

Drying and physiological quality of palheteira seeds (*Clitoria fairchildiana* R.A. Howard)

Secagem e qualidade fisiológica de sementes de palheteira (*Clitoria fairchildiana*

R.A. Howard)

El secado y la calidad fisiológica da las semillas de la palheteira (*Clitoria fairchildiana*

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Abstract

Knowledge about seed storage behavior is important to preserve plant species. *Clitoria fairchildiana* R.A.Howard is an endemic species with medicinal properties, it is used in the recovery of degraded land. The objective of this study is to evaluate the effect of drying on the physiological quality of *C. fairchildiana* seeds. Seeds were oven dried along with silica gel for 18 days and in intervals of 2 or 3 days, samples were removed to assess the seed moisture content. This was done in an oven at 105 ± 3 °C for 24h, using four replicates with 10 seeds each. After undergoing the above-mentioned treatment, a sample of seeds was stored in the freezer (-18 °C) for 3 months. Sowing was carried out in plastic trays in a sand and

sawdust mixture (1:1), previously sterilized in hot water (100 °C) for two hours. Seeds were left to germinate in a laboratory with no temperature and relative humidity control. Germination tests were performed over 14 days with daily counts of the number of emerged seedlings. The percentage of seed germination, abnormal seedlings and dead seeds were obtained 14 days after sowing. The experimental design was completely randomized with four replications of 25 seeds. The reduction of seed moisture content from 18.5% to 5.6% affected physiological seed quality causing a reduction in the germination percentage and an increase in dead seeds and abnormal seedling percentage. *C. fairchildiana* seeds are classified as intermediate and they can be desiccated up to 8.6% with no reduction in physiology quality.

Keywords: Desiccation; Storage; Vigor; Seed moisture content.

Resumo

O conhecimento acerca o armazenamento de sementes é importante para a preservação de espécies vegetais. A *Clitoria fairchildiana* R.A.Howard é uma espécie endêmica com propriedades medicinais utilizada na reconstituição de áreas degradadas. O objetivo deste estudo foi analisar o efeito da secagem na qualidade fisiológica de sementes dessa espécie. As sementes foram secadas em sílica gel por 18 dias em intervalos de 2 ou 3 dias amostras foram retiradas para determinação do grau de umidade. Este foi obtido em estufa a $105\pm 3^{\circ}\text{C}$ por 24h, com 4 repetições de 10 sementes. Após obter o último grau de umidade uma amostra das sementes foi armazenada em freezer (-18°C) por 90 dias. A semeadura foi em vasos contendo substrato de areia e serragem, na proporção de 1:1, previamente cozido em água aquecida por duas horas. Os vasos permaneceram em laboratório desprovido de controles de temperatura e de umidade relativa do ar. O teste de germinação ocorreu durante 14 dias com contagem diária das plântulas emersas e no final desse determinou-se as porcentagens de germinação, sementes mortas e plântulas anormais. O delineamento foi inteiramente casualizado com quatro repetições de 25 sementes por tratamento. A redução do grau de umidade de 18,5% para 5,6% afetou a qualidade fisiológica das sementes acarretando redução na taxa de germinação e aumento na porcentagem de sementes mortas e de plântulas anormais. Sementes de *C. fairchildiana* são classificadas como intermediária no armazenamento e podem ser secadas até 8,6% sem alteração na qualidade fisiológica.

Palavras-chave: Dessecamento; Armazenamento; Vigor; Grau de umidade da semente.

Resumen

El conocimiento sobre el almacenamiento de semillas es importante para la preservación de especies vegetales. La *Clitoria fairchildiana* R.A.Howard es una especie endémica con propiedades medicinales utilizada en la reconstitución de áreas degradadas. El objetivo de este estudio fue analizar el efecto del secado en la calidad fisiológica de las semillas de esta especie. Las semillas se secaron en gel de sílice durante 18 días y, en intervalos de 2 o 3 días, se fueron retirando muestras para determinar el grado de humedad. Este se obtuvo en invernadero a 105 ± 3 °C durante 24h, con 4 repeticiones de 10 semillas. Después de obtener el último grado de humedad, se almacenó una muestra de las semillas en frigorífico (-18°C) durante 90 días. La siembra se hizo en macetas que contenían substrato de arena y serrín, en la proporción de 1:1, previamente hervido en agua durante dos horas. Las macetas permanecieron en laboratorio desprovisto de controles de temperatura y de humedad relativa del aire. La prueba de germinación se desarrolló durante 14 días con cuenta diaria de las plántulas emergidas y, a finales de esta, se determinaron los porcentajes de germinación, las semillas muertas y las plántulas anormales. El delineamiento fue completamente casualizado con cuatro repeticiones de 25 semillas por tratamiento. La reducción del grado de humedad del 18,5% a 5,6% afectó a la calidad fisiológica de las semillas acarreando una reducción en la tasa de germinación y un aumento en el porcentaje de semillas muertas y de plántulas anormales. Las semillas de *C. fairchildiana* se clasifican como intermedias en el almacenamiento y pueden secarse hasta un 8,6% sin alteración en la calidad fisiológica.

