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Conservation and physiological quality of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) seeds

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ABSTRACT: *Handroanthus spongiosus* (Rizzini) S. Grose is an endangered tree species. However, its seed quality, storage, and conservation strategies are issues still unexplored. This study aimed to evaluate the physiological quality of *H. spongiosus* seeds subjected to different storage times, packaging, and environments for their conservation. A completely randomized experimental design was used, in a double factorial arrangement with an additional treatment (recently-harvested seeds), consisted of five storage times (up to 24 months) and six storage conditions, combining packaging types (permeable and impermeable) and environments (room, cold chamber, freezer, and liquid nitrogen conditions). Seed germination percentage and normal seedling percentage, shoot length, root length, and root to shoot dry weight ratio were evaluated. The seed germination and normal seedling percentages of *H. spongiosus* seeds conserved under room conditions decreased over the storage time. Normal seedling percentages decreased from the 12th month of storage onwards. Low and ultralow temperatures are recommended for short and medium-term conservation of *H. spongiosus* seeds, since they did not affect the growth of seedlings.

Index terms: Caatinga, dry forest, germination, longevity, storage.

RESUMO: *Handroanthus spongiosus* (Rizzini) S. Grose é uma espécie arbórea ameaçada. No entanto, a qualidade de suas sementes, o armazenamento e as estratégias de conservação ainda são questões inexploradas. O objetivo deste trabalho foi avaliar a resposta temporal a diferentes embalagens e ambientes de armazenamento na conservação da qualidade fisiológica das sementes de *H. spongiosus.* O delineamento experimental foi inteiramente casualizado em esquema fatorial duplo com tratamento adicional (sementes recém-colhidas), considerando cinco períodos de armazenamento (até 24 meses) e seis condições de armazenamento, combinando embalagens (permeável ou impermeável) e ambientes (laboratório, câmara fria, freezer e nitrogênio líquido). Germinação, plântulas normais, comprimento da parte aérea e raiz e relação entre a massa seca de raiz e parte aérea foram avaliadas. As sementes de *H. spongiosus* mantidas em ambiente de laboratório apresentaram decréscimo na porcentagem de germinação e de plântulas normais ao longo do armazenamento. A porcentagem de plântulas normais diminuiu apenas a partir de 12 meses de armazenamento. Temperaturas baixas e ultrabaixas podem ser indicadas para a conservação da qualidade das sementes em armazenamento a curto e médio prazo, uma vez que o crescimento de plântulas não foi afetado.

Termos para indexação: Caatinga, floresta seca, germinação, longevidade, armazenamento.

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INTRODUCTION

Anthropogenic actions have contributed for the degradation of the Caatinga biome through practices characterized by the exploratory process of this region, such as the removal and burning of native vegetation and agricultural and livestock activities (Ferreira et al., 2014). In addition, many plant species have been threatened around the world due to aggression to ecosystems and impoverishment of biodiversity caused by these actions (Voronkova et al., 2018).

Thus, conservationist strategies for threatened species have been developed and applied as alternatives for ex situ conservation through seed or seedling production in different times of the year (Shen et al., 2015). Therefore, determining conservation strategies for seeds of threatened wild species is required and involves the maintenance of their viability and physiological potential during storage (Araujo et al., 2017).

The storage environment and packaging affect the maintenance of viability and vigor of seeds in the short-, medium-, and long-terms. Successfully conserving forest seeds require previous information on physiological characteristics, since seeds from different species require different conditions for conservation (Vitis et al., 2020; Walters and Pence, 2021).

Determining ideal environmental conditions, such as relative air humidity and temperature, for the conservation of seeds is needed to maintain their physiological quality during storage (Veiga-Barbosa et al., 2013; Tonetto et al., 2015). An adequate storage environment can minimize the speed deterioration, allowing the maintenance of viability of seeds for a longer period than that obtained under natural non-controlled conditions (Torres et al., 2020). In addition, initial seed water content and storage packaging affect the maintenance of the seed physiological quality due to gas exchanges between seeds and the environment (Reis et al., 2012; Lúcio et al., 2016; Gomes et al., 2018; Ribeiro et al., 2018).

