EX SITU SEED CONSERVATION OF BRAZILIAN CACTI

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ABSTRACT – Evaluation of the seed germination is an important aspect to the determination of seed longevity and, consequently, plant conservation. The aim of the present study was to analyze the effect of storage time on seed germination for eight species of Cactaceae. Seeds placed in cold storage (7 ± 2°C) in periods ranging from 24 to 30 months. The effect of storage time was analyzed through a comparison of stored seeds and recently collected seeds that have been evaluated before storage. Throughout the storage period, seeds from *Pilosocereus pachycladus* subsp. *pachycladus* and *Pilosocereus tuberculatus* maintained germinability similar to that of recently collected seeds. Germinability was reduced for seeds from *Cereus albicaulis*, *Cereus jamacaru* subsp. *jamacaru* and *Pilosocereus pachycladus* subsp. *pernambucoensis,* but the seed germination remained above 50%. Drastic reductions in germinability occurred after storage for seeds from *Cereus fernambucensis* subsp. *fernambucensis*, *Pilosocereus chrysostele* and *Pilosocereus gounellei* subsp. *gounellei*. No significant differences in the other germination variables were found for any of the species studied. Based on the present findings, the *ex situ* conservation of germplasm through a seed bank in cold storage is an effective method for the conservation of seeds, as the majority of seeds maintained viability greater than de 50% after the storage period.
KEYWORDS: *Cactaceae, germinability, seed bank, storage, viability.*

CONSERVAÇÃO *EX SITU* DE SEMENTES DE CACTOS DO BRASIL

RESUMO – A avaliação da germinação de sementes é um importante aspecto para a determinação da longevidade da semente e, consequentemente, para a conservação das plantas. O objetivo do presente estudo foi analisar o efeito do tempo de armazenamento na germinação de oito espécies de Cactaceae. As sementes foram armazenadas em câmaras frias (7 ± 2°C) em períodos que variaram de 24 a 30 meses. O efeito do tempo de armazenamento foi analisado através da comparação das sementes armazenadas e sementes recém-coletadas avaliadas antes do armazenamento. Durante todo período de armazenamento, as sementes de *Pilosocereus pachycladus* subsp. *pachycladus* e *Pilosocereus tuberculatus* mantiveram a germinabilidade similar às sementes recém coletadas. A germinabilidade foi reduzida em sementes de *Cereus albicaulis*, *Cereus jamacaru* subsp. *jamacaru* e *Pilosocereus pachycladus* subsp. *pernambucoensis,* mas a germinação das sementes permaneceu acima de 50%. Reduções drásticas na germinabilidade ocorreram após o armazenamento de sementes de *Cereus fernambucensis* subsp. *fernambucensis*, *Pilosocereus chrysostele* e *Pilosocereus gounellei* subsp. *gounellei*. Não houve diferença significativa nos demais parâmetros de germinação calculados para todas as espécies estudadas. Com base nesses resultados, a conservação *ex situ* de germoplasma por meio de um banco de sementes armazenado em câmara fria é um método eficaz para a conservação de sementes, como a maioria das sementes manteve a germinabilidade maior do que de 50% após o período de armazenamento.
PALAVRAS-CHAVE: *Cactaceae, germinabilidade, banco de sementes, armazenamento, viabilidade.*

