Adv. Hort. Sci., 2019 33(3): 327-332 DOI: 10.13128/ahs-23642

AHS
Advances in Horticultural Science

Long-time storage *Pochota fendleri* seeds with different packaging

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Key words: germination, PET, plastic, seed quality, vigour.

Abstract: The aim of this study was to evaluate the vigour and physiological quality of seeds of *Pochota fendleri* in two weight classes, stored in different types of packaging over a period of 28 months. The experimental design was completely randomised, with treatments arranged in a 2 x 3 x 4 factorial scheme (2 seed sizes x 3 types of packaging x 4 storage periods), with four replications. The stored seeds were evaluated for germination percentage and germination speed index every six months up to 28 months. At 28 months, the percentage incidence of fungi on the seeds was also determined. The seeds of *Pochota fendleri* remain viable and of high quality for a period of 28 months storage. A PET bottle kept in the refrigerator is recommended to store *Pochota fendleri* seeds.

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Citation:
SOUZA A.G., SMIDERLE O.J., PEDROZO C.A., 2019 - *Long-time storage Pochota fendleri seeds with different packaging*. - Adv. Hort. Sci., 33(3): 327-332

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 13 July 2018
Accepted for publication 28 March 2019

1. Introduction

A tree species belonging to the family Malvaceae, *Pochota fendleri* (Jacq. WS Alverson) is highly valued in various countries of Central and South America; in Brazil, the State of Roraima is the only area where the species occurs naturally (Smiderle *et al*., 2017). The high rates of deforestation in areas of natural occurrence, caused mainly by the high demand for wood, have classified the species as threatened with extinction (Llamozas *et al.*, 2003; FAO, 2017). In addition to its use as wood in the production of manufactured boards, planks, panels, doors, windows and furniture, the plants of *Pochota fendleri* are also used for the shade and shelter of livestock, for planting living fences, for the manufacture of handicrafts and in the recovery of degraded areas (Briscoe, 1995; Smiderle *et al*., 2017).

Planting Brazilian native species when compared to exotic species, can contribute to the conservation of regional biodiversity, and may also present important technical and economic advantages due to the ease of acclimatising and perpetuating these species (Smiderle and Souza, 2016). However, the reduced number of seeds, the difficulty of their collection in areas of natural occurrence and the lack of basic information on native species make them difficult to cultivate.

One of the most researched and controversial aspects concerns the influence of seed weight on physiological quality; in any one batch, seeds classified as large usually give better performance when compared to small seeds as they have a greater amount of nutrient reserves, which serve as input for the initial development of the plant (Souza *et al*., 2017 a).

Therefore, based on morphological characteristics, germination tests and initial seedling vigour, it is possible to make better decisions about the quality of seed batches, resulting in a reduction in the production time of more-uniform seedlings, and the possibility of successful initial seedling establishment (Pereira *et al*., 2011; Dresch *et al*., 2013; Oliveira *et al*., 2016; Souza *et al*., 2016; Souza *et al.*, 2017 b).

Among the various stages of the seed-production process and seed technology, storage constitutes one of the critical phases and has a great influence on the viability and conservation of the seeds in the batch (Nery *et al*., 2017). Storage is important for maintaining seed quality over time, delaying the process of deterioration, so that the seeds maintain their longevity and vigour until they are sown (Carvalho and Nakawaga, 2000).

Knowing the storage capacity of seeds makes it possible to adopt the proper conditions for each species; however, due to the diversity of forest species, there is still a lack of information on the technology of such seeds in the literature, especially in relation to storage behaviour (Balouchi *et al*., 2017) for the conservation of germinating power (Smiderle *et al*., 2016; Felix *et al*., 2017).

The type of packaging used during storage is also important for maintaining both viability and vigour, since it is directly related to the physiological quality of the stored seeds (Rodrigues *et al*., 2016). The physiological quality of seeds is often evaluated by the standard germination test, which is carried out under optimal environmental conditions to determine the maximum germinating potential and establish a limit for the performance of the batch after sowing (Catão *et al*., 2016).

Conditions able to preserve seed quality for a certain period are essential; however, there is still little information available (Smiderle *et al*., 2018) on seed technology when applied to native species such as *Pochota fendleri*.

The aim of this study was to increase information on the conservation of forest seeds by evaluating the vigour and physiological quality of the seeds of *Pochota fendleri* in two weight classes stored over 28 months in different types of packaging.