Palabras clave: Desecación; Almacenamiento; Vigor; Grado de humedad de la semilla.

1. Introduction

One important way to preserve plant species is through seed storage. Consequently, knowledge of seed storage behavior is important in order to determine whether or not seed storage is suitable (Hong et al., 1998). Seed longevity is influenced by the plant species and environmental conditions (Sano et al., 2015), as well as temperature and moisture content during storage (Smaniotto et al., 2014).

Roberts (1973) classified seed storage behavior as orthodox and recalcitrant. Orthodox seeds can be dried up to 2% to 5% and stored at low temperatures without affecting germination, while recalcitrant seeds should be stored with high seed moisture content, between 12% to 31%, depending on the species (Roberts, 1973), and they cannot be stored during a long period without reducing viability (Kleinwächter et al., 2014).

Subsequently a third category intermediate between the orthodox and recalcitrant was identified (Ellis et al., 1990). The physiological quality of intermediate seeds can be affected when seed moisture content is low, between 7% to 12%, depending on the species (Hong and Ellis, 1996), and they do not tolerate low temperatures (Ellis et al., 1990). Therefore, information about seed storage behavior is essential to the conservation of plant species for a short- or long-term period.

Clitoria fairchildiana R.A.Howard, commonly known in Brazil as palheteira, is an endemic species from Brazil found naturally in the states of Amapá and Pará (Fantz, 1990) and it has spread to all states of Brazil, except Acre, Mato Grosso, Roraima and the Distrito Federal (Flora do Brasil, 2020). It occurs in areas of anthropic vegetation, cerrado, restinga and riparian forest (BFG, 2015; Rando & Souza, 2015).

According to Lorenzi (2009), this is a fast-growing species used in landscaping, civil construction, rural and urban afforestation and recovery of degraded areas. In the medicinal scope, it presents anti-tumor proprieties induced by rotenoids and lectins (Santos, 2014); this same biochemical property has insecticidal potential (Dantzger, 2015), being a promising alternative among the native species that can be used in the pharmaceutical industry.

The objective of this study is to study the effect of desiccation on the physiological quality of *C. fairchildiana* seeds.

2. Materials and Methods

The fruits were harvested from three trees at the Universidade Federal do Pará, in Belém, State of Pará, Brazil (01°28' S; 48°27' W) and the experiment was carried out at Embrapa Amazônia Oriental, in Belém, State of Pará, Brazil.

The experiment was carried out for four months, in laboratory conditions, without control of temperature and relative humidity. The experiment was carried out in a completely randomized design with 10 treatments and 4 replications of 25 seeds for each treatment.

The seeds were manually removed from the fruits and after that, the seeds damaged by insects were eliminated. The seeds were dried in an oven containing silica gel in the proportion of 1.250 g of silica to 1,700 seeds. Samples were taken every 3 days and seed moisture content was determined in four replications of 10 seeds, in an oven set at $105 \pm 3^\circ \text{C}$ for 24 h (Brasil, 2009). Moisture content percentage was expressed on a fresh weight basis (fwb). After obtaining the last treatment (18th day), a sample of 150 seeds were stored in a freezer (-18°C) for 90 days according to Hong and Ellis (1996).

The sowing was on a horizontal position at a depth 1.0 cm in pots (23 cm x 18 cm), in the substrate containing 5 kg (dry weight) of substrate. The substrate used was a mixture of sand and sawdust (1:1), previously cooked in hot water (100 °C) for two hours to reduce microbial contaminants. Seeds were left to germinate in a laboratory under no temperature control and relative humidity (minimum and maximum temperature and relative humidity were 22° C and 29 °C, and 74% and 95%, respectively).