CONSERVACIÓN EX SITU DE SEMILLAS DE CACTUS DE BRASIL

RESUMEN – La evaluación de la germinación de las semillas es un aspecto importante para determinar la longevidad de las semillas y por lo tanto para el mantenimiento de las plantas en el medio ambiente. En este estudio el objetivo fue analizar el efecto del tiempo de almacenamiento sobre la germinación de las semillas de ocho especies de Cactaceae. Las semillas se almacenaron en cámara de frío (7 ± 2°C) durante periodos de 24 a 30 meses. El efecto del tiempo de almacenamiento se analizó mediante la comparación de las semillas almacenadas y semillas recién colectadas evaluadas antes del almacenamiento. Durante todo el período de almacenamiento, la germinabilidad de las semillas de *Pilosocereus pachycladus* subsp. *pachycladus* y *Pilosocereus tuberculatus* se mantuvo similar a la germinabilidad de las semillas recién colectadas. Se observó una reducción de la germinabilidade de las semillas de *Cereus albicaulis*, *Cereus jamacaru* subsp. *jamacaru* y *Pilosocereus pachycladus* subsp. *pernambucoensis*, pero la germinación de las semillas de estas especies se mantuvo por encima del 50%. Sin embargo, se observó reducciones drásticas en la germinación después del período de almacenamiento de las semillas de *Cereus fernambucensis* subsp. *fernambucensis*, *Pilosocereus chrysostele* y *Pilosocereus gounellei* subsp. *gounellei*. No se observó diferencia significativa en los otros parámetros de germinación calculados para todas las especies. Basándose en estos resultados, la conservación *ex situ* del germoplasma por medio de un banco de semillas almacenadas en una cámara de frío es un método eficaz para la preservación de las semillas, ya que la germinabilidad de la mayoría de las especies se mantuvo mayor que 50% después del período de almacenamiento.
PALABRAS CLAVE: *Cactaceae, germinabilidad, banco de semillas, almacenamiento, viabilidad.*

INTRODUCTION

The *ex situ* conservation of plants regards the maintenance of plants outside their natural environment and is one of a set of actions employed for the management of genetic resources of different species. Successful conservation outside the original environment depends on the correct processing of the material and is characterized by the evaluation and documentation of the collection being conserved (Valois, 1996). According to Walter (2010), the incorporation of germplasm in botanical collections and sample exchanges are key elements in the conservation of plant genetic resources and have been widely used when it is not possible to conserve plants in their natural environment.

As ecosystems face climate change, strong pressure from human activities, genetic erosion and the loss of diversity, the conservation of genetic resources should be seen as a priority on a worldwide scale and an important issue for study (Draper *et al.*, 2004). Different *ex situ* conservation methods include *in vivo* banks, seed banks and *in vitro* banks, which can be used for the most diverse families of plants, including Cactaceae (Assis *et al.*, 2011). The aim of conserving seed banks in cold storage is to preserve the physical and physiological quality of seeds. When stored correctly, seeds maintain viability for a long period of time and can subsequently be used for different purposes, such as studies on species morphology and physiology, genetic diversity, reforestation and the recovery of degraded areas. Moreover, this conservation method provides information on the period in which seeds remain viable to ensure the natural regeneration of environments.

When used for any purpose, stored seeds must maintain their germination potential, which is represented by the percentage of seeds that germinate in a given period of time and under particular storage conditions (Draper *et al.*, 2004). Seeds from Cactaceae exhibit considerable diversity regarding shape, size and structure and response differently to the effects of storage time when conserved in seed banks (Assis *et al.*, 2011). Moreover, considering the current threats and pressures to natural populations of cacti due to human activities, such as deforestation, habitat fragmentation, mining and the illegal collection and trade of these plants (Zappi *et al.*, 2011), the characterization of the seeds of cacti species (germinability and the maintenance of viability) is essential to the management of natural resources and the conservation of these native species (Assis *et al.*, 2011).

According to Assis *et al.* (2011), the lack of knowledge on seed biology of native species of Cactaceae is one of the major difficulties in the conservation of cactus seeds in germplasm banks and cultivation (seedling production). Studies on the reduction in seed viability over time can provide important information to the conservation and restoration of seed banks (Oliveira *et al.*, 1984). Thus, the aim of the present study was analyze the effect of *ex situ* storage time on the seed germination from eight species of Cactaceae that occur in northeastern Brazil.