2. Materials and Methods

The research was carried out at the Seed Analysis Laboratory and in the forest sector of Embrapa Roraima. The species used in the research was *Pochota fendleri* (Seem.) WS Alverson & MC Duarte, whose seeds were collected for quality analysis from trees at 10 years of age, in the Experimental Area of Embrapa Roraima located in the district of Mucajaí, Roraima (at 2°23'45.31" N and 60°58'44.34" W) during March and April of 2014. The fruit was harvested as soon as it opened. After the seeds were extracted, they were left to dry for 24 hours on a shaded canvas on the ground, and then packed in polyethylene bags and sent to the Seed Analysis Laboratory of Embrapa Roraima located at 02°45'28N and 60°43'54" W, at an altitude of 90 m, in Boa Vista, Roraima, for the experiments to be carried out. The collected seeds were selected and sorted as to weight (small seeds being those that passed through a 4 mm diameter sieve, having a mean individual weight of 0.027 g; and large seeds, those that were retained in a 4.5 mm diameter sieve, with a mean individual weight of 0.048 g). The seeds were then packed in individual paper bags, transparent plastic containers or PET bottles, and stored in a refrigerator at 10°C and a relative humidity of 60%. The temperature and relative humidity were monitored with a thermo hygrometer to obtain a monthly average. The packs of samples were divided into sufficient quantities for later evaluation.

Four samples of 10 previously weighed seeds of *Pochota fendleri* were selected to determine the water content remaining in a drying oven at 105±3°C for 24 h (MAPA, 2009). This determination was repeated at 16 and 28 months. The moisture content was calculated based on the fresh weight of the seeds (MAPA, 2009). The experimental design was completely randomised, with treatments arranged in a 2 x 3 x 4 factorial scheme (2 seed classes x 3 types of packaging x 4 storage periods), with four replications.

Seed characteristics were determined every six months during the 28 months of storage, starting from the fourth month. The tests and methodologies to which the seeds were submitted and evaluated are described below.

The germination test was carried out on four replications of 50 seeds, in plastic boxes (gerbox®) on

germination paper (germitest®) moistened with distilled water at 2.5 times the weight of the paper and kept in a germination chamber at 25±2°C under constant light. The germination test was evaluated by daily counts until the fourteenth day, considering the number of seeds that emitted a root greater than 2 mm (Labouriau, 1983). From the data obtained with the germination test, the germination speed index (GS) was calculated as per the method recommended by Maguire (1962).

The seeds were also characterised for biometry, the small seeds showing mean values of 5.01 mm for length and 3.32 mm for diameter, and the large seeds, 5.49 mm and 4.20 mm. The values were obtained with the aid of a digital calliper.

The means of the variables were submitted to the statistical analysis utilizing the software Sisvar (Ferreira, 2014), with variance analysis and the Tukey test (p≤0.05%). Regression analysis was performed for the time factor (months). The statistical analysis was carried out using the Sisvar software.

3. Results and Discussion

At the time of storage, the seeds of *Pochota fendleri* presented a mean water content of 10.5% for the seeds classified as small, and 8.1% for those classified as large. The results for seed water content after 4, 16 and 28 months storage in the different treatments can be seen in Table 1. The water content of the seeds was determined by the storage conditions, and no statistical analysis was applied to the values. The relative humidity of the three storage containers varied during the 28 months, which contributed to maintaining the seed water content.

The packages prevented or decreased the exchange of water vapour between the seeds and the storage environment. It is worth noting that before storage the seeds presented 98% germination for those classified as large and 85% for those classified as small.

The results obtained in the present study demonstrated a strong relationship R^2= 0.88, for germination percentage in both classes of seed packed in the different containers. For the study under analysis, the seeds classified as small showed inferior performance in relation to germination percentage when compared to the large seeds (Fig. 1A and B), irrespective of container or storage period.

Table 1 - Mean water content (%) in seeds of *Pochota fendleri* for different weight classes, packaging and storage

Packaging	Months			Mean
	4	16	28	
Large seeds				
Paper bag	8.0 a B	7.8 a B	7.7 a A	7.8
Plastic bag	8.1 a B	8.0 a B	7.8 a B	7.9
PET bottle	8.1 a B	8.0 a B	7.8 a B	7.7
Mean	8.05	7.96	7.77	
Small seeds				
Paper bag	10.0 a A	9.2 b A	8.0 b A	9.0
Plastic bag	10.5 a A	10.0 a A	9.0 a A	9.7
PET bottle	10.5 a A	10.0 a A	9.5 a A	10.0
Mean	10.25	9.90	8.79	

In the column, means followed by different letters, lowercase letters between packages and uppercase between sizes, differ by Tukey test at 5%.

