, 10 and 12 months) of storage, and two physical conditions of the endocarp (intact and scarified). The study analyzed water content, emergence, speed index and mean emergence time, length and dry mass of the aerial part and root system, and collar diameter of the seedlings. The 12 month storage did not overcome the tegument dormancy of *S. brasiliensis* diaspores. After storage in the natural environment, manual scarification favored emergence and the seedlings' speed of establishment. The diaspores presented increased germination capacity at five months and decreased seedling development before losing their germination capacity, regardless of storage conditions.

Key words: *Ex situ* conservation. Deterioration. Dormancy.

RESUMO - O armazenamento de diásporos possibilita disponibilizar mudas para atender os programas de reflorestamento ou de recuperação de áreas degradadas, principalmente para espécies perenes que não produzem diásporos regularmente ou quando a germinação dos mesmos é difícil e demorada. Objetivou-se verificar o potencial fisiológico de diásporos de *Schinopsis brasiliensis* armazenados em dois ambientes por 12 meses, com posterior escarificação manual do endocarpo. O delineamento experimental adotado foi o inteiramente casualizado, em esquema fatorial 2 × 7 × 2, com dois ambientes (natural e geladeira) e sete períodos (0; 2; 4; 6; 8; 10 e 12 meses) de armazenamento, e duas condições físicas do endocarpo (intacto e escarificado). Foram avaliados o teor de água, emergência, índice de velocidade e tempo médio de emergência, comprimento, massa seca da parte aérea e do sistema radicular e diamêtro do coleto das plântulas. O armazenamento por 12 meses não supera a dormência tegumentar dos diásporos de *S. brasilensis*. A escarificação manual após o armazenamento em ambiente natural favorece a emergência e a velocidade de estabelecimento das plântulas. Independente da condição de armazenamento, os diásporos apresentam maior capacidade germinativa aos cinco meses. O declínio no desenvolvimento das plântulas, independente da condição de armazenamento dos diásporos, ocorre antes da perda da capacidade germinativa.

Palavras-chave: Conservação *ex situ*. Deterioração. Dormência.

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Editor-in-Chief: Alek Sandro Dutra - alekdutra@ufc.br
*Author for correspondence
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[1]Parte da dissertação do segundo autor, apresentada ao Programa de Pós Graduação em Produção Vegetal, Universidade Federal Rural de Pernambuco (UFRPE), Unidade Acadêmica de Serra Talhada (UAST)
[2]Departamento de Agronomia, Universidade Federal Rural de Pernambuco (UFRPE), Unidade Acadêmica de Serra Talhada (UAST), Serra Talhada-PE, Brasil, monalisa.diniz@ufrpe.br (ORCID ID 0000-0001-9052-7380)
[3]Autônomo, Mestre em Produção Vegetal, Universidade Federal Rural de Pernambuco (UFRPE), Unidade Acadêmica de Serra Talhada (UAST), Serra Talhada – PE, Brasil, vanessarenatasousa@gmail.com (ORCID ID 0000-0002-9587-0855)
[4]Departamento de Fitotecnia e Ciências Ambientais, Universidade Federal da Paraíba, Programa de Pós Graduação em Agronomia, Areia-PB, Brasil, joicenaiara@hotmail.com (ORCID ID 0000-0002-3260-8745)
[5]Departamento de Agronomia, Universidade Federal do Agreste de Pernambuco (UFAPE), Programa de Pós-Graduação em Produção Agrícola, Garanhuns-PE, Brasil, edilma.goncalves@ufape.edu.br (ORCID ID 0000-0001-5150-7088), jeandson.viana@ufape.edu.br (ORCID ID 0000-0002-8958-2873)

INTRODUCTION

Germination is a vital ecophysiological process for forest regeneration and maintenance of vegetation diversity. Thus, knowledge about this aspect is essential for the recovery of degraded areas to occur satisfactorily (ARAÚJO; CASTRO; ALBUQUERQUE, 2007). The literature is still deficient in information on seeds and diaspores of many native species, especially concerning behavior during storage (OLIVEIRA; ALVES; FERNANDES, 2018).