Material and Methods

Species studied

The family Cactaceae is represented in Brazil by 260 species distributed throughout all ecosystems in the country. Approximately 90 species of cactus are found in the *Caatinga* ecosystem, a Tropical Dry Forest that dominates a large portion of the northeastern region of Brazil (Taylor *et al.*, 2015). The present study was conducted with eight species of the family Cactaceae belonging to the genera *Cerus* Mill. and *Pilosocereus* Byles & Rowley. In Brazil, the genus *Cereus* is represented by 15 species distributed throughout the country. The three species with the largest geographic distribution in the northeastern region are *Cereus albicaulis* (Britton & Rose) Luetzelb., *Cereus fernambucensis* Lem. subsp. *fernambucensis* and *Cereus jamacaru* DC. subsp. *jamacaru* (Meiado *et al.*, 2012a), the seeds of which were evaluated in the present study. Species of this genus are characterized by salient ribs, strong, pungent spines generally of a brownish color and large, attractive, white, nocturnal flowers that can reach up to 20 cm (Cavalcante *et al.*, 2013). The genus *Pilosocereus* is represented by 29 species distributed throughout Brazil, except the southern region of the country (Taylor *et al.*, 2015). This genus is the most diverse among Brazilian cacti and many species have similar morphology, which hinders their identification (Cavalcante *et al.*, 2013). The seeds of five species of this genus that are widely distributed in northeastern Brazil were analyzed in the present study: *Pilosocereus chrysostele* (Vaupel) Byles & Rowley, *Pilosocereus gounellei* (F.A.C. Weber) Byles & Rowley subsp. *gounellei*, *Pilosocereus pachycladus* F. Ritter subsp. *pachycladus*, *Pilosocereus pachycladus* F. Ritter subsp. *pernambucoensis* (F. Ritter) Zappi and *Pilosocereus tuberculatus* (Werderm.) Byles & Rowley (Meiado *et al.*, 2012a).

The seeds used in this study were collected between 2009 and 2012 in different areas of northeastern Brazil **(Table 1)** through expeditions to priority conservation areas. We sent the seeds to the Seed Laboratory of the Reference Center for the Recovery of Degraded Areas of the Caatinga of the Federal University of the São Francisco Valley for processing and cold storage (7 ± 2°C) for periods ranging from 24 to 30 months, depending on the species **(Table 2)**. Moisture content of the seed was uniformly low, averaging 8.0%. The seeds were stored in paper bags, and moisture content of the seed did not change after the storage period.

Seed germination test

To evaluate the effect of storage period on seed germination, we distributed 100 seeds from each species among four replicates of 25 seeds each put to germinate in Petri dishes measuring 5 cm in diameter containing filter paper moistened with 3 mL of distilled water. The experiments were conducted in germination chambers with the ideal photoperiod and temperature corresponding to each species for a period of 30 days (Meiado, 2012; Meiado *et al.*, 2015). This procedure was also performed with recently collected seeds from the same seed lot prior to storage and repeated after the storage period.

Germination parameters and statistical analysis

The emergence of the radicle was the criterion for the determination of germination (Meiado *et al.*, 2010, 2015). For each treatment, germinability (%), mean germination time (days) [$t = \sum ni.ti / \sum ni$, in which ti is the time since the onset of the experiment to the nth observation (days) and ni is the number of seed germinated in time i], emergence rate index (ERI) adapted from Maguire (1962) [$ERI = (G_1/N_1) + (G_2/N_2) + \ldots + (G_n/N_n)$, in which G_1, G_2 and G_n correspond to the number of seeds germinated at the first, second and last count, respectively, and N_1, N_2 and N_n represent the number of days elapsed to the first, second and last count, respectively] and synchronization index [$E = - \sum fi.\log_2 fi$, in which fi is the relative germination rate (*i.e.*, the proportion of seeds germinated in a

TABLE 1. Photoblastism, optimum temperature for seed germination (°C) and collection site of seeds of eight species of Brazilian cacti.

Species	Photoblastism	Temperature (°C)	Collection site of seeds (geographic coordinates)
C. albicaulis	Positive	30	Sento Sé, Bahia (09°50'43.62''S, 41°05'27.73''W)
C. fernambucensis ssp. *fernambucensis*	Positive	25	Aracaju, Sergipe (11°01'30.80''S, 37°04'37.80''W)
C. jamacaru ssp. *jamacaru*	Positive	30	Parnamirim, Pernambuco (08°09'31.27''S, 39°35'55.66''W)
P. chrysostele	Positive	30	Serra Talhada, Pernambuco (07°59'09.00''S, 38°17'45.00''W)
P. gounellei ssp. *gounellei*	Positive	30	Parnamirim, Pernambuco (08°04'26.30''S, 39°33'51.10''W)
P. pachycladus ssp. *pachycladus*	Positive	25	Tanquinho, Bahia (11°57'38.66''S, 39°08'24.60''W)
P. pachycladus ssp. *pernambucoensis*	Positive	30	Parnamirim, Pernambuco (08°05'26.10''S, 39°34'42.00''W)
P. tuberculatus	Positive	30	Buíque, Pernambuco (08°37'24.80''S, 37°09'23.00''W)

time interval)] were calculated based on Ranal and Santana (2006).