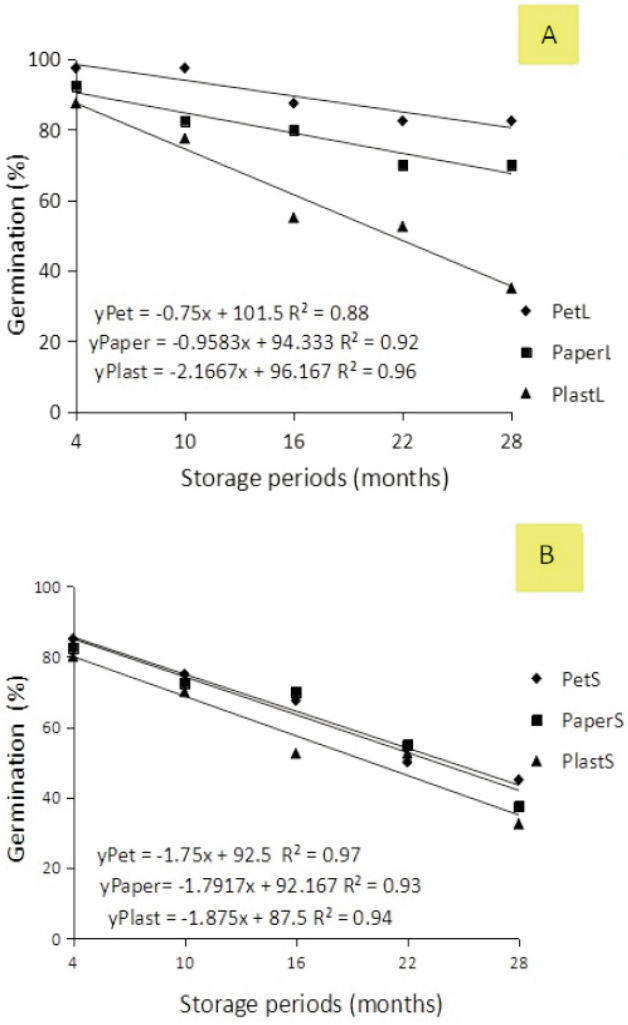

Fig. 1 - Mean values for germination (%) of large (A) and small (B) seeds of *Pochota fendleri* for different packaging and storage periods (months). Pet L= Pet bottles, Large; Paper L= Paper bags, large; Plast L= transparent plastic containers, Large; Pet S= Pet bottles, Small; Paper S= Paper bags, Small; Plast S= transparent plastic containers, Small.

In Table 2, the means of the squares and the levels of significance can be seen by the F test for the characteristics evaluated, at a level of 0.05% probability for all variables studied.

At 28 months storage, the large seeds packed in the PET bottles presented on average a 68% yield in germination percentage when compared to those in the plastic packaging (Fig. 1A).

It was found however, that even after 28 months storage, the large seeds storage Pet bottles (Fig. 1A) maintained a germination percentage 83% below the minimum established for commercialisation, which is 85% (MAPA, 1992).

According to Souza *et al*. (2017 b), for the same species the seeds of greater weight presumably have more reserves, a higher level of hormones and well-formed embryos, and are considered to have greater vigour.

With the small seeds however, there was an expressive linear loss in germination percentage in the three containers during storage. The same results were seen for germination speed index in the small seeds (Fig. 2B), demonstrating that this characteristic can be efficient in detecting differences in vigour among seed size classes in storage.

Evaluating germination capacity in seeds of *Enterolobium schomburgkii*, Horing *et al*. (2012) found that there was a linear decrease in germination percentage after 30 months of storage for seeds packed in waterproof plastic pots but stored under laboratory conditions. Seeds of *Caesalpinia leiostachya* (Benth.) Ducke (pau-ferro) maintained a higher germination percentage when stored for a period of eight months inside the fruit in a natural environment (Biruel *et al*., 2007).

The germination speed index obtained for large seeds stored for up to 10 months in PET bottles was 18.0 (Fig. 2A); however, a greater reduction was found when the seeds were kept in paper or plastic bags (Fig. 2A).