The importance of determining the storage conditions is unquestion able since they can cause degenerative changes of biochemical, physiological, and physical origin (OLIVEIRA; ALVES; FERNANDES, 2018). Storage under appropriate conditions can attenuate the speed of seed deterioration. Thus, learning the behavior of seeds when subjected to different storage conditions is essential for managing the species (FERREIRA; BORGHETTI, 2004).

The species *Schinopsis brasiliensis* Engl. (known by the common names braúna or baraúna) is native to the Caatinga and Atlantic Forest and is found in semi-arid regions of Brazil (FERNANDES *et al*., 2015). Because of its high wood quality and relative resistance to decomposition, excessive and unsustainable exploitation has resulted in the near depletion of its populations (MARTINELLI; MORAES, 2013). The species is included in the vulnerable category of the official list of endangered species of Brazilian flora of the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) but may move to the endangered category (BRASIL, 1992). In addition to biological studies that enable the production of seedlings, both the legalization of exploitation and the subsequent commercialization go through market studies.

The main form of propagation of *S. brasiliensis* is through diaspores, which is a difficult and slow process. Therefore, the evaluation of diaspore quality is fundamental for raising seedlings in nurseries. The fruit is a samara with markedly differentiated pericarp layers: a membranous epicarp, a spongy mesocarp, and a woody endocarp that is "bony" and impermeable to water (LORENZI, 2014). The endocarp surrounds the seed and does not detach easily, forming the pyrene (BARROSO *et al.*, 1999). This layer increases seed longevity by preventing germination under favorable conditions as a survival strategy (SANTOS *et al.*, 2018).

Alves *et al*. (2007) stated that mechanical scarification was the most used procedure for overcoming dormancy of *S. brasiliensis* diaspores. In contrast, Oliveira and Oliveira (2008) found that the best way to obtain more regular, rapid, and complete germination of the diaspores consisted of removing the epicarp and mesocarp to eliminate germination inhibitors, pre-dryingduring storage for 25 to 30 days to cause cracks in the endocarp and sowing in moist sand to soften the endocarp. However, Coelho *et al*. (2016) observed that removing the epicarp and mesocarp, soaking in running water for 12 hours, and subsequent scarification near the micropyle region using sand paper resulted in a higher emergence percentage.

Currently, the literature has information on the behavior of diaspores or seeds of many native forest species during storage. However, there is still a lack of knowledge on some species, including *S. brasiliensis* (BRASIL, 2013). Within this context, the objective of this study was to evaluate the physiological potential of *S. brasiliensis* diaspores stored in two environments for 12 months and verify how the diaspores respond to manual scarification after storage.

MATERIAL AND METHODS

Diaspores of *S. brasiliensis* were collected at a uniform stage of visual maturity, i.e., complete brown coloration, in the city of Custódia, state of Pernambuco, Brazil, from two trees (08°17'54.8"S and 37°43'49"W; 08°20'56.2"S and 37°45'52"W). The diaspores were kept in plastic bags under refrigeration at 7 °C between the collection period and the start of the experiment.

Storage: After processing — removal of the epicarp and mesocarp — the diaspores were placed in kraft paper containers and stored in natural and refrigerated environments, where the temperature and relative humidity of the air were monitored with an INCOTERM® digital thermo-hygrometer.

After each storage period, half of the diaspores were scarified, while the other remained intact. The diaspores were manually scarified with 80-grit sand paper in the region near the micropyle (ALVES *et al*., 2007).

The evaluations of the experiment occurred every other monthfor 12 months, and the following variables underwent analysis:

Water content: It was determined by drying in an oven at 105 ± 3 °C for 24 hours (BRASIL, 2009), using two sample sets with 25 diaspores per set. The results were expressed on a wet basis.