The plant species *Handroanthus spongiosus* (Rizzini) S. Grose, popularly known in Brazil as *cascudo, ipê-cascudo* or *sete-cascas*, belongs to the Bignoniaceae family. It is endemic to the Caatinga biome and is classified as an endangered species, according to the official national list of threatened flora species (Lohmann et al., 2013; Lohmann, 2020).

Seeds from plants of the *Handroanthus* genus present a relatively short natural viability period, which hinders the conservation and, consequently, the production of seedlings for these species (Cabral et al., 2003). Researchers have developed and published works involving the storage of different *Handroanthus* species over the last years due to the importance of these species (Shibata et al., 2012; Abbade and Takaki, 2014; Martins and Pinto, 2014; Tonetto et al., 2015; Maciel et al., 2020; Araujo et al., 2021). However, information on longevity, storage, or conservation is not available for *H. spongiosus* seeds. Moreover, there is no information on the quality of *H. spongiosus* seeds stored under room, low, and ultralow temperatures. Thus, this study aimed to evaluate the physiological quality of *H. spongiosus* seeds subjected to different storage times, packaging, and environments for their conservation.

MATERIAL AND METHODS

Seed collection

Handroanthus spongiosus (Rizzini) S. Grose (HUEFS-259093) seeds were obtained by harvesting mature fruits (brownish) at the seed dispersion stage from nine plants in Lagoa Grande, PE, Brazil (8°34'4''S, 40°10'18''W), in December 2017. The fruits were processed to remove branches, leaves, damaged seeds, and other impurities, generating the recently-harvested seed lot.

Seed storage

The experiment was conducted in a completely randomized experimental design, in a double factorial arrangement, with an additional treatment (5×6+1). The factors consisted of storage times (6, 9, 12, 18, and 24 months) and storage environmental conditions, considering the packaging (permeable or impermeable) and environments (room, cold chamber, freezer, and liquid nitrogen conditions).

Recently-harvested seeds were placed in polyethylene bags (PB) and cotton bags (CB) for room condition storage (RC),

with mean temperature of 25 ± 4 °C and 45 ± 3% relative air humidity, or in cold chamber (CC), set to 10 ± 3 °C and 60 ± 4% relative air humidity. Seeds were placed in PB for the freezer storage (FS; -20 °C and 66% relative air humidity). The seeds stored in liquid nitrogen (LN) (-196 °C) were kept in polyethylene cryogenic tubes. Thus, six storage conditions were tested: polyethylene bag and room condition storage (PB-RC), cotton bag and room condition storage (CB-RC), polyethylene bag and cold chamber storage (PB-CC), cotton bag and cold chamber storage (CB-CC), polyethylene bag and freezer storage (FS), and cryotube and liquid nitrogen storage (LN).

The recently-harvested seeds and seeds stored for 6, 9, 12, 18, and 24 months were evaluated for physiological quality (seed water content, germination, and vigor). The seeds stored in FS and LN were subjected to slow thawing for 4 hours in a refrigerator (10 °C) and 1 hour at room temperature (Alencar et al., 2018), before evaluating the seed quality.

Seed physiological quality evaluations

The water contents of recently-harvested and stored seeds were obtained by the oven method at 105 ± 3 °C for 24 hours using two samples of 50 seeds (Brasil, 2013).

The physiological quality of recently-harvested and stored seeds were evaluated through germination tests carried out using four replications of 50 seeds for each treatment. The seeds were distributed between three Germitest paper sheets, moistened with distilled water at the proportion of 2.5-fold the dry paper weight, individually placed in polyethylene bags, and incubated in a BOD (biochemical oxygen demand) chamber at constant temperature of 25 ± 1 °C and photoperiod of 12 hours (adapted from Brasil, 2013) for 14 days. Then, the germination percentage (%G) and normal seedling percentage (%NS) were obtained, considering seeds with radicle lengths equal to or higher than 2.5 mm as germinated.

The seed vigor was evaluated by the performance of ten normal seedlings of each replication, considering the root and shoot lengths (cm) and root and shoot dry weights (mg), according to Nakagawa (2020).