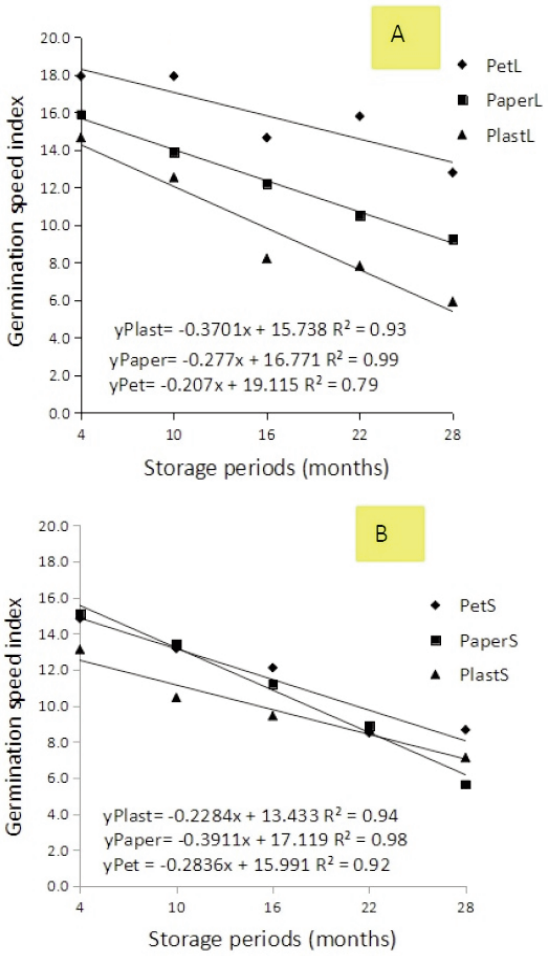

Fig. 2 - Mean values for the germination speed index of large (A) and small (B) seeds of *Pochota fendleri* submitted to different packaging and months of storage. Pet L= Pet bottles, Large; Paper L= Paper bags, large; Plast L= transparent plastic containers, Large; Pet S= Pet bottles, Small; Paper S= Paper bags, Small; Plast S= transparent plastic containers, Small.

Table 2 - Summary of the variance analysis (Mean Squares and significance by the F test), coefficients of variation, and general means obtained for germination (G) germination speed index (GSI) and water content (U%) of *Pochota fendleri* for different packaging and storage periods (months)

Source of variation	DF	Germination (%)	Germination speed index	Source of variation	DF	Water content (%)
Storage periods (SP)	4	6481.7708 **	232.8101 **	Storage periods (SP)	2	4918472 **
Packing (P)	2	3954.1666 **	183.7151 **	Packing (P)	2	2040139 **
Size (S)	1	8066.6666 **	140.9604 **	Size (S)	1	53388889 **
SP x P	8	288.8020 **	3.3093 NS	SP x P	4	0.270347 NS
SP x S	4	272.3958 NS	3.8119 NS	SP x S	2	2287639 **
P x S	2	1379.1666 **	60.6049 **	P x S	1	1075972 **
SP x P x S	8	159.1145 NS	8.0687 NS	SP x P x S	2	0.186597 NS
Error	120	1.237.500	40.788	Error	54	0.183426
Total	149			Total	71	
Coefficient of variation (%)		16.08	17.31	Coefficient of variation (%)		4.87

*significant difference in 0.05 levels; NS= not significant.

It is therefore interesting to classify seeds by size, since the procedure is quick, and can be carried out manually or with the use of specific sieves or even a densimetric table. The use of heavier seeds gives the consumer or producer a greater guarantee of the physiological quality of the material purchased and used in producing seedlings of *Pochota fendleri*, since seed quality assumes a prominent role in the cultivation of forest species, and can be considered one of the principal bottlenecks, especially in the area of seedling production.

In turn, seeds classified as small and packed in paper bags presented an index that was 35% smaller at 28 months than seeds stored in PET bottles. According to Marcos Filho (2005), a reduction in the germination speed index is the first symptom of a fall in seed performance, generally determined by disorganisation of the membrane system. Bello (2005) found an increase in the mean germination time and a decrease in the germination speed index in seeds of *Torresea acreana* (Mirtaceae) over a storage period of 12 months, indicating a reduction in germination speed.

The results obtained for the variables evaluated in the present study indicate that seeds should be stored in PET bottles in a refrigerated environment maintained at 10°C for different periods (months).

There are currently no studies that might support the staggered production of *Pochota fendleri* seedlings in the field or in the nursery. The practice of staggered sowing is not yet carried out by producers of *Pochota fendleri*, but such a method, if based on scientific principles, could make a positive contribution to seedling production, making it possible to produce seedlings at different times throughout the year, as well as providing seedlings of differing quality standards, which would depend on the investment capacity of the producer.

4. Conclusions

The seeds of *Pochota fendleri* remain viable with high physiological quality for up to 28 months storage in PET bottles. Seeds of *Pochota fendleri*, stored in a refrigerator at 8 to 10°C in PET bottles, retain their physiological quality. The vigour of large seeds of *Pochota fendleri* is preserved for a longer time in PET bottles. The use of heavier seeds gives the consumer or producer a greater guarantee of the physiological quality of the material purchased and used in producing seedlings of *Pochota fendleri*, since seed quality assumes a prominent role in the cultivation of forest species.

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