Emergence: The experiment was executed in a nursery (50% light interception), with four replications of 25 diaspores sown in expanded polystyrene trays with 128 cells (40 cm^3). The trays contained commercial coconut fiber substrate and were irrigated daily to maintain substrate humidity. Daily counts were performed from the day of sowing until the 25 th day, using the emergence of the first pair of true leaves as the criteria. The results were expressed as a percentage. The emergence speed index

(ESI) (MAGUIRE, 1962) and mean time of emergence (MTE) (LABOURIAU, 1983) were performed along with the emergence analysis.

Seedling length: At the end of emergence (after 25 days), the lengths of the aerial part (LAP) and root system (LRS) of the normal seedlings of each treatment were measured. The seedlings were cut at the collar region and from the collar, measured with a graduated ruler to the leaf apex (LA) and root apex (RA), and the results were expressed in cm seedling^{-1}.

Collar diameter (CD)*:* The diameter of the collar was measured using a digital pachymeter, and the results were expressed in cm seedling^{-1}.

Dry mass of seedlings: The aerial part and root system were conditioned in kraft paper bags and placed in a drying oven regulated at 80 ºC for 24 hours to obtain the dry mass of the aerial part (DMAP) and root system (DMRS). After this period and appropriate cooling, they were weighed on an analytical balance with a precision of 0.0001 g, and the results were expressed in g seedling^{-1}.

The treatments were distributed in an entirely randomized experimental design, with four replications of 25 diaspores. The experiment used a 2 × 7 × 2 factorial scheme, with two environments (natural and refrigerator) and seven periods (0, 2, 4, 6, 8, 10 and 12 months) of storage and two physical conditions of the endocarp (intact and scarified).

The data were subjected to analysis of variance, the means were compared using Tukey's test at 5% probability level, and the quantitative variables were determined by regression analysis. The statistical analyses were performed using the Sisvar 5.6 software (FERREIRA, 2014).

RESULTS AND DISCUSSION

The initial water content for *S. brasiliensis* diaspores was 15.8 and 16.9% for intact and scarified, respectively (Table 1). The study identified variations in water content after storage according to the storage environments and treatments for overcoming dormancy. Batista *et al*. (2011) observed similar results when they found a reduction in the water content *Cedrela odorata* seeds stored in a natural environment and a refrigerator for nine months in relation to the initial humidity.

The water content presented distinctly in the storage environments. The natural environment showed lower values, ranging from 9.3 to 13.5% for intact and 9.5 to 15.2% for scarified diaspores. The refrigerator environment showed a variation of 11.2 to 14.9% for intact and 9.6% to 15.5% for scarified diaspores (Table 1).

The natural environment condition presented variations in temperature and relative humidity (RH) of the air, with mean values of 27.6 ºC and 38.5% RH. In the refrigerator environment, the means were7.2 ºC and 47.6% RH (Figure 1).

Temperature and relative humidity of the air at the storage site are the main factors that influence the physiological quality of the seed. Relative humidity controls the water content of the seed, while temperature affects the speed of biochemical processes (GOLDFARB; QUEIROGA, 2013).

In the analysis of variance concerning the percentage and ESI (Table 2), a significant interaction was observed between storage environments and treatments for overcoming dormancy, while the interaction between environments and storage periods was significant for the speed index and mean time of emergence.

Figure 1 - Temperature (T ºC) and relative humidity (RH%) monitoring during storage of Schinopsis brasiliensis diaspores for 12 months

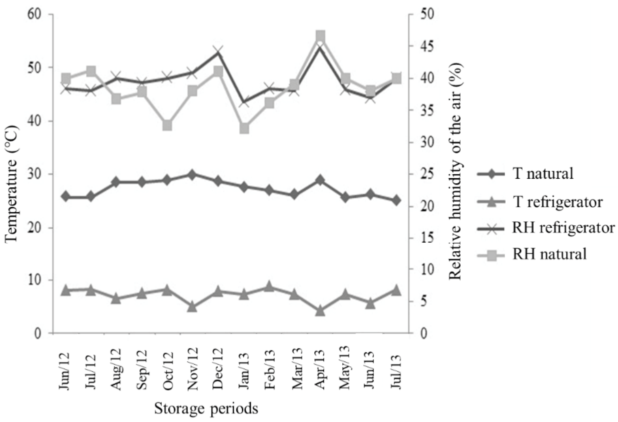


Table 1 - Water content (%) of *Schinopsis brasiliensis* diaspores, stored for 12 months and subjected to subsequent manual scarification of the endocarp (intact - I; scarified - S)