Statistical analysis of the data

The data were analyzed to verify the assumptions of analysis of variance through the normality of residues and homogeneity of variances by the Shapiro-Wilk (Shapiro and Wilk, 1965) and Levene (1960) tests, respectively, at 0.05 probability level. The data did not meet the assumptions of ANOVA and it was chosen not to use the angular transformation for the dependent variable; thus, they were fitted to Generalized Linear Models (GLM). The GLM were analyzed and the significant differences within each storage time, storage condition, and variables studied were analyzed through comparisons of means pairs by the post-hoc Tukey's test at 5% significance. The means were fitted by the method of Šidák (1967). The results found for the stored and recently-harvested seeds were compared by the Dunnett test (Dunnett, 1955) at 0.05 probability level. The analyses were carried out using the R program (R Core Team, 2020).

RESULTS AND DISCUSSION

The data of germination percentage (%G) ($p = 0.0814$) and percentage of normal seedlings (%NS) ($p = 0.6037$) presented normality of residues by the Shapiro-Wilk test, but only %NS presented homogeneous variance ($p = 0.0504$) by the Levene test. The GLM showed that the interaction between the studied factors was significant for all variables, except for shoot length ($p = 0.2473$), for which only the effects of isolated factors were significant.

The recently-harvested seeds presented initial water content of 5.67%, which varied from 3.18% to 8.94% in the different storage conditions, mainly considering the packaging used (Table 1). Different seed structures presented different water levels; the water content obtained represents the mean of the whole seed (McDonald et al., 1994; Bewley et al., 2013). Water levels lower than 10% represent the water responsible for maintaining the structural integrity and property of macromolecules and are affected by the seed chemical composition and temperature (Vertucci, 1993; Bewley et al., 2013; Marcos-Filho, 2015). Oscillations in these levels depend on the species and storage environment, which can affect the cell physiological status, including the conformation of proteins and organic compounds consisted

of polymers of amino acids, which are water sorption sites (Bewley et al., 2013; Marcos-Filho, 2015).

Seeds conserved under CB-RC presented a lower water content than the recently-harvested seed lot up to the 12nd month, followed by a significant increase of 3.27% in the 18th, and 0.65% in the 24th month of storage, different from the polyethylene bag packaging and room condition storage (PB-RC), which presented a 1.34% decrease in these two last times. In the cold chamber (CC) and freezer storage (FS) environments the seed water content decreased only in the 20th month; the cotton bag packaging and cold chamber storage (CB-CC) stood out with a 2.49% decrease. However, the liquid nitrogen storage (LN) kept the water content above that of the recently-harvested seed lot over the storage time (Table 1).

Regarding the physiological quality of seeds, the cryostorage in liquid nitrogen (LN) was the only condition that prevented the deterioration of *H. spongiosus* seeds up to 24 months, presenting similar results or superior results to those of recently-harvested seeds. However, the storage in permeable packaging and room temperature (CB-RC) presented the greatest seed deterioration after 24 months, with lower seed germination and seedling performance (Table 2). This is due the non-controlled conditions in the CB-RC, with seeds exposed to oscillations of temperature and relative air humidity, that can increase deterioration and loss of integrity of membranes, compromising RNA and protein syntheses, causing degradation of RNA and even disintegration of cell nuclei (Corbineau, 2012; Jyoti and Malik, 2013; Demidchik, 2015; Capilheira et al., 2019).

Table 1. Water contents (%) in *Handroanthus spongiosus* seeds subjected to different storage conditions and times.

RHS	5.67	Storage time (months)				
Storage conditions		6	9	12	18	24
CB-RC		*4.90	*4.84	5.59	*8.94	*6.32
PB-RC		5.49	5.09	*4.98	*4.33	*4.33
CB-CC		*6.52	*6.96	*6.32	*8.75	*3.18
PB-CC		5.91	*6.38	*6.37	*8.84	5.35
FS		*6.34	5.99	5.59	*7.59	5.11
LN		*7.06	*8.52	*7.82	*8.24	*7.18

*Seed water contents significantly different from recently-harvested seeds (RHS) by the Dunnett test at 0.05 probability level. CB-RC = seeds placed in cotton bags and stored under room conditions; PB-RC = seeds placed in polyethylene bags and stored under room conditions; CB-CC = seeds placed in cotton bags and stored in cold chamber; PB-CC = seeds placed in polyethylene bags and stored in cold chamber; FS = seeds stored in a freezer; LN = seeds stored in liquid nitrogen (-196 °C); RHS = recently-harvested seeds. n= 100.

Table 2. Physiological quality of *Handroanthus spongiosus* seeds subjected to different storage conditions and times.