All germination variables were analyzed using the Student's t-test. The Shapiro-Wilk and Levene tests were used to determine the normality of the data and equal variance, respectively (Zar, 2010). All statistical analyses were performed with the aid of the STATISTICA 10.0 program (Stat Soft, 2012), with the level of significance set to 5% ($P < 0.05$).

RESULTS

The seeds from the eight species of cactus in cold storage remained intact with no signs of deterioration after *ex situ* conservation. However, three different types of germination responses were found among the seeds stored for 24 months or more. The first type of germination response was found in *P. pachycladus* subsp. *pachycladus* and *P. tuberculatus*, which maintained germinability similar to recently collected seeds after the storage period, with no significant differences ($t = 1.0136$, $df = 6$, $P = 0.3498$ and $t = 0.1924$, $df = 6$, $P = 0.8537$, respectively, **Figure 1**).

Germinability was reduced for seeds from *C. albicaulis*, *C. jamacaru* subsp. *jamacaru* and *P. pachycladus* subsp. *pernambucoensis* ($t = 9.1088$, $df = 6$, $P < 0.0001$; $t = 11.0000$, $df = 6$, $P < 0.0001$; and $t = 3.8311$, $df = 6$, $P < 0.0001$, respectively), but the germination rate remained above 50% **(Figure 1)**, which is considered satisfactory for the storage time (≥ 24 months) and represents the second type of germination response found in the present study. Drastic reductions in germinability (< 20%) occurred after storage for seeds from *C. fernambucensis* subsp. *fernambucensis*, *P. chrysostele* and *P. gounellei* subsp. *gounellei* ($t = 14.1000$, $df = 6$, $P < 0.0001$; $t = 19.5500$, $df = 6$, $P < 0.0001$; and $t = 12.1243$, $df = 6$, $P < 0.0001$, respectively), representing the third type of response **(Figure 1)**.

Mean germination time ranged from five to ten days, with no significant differences between the stored seeds of any of the plants and recently collected seeds **(Table 2)**. Moreover, we found no significant differences regarding the emergence rate index in any of the species studied. This indicates that, although germinability was reduced in some species, the other germination variables remained similar after the period of cold storage **(Table 2)**.

TABLE 2. Mean germination time (MGT – days), emergence rate index (ERI) and synchronization index (E) of seed germination of eight species of Brazilian cacti submitted to cold storage (7 ± 2°C) at different time intervals (months).

Species	Storage (Months)	MGT		ERI		E	
		Recently collected seeds	Stored seeds	Recently collected seeds	Stored seeds	Recently collected seeds	Stored seeds
C. albicaulis	24	9.40 ± 0.46	9.95 ± 0.70	3.78 ± 0.73	3.57 ± 0.77	3.38 ± 0.63	3.14 ± 0.54
C. fernambucensis ssp. *fernambucensis*	24	9.75 ± 0.21	9.47 ± 0.92	2.60 ± 0.44	2.49 ± 0.50	2.50 ± 0.41	2.38 ± 0.47
C. jamacaru ssp. *jamacaru*	30	9.89 ± 0.15	9.81 ± 1.07	4.49 ± 0.67	4.62 ± 0.60	3.54 ± 0.49	3.58 ± 0.23
P. chrysostele	25	5.40 ± 0.97	5.75 ± 1.70	1.92 ± 0.32	1.42 ± 1.39	1.04 ± 0.36	0.69 ± 0.42
P. gounellei ssp. *gounellei*	27	8.17 ± 0.20	8.00 ± 0.49	2.74 ± 0.23	2.37 ± 1.04	2.02 ± 0.30	1.83 ± 0.58
P. pachycladus ssp. *pachycladus*	30	10.05 ± 0.65	9.95 ± 0.83	6.05 ± 0.66	6.39 ± 1.01	4.82 ± 0.19	4.63 ± 0.34
P. pachycladus ssp. *pernambucoensis*	26	9.35 ± 0.32	9.30 ± 0.46	5.42 ± 0.37	5.04 ± 0.87	3.90 ± 0.04	3.78 ± 0.56
P. tuberculatus	24	9.18 ± 0.31	9.61 ± 1.00	8.31 ± 1.18	7.86 ± 0.90	4.56 ± 0.38	4.67 ± 0.23