	Storage (months)													
Storage environments	0		2		4		6		8		10		12	
	I	S	I	S	I	S	I	S	I	S	I	S	I	S
Natural	15.8	16.9	13.5	15.2	12.3	15.1	11.4	14.8	11.6	14.9	9.5	10.8	9.3	9.5
Refrigerator	15.8	16.9	14.9	15.5	14.1	15.3	13.5	14.8	13.9	15.4	13.2	10.2	11.2	9.6

Table 2 - Mean square values of the analysis of variance for the physiological potential characteristics of *Schinopsis brasiliensis* diaspores stored for 12 months

Sources of variation	Mean Square			
	GL	E	ESI	MTE
SP (F1)	6	887.42**	0.36**	32.86**
OD (F2)	1	6993.08**	2.59**	30.89**
SE (F3)	1	305.58ns	0.17ns	0.02ns
F1 x F2	6	403.49ns	0.13ns	11.04*
F1 x F3	6	225.37ns	0.14*	18.42**
F2 x F3	1	1255.58*	0.32*	0.91ns
F1 x F2 x F3	6	227.45ns	0.04ns	1.01ns
Residue	84	242.03	0.061	4.08
CV(%)		36.49	39.40	12.70

** Significant at 1% probability level; *: significant at 5% probability level; ns: not significant; SP: storage periods; OD: overcoming dormancy; SE: storage environment; CV: coefficient of variation; GL: degree of freedom; E: emergence; ESI: speed of emergence index; MTE: mean time of emergence

Table 3 - Emergence (E) and emergence speed index (ESI) of *Schinopsis brasiliensis* seedlings from diaspores stored for 12 months and subjected to manual scarification of the endocarp

Storage environments	E (%)		ESI	
	Physical condition of the endocarp			
	Intact	Scarified	Intact	Scarified
Natural	33 Ab	55 Aa	0.46 Ab	0.87 Aa
Refrigerator	36 Ab	46 Ba	0.49 Ab	0.69 Ba

Means followed by the same letters, lowercase in the rows and uppercase in the columns, do not differ by the Tukey's test at 5% probability level

For emergence and ESI (Table 3), the diaspores that were scarified provided the highest values when compared to those that did not undergo any treatment (intact), corroborating the results found by Coelho *et al.* (2016) and Alves *et al*. (2007). As the seeds present tegument impermeability resulting from the pyrene layer (BARROSO *et al*., 1999; SANTOS *et al*., 2018), scarification with sand paper facilitated the entry of water favoring the germination process.

Regarding storage conditions (Table 3), a difference was found only for the scarified diaspores, where both seedling emergence and emergence speed index were statistically superior for the diaspores stored in the natural environment, compared to those stored in the refrigerator. Despite the lower temperature in the refrigerator, the relative humidity was higher (Figure 1), which culminated with a higher water content of the diaspores stored in this condition (Table 1). The water content of seeds is considered the main factor affecting seed longevity since it can induce a high respiration rate and, consequently, the consumption of energy reserves. This was one of the main characteristics observed in previous studies (MARTINS; PINTO, 2014; WANG *et al*., 2018).

When evaluating different packaging, periods, and storage environments, Borba Filho and Perez (2009) found *Tabebuia roseo-alba* (Ridl.) Sandwith (Bignoniaceae) seeds, regardless of the type of packaging, showed no reduction in germination percentage when stored in a refrigerated environment. However, those stored in the laboratory environment showed reduced viability after 120 days. Diaspores of *Myracrodruon urundeuva* Allemão (Anacardiaceae) showed a slight reduction in vigor when stored for ten months in a refrigerated chamber (BARBOZA *et al*., 2018).