The emergence test was carried out for 14 days with a daily counting of emerged seedling. A seedling was considered emerged when it showed the cotyledon above the substrate surface. At the end of emergence test, the substrate was washed and the germination percentages (normal seedlings), dead seeds and abnormal seedlings were determined according to the Rules for Seeds Analysis (Brasil, 2009). The seedling emergence index was calculated by counting the number of emerged seedlings quantified during the emergence test (Maguire, 1962).

The days to germination emergence onset were quantified by the number of days from sowing until germination of the first seed. At the end of emergence test, the seedlings were removed from substrate and divided into root, stem, and leaf, and then packaged in a paper bag and dried at 68 °C in an oven with forced air for 48 h. Finally, the samples were weighed to determine the dry mass of the roots, stems, and leaves.

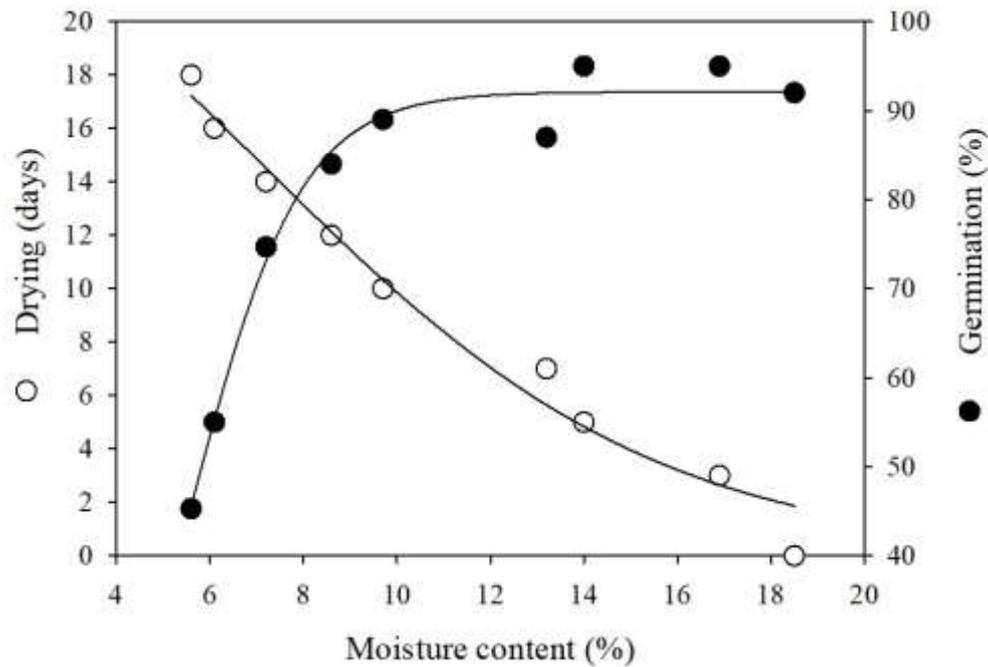
This methodology was described by Hong and Ellis (1996) and it is used in tropical species as *Manilkara huberi* (Ducke) A. Chev (Barros et al., 2019) and *Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum. (Cruz & Cicero, 2008) to classified seeds according to tolerance, to desiccation and storage behavior.

The experimental design was completely randomized with four replications of 25 seeds for each moisture content. Data were subjected to Levene test for homogeneity of variance (Zar, 1996). The variables stem dry mass and root dry mass were transformed using $\text{Log}(x+1)$. Data were subjected to analysis of variance and means compared by Tukey's test ($p \leq 0.05$) using Statistica software (Statsoft, 2016) to perform all analyses. Data were back transformed for presentation.

3. Results and Discussion

In Figure 1 was observed equations not linear of the drying and seed germination of palheteira. The initial moisture content of palheteira seeds was higher than that reported by Cruz and Pereira (2019).

Figure 1. Drying ($y=29.131(1+\exp(-(x-7.169)/-4.231); R^2=0.98$) and germination ($y=92.109(1+\exp(-(x-5.624)/-1.167)); R^2=0.98$) curve palheteira (*Clitoria fairchildiana*) seeds.



Source: Authors.

In Table 1 was observed the effect of moisture content reduction in physiological quality of seeds. Statistically significant differences ($P \leq 0.05$) between treatments were observed for all the variables. Reduction of the seed moisture content to 8.6% did not affect emergence, emergence speed index, germination, dead seed and abnormal seedlings.

Table 1. Desiccation time (DT), seed moisture content (U), days to germination emergence onset (DGO), emergence (E), emergence speed index (ESI), germination (G), dead seeds (DS) and abnormal seedling (AS) in *C. fairchildiana* seeds during drying.