DISCUSSION

The present findings demonstrate that the majority of seeds from the species of cactus studied herein can undergo cold storage, which represents an important strategy for the *ex situ* conservation of cacti that occur in Brazilian ecosystems. According to Rojas-Aréchiga and Vázquez-Yanes (2000), few studies have evaluated the longevity of Cactaceae seeds. Although some older studies state that the majority of cactus seeds exhibit an orthodox behavior that ensures storage from many years (Fearn, 1977), most recent studies have evaluated germinability in periods of up to only seven months (Veiga-Barbosa *et al.*, 2010; Abud *et al.*, 2012; Goodman *et al.*, 2012; Salazar *et al.*, 2013), which may not be enough time to

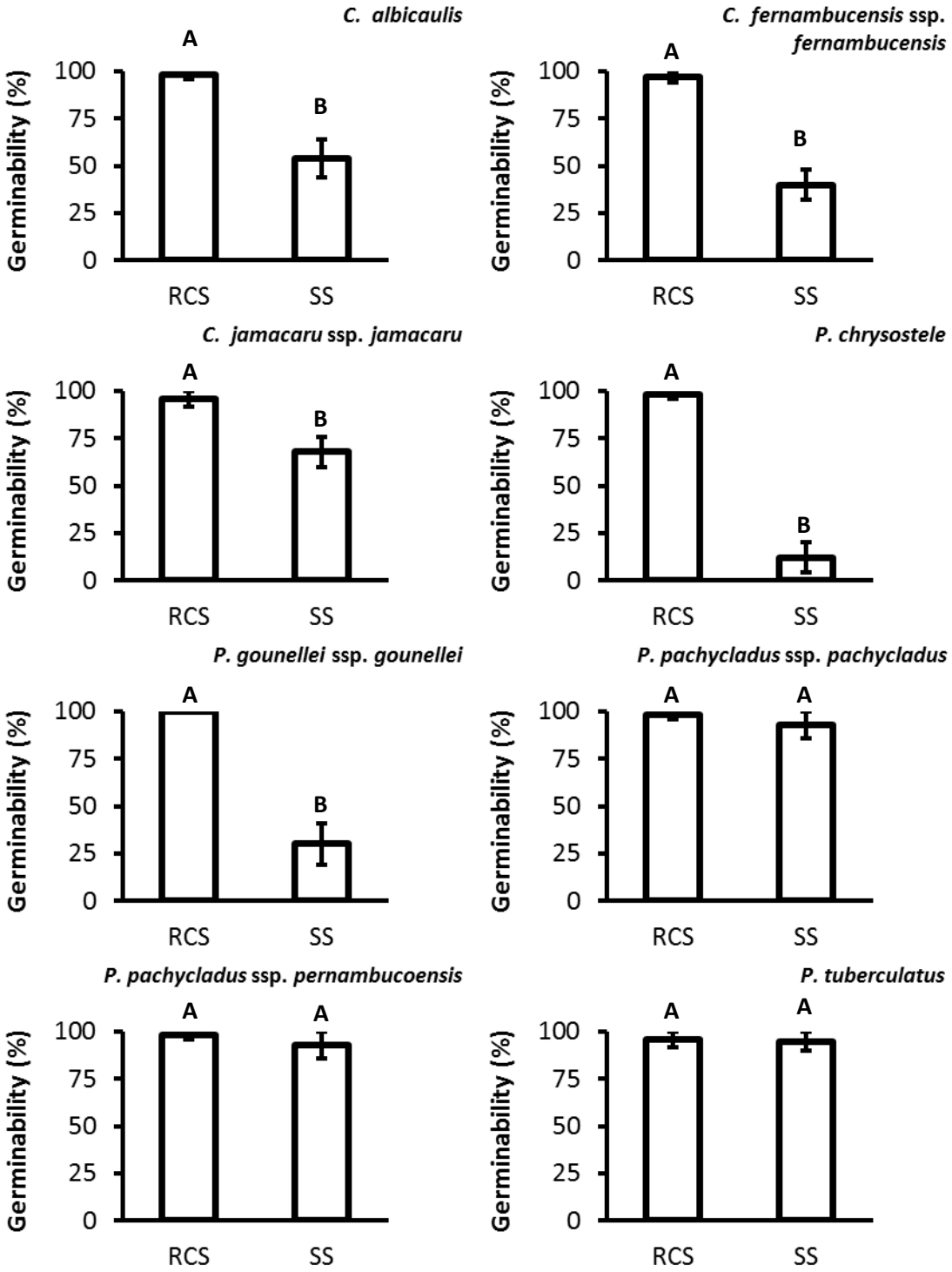

FIGURE 1. Germinability (%) of eight species of Brazilian cacti submitted to cold storage (7 ± 2°C) at different time intervals. RCS: recently collected seeds. SS: Stored seeds. Different letters indicate significant differences at $P < 0.05$ (Student's t-test).

determine the loss of viability of stored seeds and could compromise the interpretation of the results regarding seed longevity. The ideal conditions for long-term storage remain unknown for the majority of seeds from cacti in Brazil (Meiado *et al.*, 2012b). However, the viability of seeds from species of the genera *Frailea* Britton & Rose and *Gymnocalycium* Pfeiff. ex Mittler is known to be lost rather quickly (Rojas-Aréchiga and Vázquez-Yanes, 2000) and the viability of seeds from some species of the genus *Pilosocereus* is compromised by the adherence of the funicular pulp to the seed (Meiado *et al.*, 2012b).

Among the eight species of cacti analyzed, five exhibited germination greater than 50% after a storage period of 24 months or more. In contrast, *C. fernambucensis* subsp. *fernambucensis* and *P. gounellei* subsp. *gounellei* had germinability lower than 20% after the period of cold storage. The seeds

from all species analyzed maintained their physical and physiological integrity in cold storage. Even when the storage period had passed, the majority of species exhibited viable seeds suitable for planting. According to Abud *et al.* (2012), seed conservation is favored by cold storage, which allows controlling the temperature and humidity, thereby reducing the degree of deterioration of the reserve compounds found in seeds.