The *S. brasiliensis* diaspores presented an initial emergence of 38% (Figure 2), reaching the maximum estimated emergence in the fifth month (49%). After this period, a gradual reduction was observed with the longer storage periods, suggesting aloss of viability by the diaspores. Chemical degradation of seed components during storage occurs due to damage caused by oxidizing agents. However, the speed of such reactions is defined by seed properties, which in turn are affected by temperature and humidity (MARCOS FILHO, 2015). Therefore, the intensity and speed of the degradation processes directly influence seed germination and vigor.

The ESI of *S. brasiliensis* diaspores subjected to different environments and storage periods showed a quadratic behavior (Figure 3). The highest estimated speed indices were observed in the fifth and sixth months for diaspores stored in the refrigerator and natural environment, respectively, with subsequent decline.

As for the data concerning MTE (Figure 4A), a reduction in the days of emergence was observed for up to six and eight months of storage under refrigeration and natural conditions, respectively, and subsequently increased. According to Marcos Filho (2015), decreased germination speed, verified by an increased MTE, is the first sign of performance decline and is generally caused by the breakdown of the membrane system.

Regarding MTE, a quadratic tendency was observed for the treatments overcoming dormancy (Figure 4B), with the minimum value estimated at six and eight months of storage for intact and scarified diaspores, respectively. Marcos Filho (2015) noted that the intensity of seed dormancy is independent of its cause and is usually inversely proportional to age. The normal trend is the gradual overcoming of dormancy as the seed ages.

Seeds deteriorate naturally over time and lose vigor due to biochemical and physiological damage (DONADON *et al*., 2015), as observed in the above ESI and MTE values. When evaluating the vigor of *Vochysia divergens* Pohl (Vochysiaceae) seeds after different storage periods (0, 30, 60, 90, 120, and 150 days) in three environments (refrigerator, laboratory, and humidity chamber), Oliveira; Alves and Fernandes (2018) observed a gradual reduction of germination and speed index over the periods, regardless of the environment, reaching total loss of vigor after 150 days.

In the analysis of variance for the variables related to the initial development of *S. brasiliensis* seedlings (Table 4), a significant interaction was observed between the environments and storage periods for CD, DMAP, and DMSR. In contrast, the interaction between storage periods and treatments for overcoming dormancy

Figure 2 - Emergence of Schinopsis brasiliensis seedlings from diaspores stored for 12 months

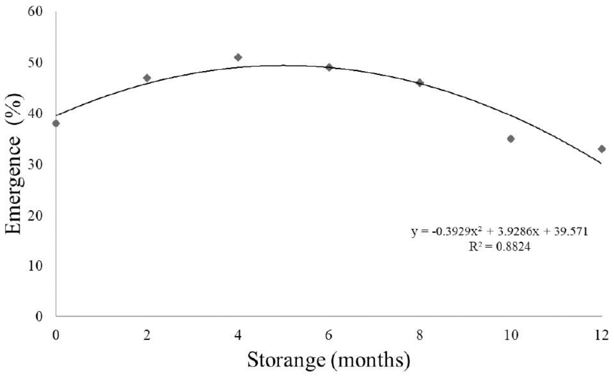


Figure 3 - Emergence speed index (ESI) of *Schinopsis brasiliensis* seedlings from diaspores stored for 12 months