Seed germination (%)							
Storage times (months)		Storage conditions					
		CB-RC	PB-RC	CB-CC	PB-CC	FS	LN
0	90						
6		*73.5 aB	87.5 aA	88.0 aA	86.0 abA	86.0 bA	91.0 aA
9		80.0 aB	87.0 aA	90.0 aA	91.5 aA	91.5 aA	88.0 bA
12		*49.5 bC	*78.0 aB	89.5 aA	90.5 aA	90.5 aA	87.0 bA
18		*22.5 cC	*46.0 bB	87.0 aA	94.0 aA	94.0 aA	92.0 aA
24		*4.0 dD	*32.0 cC	*64.0 bB	*76.5 bB	*76.5 cB	91.5 aA
CV%		18.1					

Continue...

Table 2. Continuation.

Storage times (months)		Seed germination (%)					
		Normal seedlings (%)					
0	73.5						
6		*28.0 bC	68.0 abB	82.5 aA	80.5 aA	67.0 bB	75.0 aA
9		67.0 aB	79.5 aA	80.0 aA	84.0 aA	*86.5 aA	75.0 aB
12		*20.0 bE	*59.5 bC	80.5 aA	83.5 aA	77.5 abA	74.0 aA
18		*10.0 cC	*31.0 cB	71.5 abA	80.0 aA	76.0 abA	75.5 aA
24		*2.0 dD	*18.5 dC	*46.0 cB	*58.5 bB	78.0 abA	74.0 aA
	CV%			19.8			
		Shoot length (cm)					
0	2.42						
6		2.72 aA	2.84 aA	2.80 aA	*3.50 aA	2.85 aA	2.90 aA
9		*1.83 abA	*1.89 abA	2.33 aA	2.23 aA	2.12 aA	2.54 aA
12		*1.86 abA	*1.75 abA	2.75 aA	2.16 aA	2.19 aA	2.26 abA
18		*1.56 bcA	*1.90 abA	2.48 aA	2.25 aA	2.23 aA	2.58 abA
24		*1.02 cB	*1.27 bB	2.27 aA	2.20 aA	2.42 aA	2.46 abA
	CV%			23.4			
		Main root length (cm)					
0	2.73						
6		*3.00 aB	*3.56 aA	*3.80 aA	*3.23 aB	2.97 aB	*3.24 aB
9		2.12 aB	2.32 bB	2.51 aB	2.84 aA	2.85 aA	2.51 aB
12		*1.85 bB	2.03 bB	2.67 aA	2.60 aA	2.84 aA	2.66 aA
18		*1.11 bC	*1.79 bC	2.69 aB	*3.63 aA	*3.63 aA	*3.64 aA
24		*1.02 cB	*1.00 cB	2.90 aA	*4.34 aA	*3.26 aA	*3.45 aA
	CV%			28.7			
		Root to shoot dry weight ratio					
0	0.22						
6		*3.04 aA	0.52 aC	*1.81 aB	*1.54 bB	0.16 cC	0.20 aC
9		0.19 of	0.25 bA	0.26 bA	0.29 cA	0.27 bA	0.21 aA
12		*1.57 bB	0.24 bC	0.25 bC	*2.04 aA	0.24 bC	0.28 aC
18		*0.64 cA	0.16 bA	0.26 bA	0.28 cA	0.26 bA	0.24 aA
24		0.25 dB	*0.07 dC	0.19 cB	0.26 cB	*1.04 aA	0.26 aB
	CV%			29.3			

Means fitted by the Šidák method followed by different letters lowercase in the columns are significantly different from each other and means followed by the same uppercase letters in the rows are not statistically different from each other by the Tukey's test at 0.05 of probability. * Root to shoot dry weight ratio significantly different by the Dunnett test at 0.05 probability level. CB-RC = seeds placed in cotton bags and stored under room conditions; PB-RC = seeds placed in polyethylene bags and stored under room conditions; CB-CC = seeds placed in cotton bags and stored in cold chamber; PB-CC = seeds placed in polyethylene bags and stored in cold chamber; FS = seeds stored in a freezer; LN = seeds stored in liquid nitrogen (-196 °C).

After 12 months of storage, decreases in %G and %NS of seeds stored in permeable packaging was higher than 50%. However, the packaging did not affect the quality of seeds stored in cold chamber (Table 2).