DT (days)	U (%)	DGO	E (%)	ESI	G	DS (%)	AS
0	18.5	6.0 b	93.0 a	3.31 a	92.0 ab	4.0 ab	4.0 a
3	16.9	6.3 b	96.0 a	3.21 a	95.0 a	2.0 a	3.0 a
5	14.0	6.8 bc	97.0 a	3.25 a	95.0 a	3.0 ab	2.0 a
7	13.2	6.0 b	90.0 a	3.12 a	87.0 ab	9.0 ab	4.0 a
10	9.7	6.0 b	93.0 a	3.09ab	89.0 ab	6.0 ab	5.0 a
12	8.6	6.8 bc	90.0 a	2.93ab	84.0 ab	9.0 ab	7.0 ab
14	7.2	7.0 c	84.0 ab	2.57 bc	74.7 b	10.6 ab	14.7 ab
16	6.1	6.8 bc	70.0 b	2.12 c	55.0 c	17.0 b	28.0 b
18	5.6	5.0 a	50.7 c	1.45 d	45.3 c	45.4 c	9.3 ab
SS	5.9	8.0 d	47.0 c	1.36 d	42.0 c	42.0 c	16.0 ab

Differences in means followed by the same letter are not statistically significant by Tukey test ($P \geq 0.05$). Source: Authors.

According to Hong and Ellis (1992), seeds show reduced viability when their content reaches a critical level. For palheteira seeds, the critical level of moisture content was 7.2% with a decrease in the germination percentage. The decrease in water content resulted in a decreased physiological quality of the seeds, considering an increase in the number of dead seeds and abnormal seedlings (Table 1).

The dry mass of root, stem, leaf, and total are showed in Table 2. Reduction the moisture content decreased the dry mass production in different components of seedlings, with higher regarding the seedling dry mass, the reduction was more accentuated from 9.7%.

Table 2. Moisture content (U), leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) in *C. fairchildiana* seeds during drying.

U (%)	LDM	SDM (mg/seedling)	RDM	TDM
18.5	38.7 a	98.7 ab	63.2 a	200.5 a
16.9	24.4 bc	103.0 a	42.7 bc	170.1 bc
14.0	32.7 ab	92.9 ab	50.4 ab	176.0 ab
13.2	42.9 a	85.9 bc	41.6 bc	170.4 bc
9.7	17.6 cd	72.4 cd	31.0 cde	121.0 d
8.6	14.2 d	67.6 d	28.6 cde	110.4 d
7.2	19.4 cd	83.2 bcd	35.8 bcd	138.4 cd
6.1	6.7 e	46.7 e	18.9 de	72.3 e
5.6	7.7 e	36.6 ef	12.9 e	57.1 e
5.9	4.5 e	27.6 f	23.5 de	55.7 e

Differences in means followed by the same letter are not statistically significant by Tukey ($p < 0.05$).
 Source: Authors.

When seeds were frozen at -18°C for 90 days it was observed statistically that freezing did not modify the emergence, emergence speed index, germination, dead seeds and abnormal seedlings, showing that the seed physiological quality is affected by the reduced water content, not the freezing.

Alves et al. (2015) and Pelissari et al. (2018) studying seed desiccation classified palheteira seeds as orthodox, diverging from the present study that classified palheteira seeds as intermediate in storage. Alves et al. (2015) dried palheteira seeds around 8% and 78% of germination, but according to Hong and Ellis (1996), this water content is a characteristic of intermediate species, and orthodox species have a desiccation of at least 2% to 6% moisture content depending on the species.

Mayrinck et al. (2016) studying seeds from native species from Brazil observed 59.1% were classified as intermediate species according to the Hong and Ellis protocol (1996). Baskin and Baskin (2004) and Kleinwächter et al. (2014) explained that the majority of tropical seeds are classified as recalcitrant or intermediate due to the favorable climatic conditions in tropical rainforests; that is, seeds of most tropical plants germinate as soon as fruits are mature, so there is no need to develop outlasting mechanisms.

In other tropical species, whose seeds are classified as intermediate in storage, *Genipa*

americana L., the maintenance of the physiological seed quality is possible when seed moisture content is reduced to 10%, close to the value obtained for palheteira seeds (Magistrali et al., 2013).

4. Conclusions

Clitoria fairchildiana seeds exhibit intermediate characteristics in storage, and it possible to maintain seed quality with seed moisture content equal to or higher than 8.6%. In the next phase of this research, it will be evaluated the effect of temperature and storage time on the physiological quality of seeds.

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