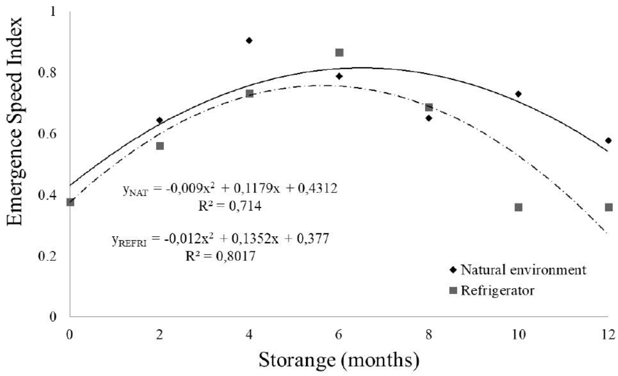
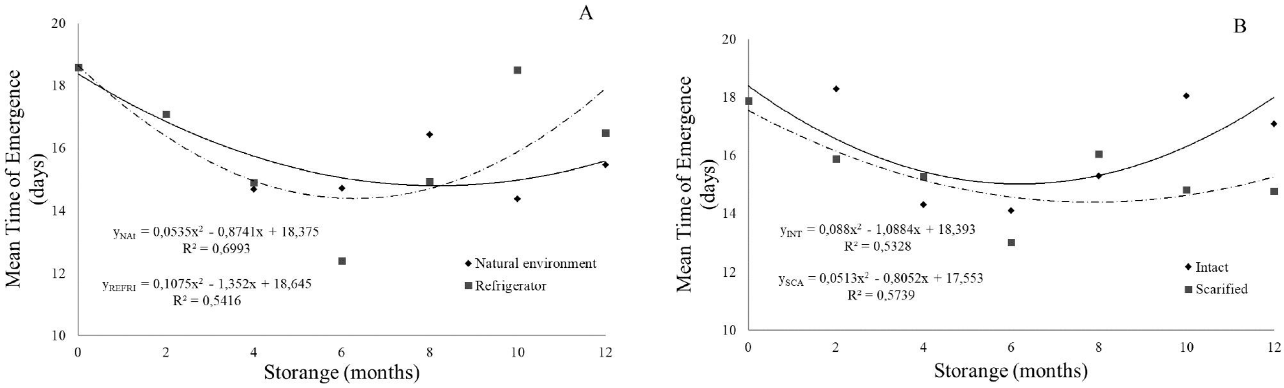


Figure 4 - Mean time of emergence of *Schinopsis brasiliensis* seedlings from diaspores stored for 12 months (A) and subjected to subsequent manual scarification of the endocarp (intact, scarified) (B)



was significant only for CD. Moreover, although the interactions were not significant, the storage periods influenced the LAP and LRS variables.

As for *S. brasiliensis* seedlings LAP, in the initial period, without storage of the diaspores, the seedlings presented 4.6 cm of length, reaching the maximum estimated value in the third month of storage (4.90 cm seedling^{-1}). There was a reduction this period, reaching values lower than the initial (Figure 5A).

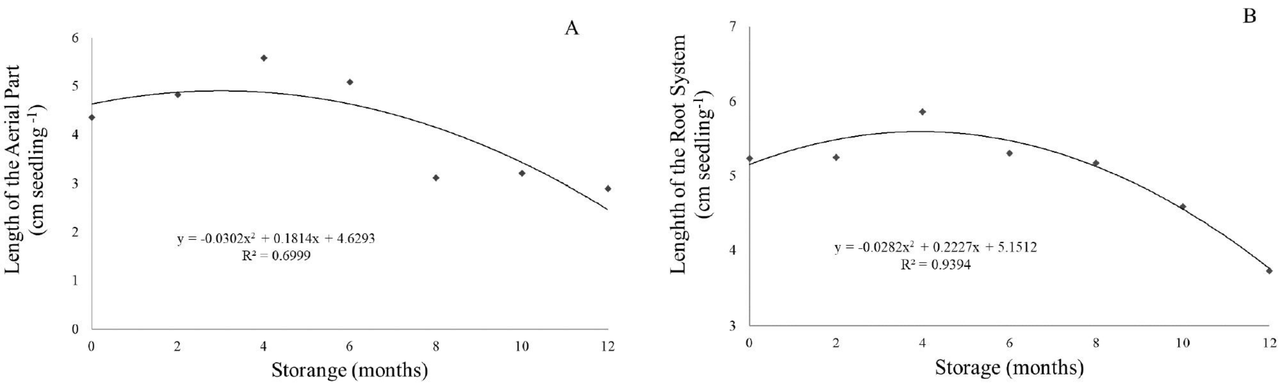
Regarding LRS (Figure 5B), a greater development was observed at four months of storage, decreasing thereafter. Among the most evident physiological symptoms during the process of seed deterioration are those related to germination and initial seedling growth (DONADON *et al*., 2015), which initially arise from damage to the membrane system due to the attack of its cellular constituents by free radicals (BEWLEY *et al*., 2013).