Seeds conserved under low temperatures in CC and FS presented germinations above 80% until the 18th month of storage, and did not differ from recently-harvested seeds. In addition, the %NS and performance (shoot and root

lengths) of seedlings from seeds stored in these conditions were statistically equal to or better than those of recently-harvested seeds (Table 2).

The results found for root to shoot dry weight ratio, calculated using the dry biomass of seedlings, where similar those found for the other evaluated variables. The root to shoot dry weight ratio of seedlings from seeds stored in LN for up to 24 months were similar to that of recently-harvested seeds and, according to the seed deterioration, it was higher, denoting a higher investment in roots by the seedlings. The cold storage (CC and FS) presented intermediate responses and the CB-RC condition resulted in higher deterioration of seeds and higher root to shoot dry weight ratio than the other treatments (Table 2).

Seeds can be classified by their tolerance to desiccation and low-temperature storage into three groups, orthodox, intermediate, and recalcitrant (Walters, 2015). Seeds of several species of the *Handroanthus* and *Tabebuia* genera are orthodox, they can disperse and be dried to water contents from 14.17% to 5.6% (Martins et al., 2009a; Silva et al., 2011; Guedes et al., 2012; Martins and Pinto, 2014; Gonçalves et al., 2015; Alencar et al., 2018), which allows their storage under low (-20 °C) and ultralow (-196 °C) temperatures, maintaining them viable for many years (Walters et al., 2013; Ballesteros et al., 2021).

Seed water content is one of the most important factors for seed storage in LN; high water contents in the cells can disrupt membranes during freezing (Panis et al., 2005). *H. spongiosus* seeds with water content of 7.18% stored in liquid nitrogen for two years maintained a high physiological quality, which was a similar result to that of recently-harvested seeds (Table 2). This denotes an advantage of this species, since not all seeds tolerate temperatures below zero, as found for *Handroanthus impetiginosus* (Mart. ex DC) (= *T. impetiginous*) seeds with 4.2% water content stored in liquid nitrogen, whose physiological quality decrease after 360 days (Martins et al. 2009b).

Seeds of some species of the Caatinga biome tolerate liquid nitrogen storage for periods above 24 months without losing their physiological quality, as is the case of *Amburana cearensis* (Allemão) AC Sm. (Araujo et al., 2017). This is probably because ultralow temperatures practically cease cell metabolism (Garcia et al., 2014). The storage of seeds in LN is an alternative for medium- and long-term conservation, since it is possible to maintain the seed viability and vigor for several years under low temperatures (-196 °C), although this process has a high cost (Kaviani, 2011; Walters et al., 2013).

Despite the water content of *H. spongiosus* seeds conserved in LN increase after six months, no loss in seed or seedling quality was found. This increase in seed water content after the storage period in low temperatures (CC, FS, and LN) was caused by the water vapor condensation process that occurs between the seed contact surface (lower temperature) and the surrounding air at thawing (higher temperature) when the seeds were removed from the cold storage (Delouche, 1968). These seeds are hygroscopic, they absorb or lose water to the environment until a balance is established between the seed water content and relative air humidity (Delouche, 1968; Oliveira et al., 2014); the period between the thawing and weighing of seeds was enough for them to absorb the water.

A test for measuring water content of cryo-conserved seeds was carried out using two replications of 50 seeds, which were directly placed in the oven after the LN or subjected to a thawing process. The water contents were similar to those obtained during the storage: 7.15% for seeds directly placed in the oven and 7.24% for those subjected to a thawing process, confirming that they absorb water soon after their removal from the LN.

Despite some species of the Caatinga biome conserve their seed physiological quality for more than 12 months under room conditions (>25 °C) (Lúcio et al., 2016; Gomes et al., 2018; Ribeiro et al., 2018), in general, storing under temperatures above 20 °C and relative air humidity higher than 70% are not recommended, as they compromise the seed physiological quality and promote the action of microorganisms and insects (Carvalho and Nakagawa, 2012). The combination of high temperatures with great water contents (usually above 12%) accelerates cell respiration, which leads to the consumption of the reserve material, oxidation of cell membranes, and degeneration of biological systems. Thus, the seeds rapidly lose their vigor and viability (Smaniotto et al., 2014).