Besides this type of *ex situ* conservation, alternatives for the conservation of plant genetic resources have been tested on species of the family Cactaceae, such as cryopreservation of seeds (Veiga-Barbosa *et al.*, 2010; Meiado *et al.*, 2012b, Marchi *et al.*, 2013). According to Veiga-Barbosa *et al.* (2010), different species of cactus, some of which are endangered, such as *Melocactus paucispinus* Heimen & R.J. Paul, maintain seed viability for up to 120 days when stored at a temperature of -196°C. Other species, such as *Melocactus albicephalus* Buining & Brederoo, undergo a significant reduction in germinability, but with rates greater than 50% when stored in liquid nitrogen for the same period of time. Moreover, the germinability of seeds from some endangered species, such as *Discocactus zehntneri* Britton & Rose subsp. *zehntneri*, can increase significantly after 30 days of storage in liquid nitrogen (Marchi *et al.*, 2013).

According to Rojas-Aréchiga and Vázquez-Yanes (2000), *ex situ* conservation through a seed bank is an effective way to conserve endangered species by maintaining seeds viable for a longer time under controlled conditions. This type of *ex situ* conservation has been evaluated for other endangered species of the genus *Pilosocereus*, such as *Pilosocereus robinii* (Lem.) Byles & G.D. Rowley, which maintains seed viability after 28 weeks in cold storage (Salazar *et al.*, 2013).

Knowledge on the biology of seeds from native species of Cactaceae through physical and physiological characterization with the evaluation of germinability and longevity is essential to establishing the adequate management of natural resources, genetic resources and the conservation of species that can be used in ecological restoration programs. Thus, seed banks are a viable tool for the long-term conservation of these species of cactus by allowing the reintroduction of plants to priority areas that need to be enriched to maintain their native populations and ecological interactions between local flora and fauna (Meiado *et al.*, 2012a; 2012b).

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