The CD progressively decreased as the period of storage increased, regardless of the storage condition (Figure 6A) and the endocarp scarification (Figure 6B). The seedlings from diaspores stored in the natural environment and refrigerator for 12 months presented a CD 44.32 and 49.83% lower than the seedlings from diaspores evaluated before storage, respectively (Figure 6A). Moreover, at the end of storage, this study observed a decrease in CD of 44.89 and 48.11% for seedlings from intact and scarified diaspores, respectively, compared to those formed from diaspores that were not stored (Figure 6B). Gomes and Paiva (2011) reported that the CD was one of the best characteristics to evaluate the quality of seedlings. However, the same was not observed for storage during this study.

Table 4 - Mean square values of the analysis of variance for the physiological potential characteristics of *Schinopsis brasiliensis* diaspores stored for 12 months and subjected to manual endocarp scarification

Source of variation	Mean Square					
	GL	LAP	LSR	CD	DMAP	DMRS
SP (F1)	6	18.56**	7.30**	0.369**	0.10**	0.05**
OD (F2)	1	0.41^{ns}	0.51^{ns}	0.023**	0.003^{ns}	0.005^{ns}
SE (F3)	1	0.04^{ns}	0.40^{ns}	0.085**	0.001^{ns}	0.000^{ns}
F1 x F2	6	0.47^{ns}	0.27^{ns}	0.022**	0.008^{ns}	0.004^{ns}
F1 x F3	6	0.31ns	0.25^{ns}	0.003**	0.01*	0.008*
F2 x F3	1	0.32^{ns}	0.001^{ns}	0.001^{ns}	0.000^{ns}	0.002^{ns}
F1 x F2 x F3	6	0.21^{ns}	0.08^{ns}	0.002^{ns}	0.006^{ns}	0.002^{ns}
Waste	84	0.28	0.14	0.0009	0.006	0.002
CV(%)		12.85	7.56	5.08	40.02	56.27

Figure 5 - Length of the aerial part (A) and root system (B) of *Schinopsis brasiliensis* seedlings from diaspores stored for 12 months



** Significant at 1% probability level; * significant at 5% probability level; ^{ns}: not significant; SP: storage periods; OD: overcoming dormancy; SE: storage environment; CV: coefficient of variation; GL: degree of freedom; LAP: length of the aerial part; LRS: length of the root system; CD: collar diameter; DMAP: dry mass of the aerial part; DMRS: dry mass of the root system

Figure 6 - Collar diameter of *Schinopsis brasiliensis* seedlings from diaspores stored for 12 months (A) and subjected to subsequent manual scarification of the endocarp (intact, scarified) (B)