The storage of seeds under room conditions (RC) presented the lowest seedling sizes, regardless of the type of

packaging, denoting that this environment favored the maintenance of their respiratory metabolism and consumption of reserves, affecting the vigor and formation of seedlings (Dias et al., 2016; Araujo et al., 2021). This combination of factors involving continuous and not controlled vapor exchange between the medium and the seed with high temperatures can increase the respiratory rate and affect the development of structures and biomass allocation, even when the water contents do not oscillate. Similar results were found for *H. chrysotrichus* seeds*,* which could not be stored at room temperature for periods longer than 30 days, whereas *H. impetiginosus* and *Handroanthus serratifolius* (Vahl) S.Grose seeds did not germinate after four and nine months of storage, respectively (Silva et al., 2011; Maciel et al., 2020; Araujo et al., 2021).

Handroanthus heptaphyllus (Vell.) Mattos and *H. impetiginosus* seeds presented germination above 70% after 6 months of storage in CC and refrigerator, respectively (Maciel et al., 2020; Araujo et al., 2021), and *Handroanthus chrysotrichus* (Mart. ex dc.) stored in FS presented 54% germination after 10 months (Tonetto et al., 2015). *H. spongiosus* seeds presented germination of approximately 90% when stored in CC and FS after 12 months (Table 2), higher than those obtained for other species of the same genus that present very similar morphological characteristics. These environments (CC and FS) can be used by seedling and plant growers as viable strategies for ex situ conservation of seeds, since they are more accessible.

The root system can be significantly compromised depending on the storage conditions and time, with negative consequences to seed quality and seedling vigor (Table 2). It was reported by Mucha et al. (2015), who found that the seed storage temperature affected the root anatomy of *Populus nigra* L. plants and reported that the storage at high temperatures decreased the proportion of roots with absorptive function (with primary development). Seedlings with higher root systems can explore a greater soil volume and have greater potential to absorb water and promote nutrient cycling and absorption (Finér et al., 2007; Betegón-Putze et al., 2019; Thorup-Kristensen et al., 2020).

The *H. spongiosus* seedlings presented higher dry matter allocation in the shoots during the post-seeding development. This can be attributed to higher investment of seedlings in thin roots at this initial stage of development as a strategy to explore a greater volume of the substrate and maximize water absorption for their development (Gransee and Führs, 2013; Yuan et al., 2016). However, these thinner roots little contribute to the root to shoot dry weight ratio. Species adapted to seasonally dry environments present a genetic trend of allocating greater biomass in the root system as a form to reach deeper soil waters faster and efficiently while using the variable water from rainfall events (Markesteijn and Poorter, 2009; Tomlinson et al., 2012; Qi et al., 2019), but it was not found for *H. spongiosus* at the seedling stage.

Seeds with low water content (lower than 10%) placed in impermeable packaging and stored at low temperatures (< 10 °C) presented higher longevity, as also found for *Tabebuia aurea* (Silva Manso) Benth & Hook ex S. Moore, *Tabebuia caraiba* (Mart.) Bureau, *H. chrysotrichus* (= *T. chrysotricha*), *H. impetiginosus* (= *T. impetiginous*), and *Handroanthus umbellatus* (Sond.) Mattos (Martins et al., 2009a; Guedes et al., 2012; Martins and Pinto, 2014; Neves et al., 2014; Araujo et al., 2021). In these conditions, there is a resistance of the packaging to water vapor exchanges between the seeds and the medium, and a low promotion of development of microorganisms that produce heat through their many metabolic reactions, thus avoiding energetic losses (Cardoso et al., 2012; Lopes and Lima, 2015).

The conservation of physiological quality of *H. spongiosus* seeds during the storage is connected to the conditions used; environments with low temperatures can conserve seed viability and vigor for longer periods. This information could be used to subsidize the development of appropriate strategies for ex situ seed conservation for *H. spongiosus* and other related species from different origins.

CONCLUSION

Storing *Handroanthus spongiosus* seeds under room conditions is not recommended, since it causes losses in seed germination and vigor. The most adequate conditions for their conservation are provided by cold chamber, freezer, and

liquid nitrogen environments. The use of impermeable packaging and low temperatures is the most indicated method for the maintenance of the physiological quality of *H. spongiosus* seeds.

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