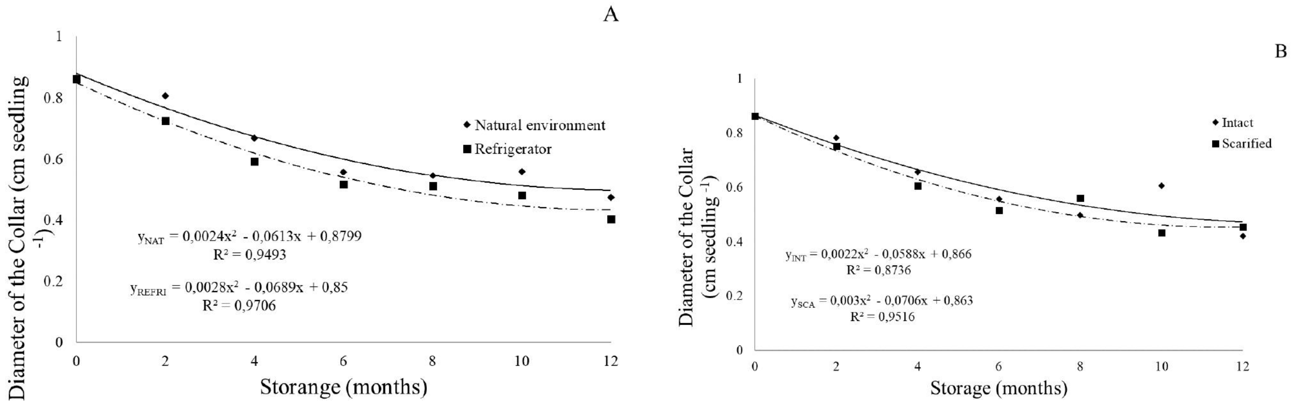
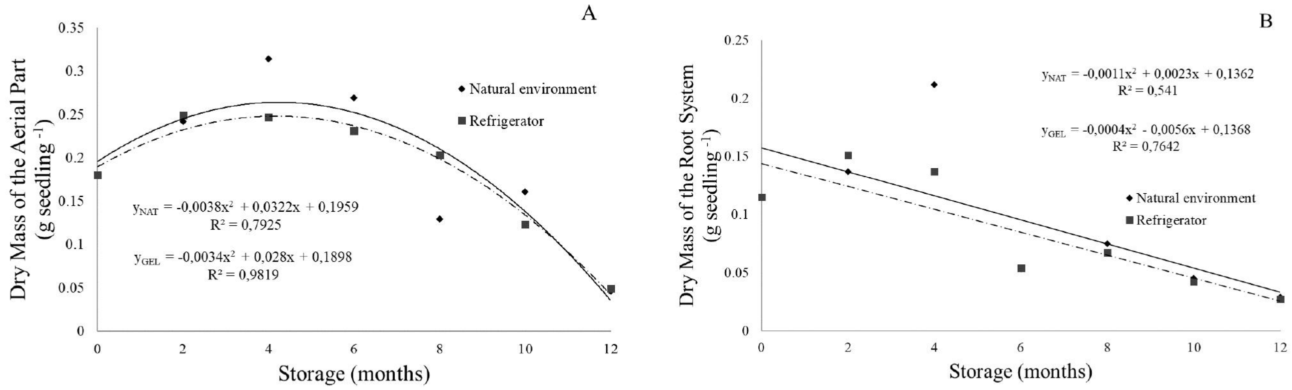


Figure 7 - Dry mass of the aerial part (A) and root system (B) of *Schinopsis brasiliensis* seedlings from diaspores stored for 12 months



A similar performance to seedling length was observed for dry mass. As indicated in Figure 7A, the seedlings from *S. brasiliensis* diaspores subjected to different environments and storage periods showed greater DMAP at four months of storage, with 0.26 and 0.25 g seedling^{-1} in seedlings from diaspores stored in natural environment and refrigerator, respectively.

The data of DMRS (Figure 7B) fitted the linear model. The dry mass decreased as the storage period increased, regardless of the storage environment. When evaluating different conditions and storage periods on the physiological quality of seeds of *Colubrina glandulosa* Perkins (Rhamnaceae), Melo Junior *et al*. (2019) observed a significant reduction in the total dry mass of seedlings from seeds stored in the laboratory for six months or longer (normal environmental conditions).

CONCLUSIONS

1. Storage for 12 months does not overcome tegument dormancy of *S. brasiliensis* diaspores;

2. Manual scarification of *S. brasiliensis* diaspores after storage in a natural environment favors seedling emergence and establishment speed;

3. Regardless of the storage condition, *S. brasiliensis* diaspores presented higher germination capacity at storage for five months;

4. Development decline of *S. brasiliensis* seedlings, regardless of the storage condition of diaspores, occurs before the loss of germination capacity.

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