

DETERMINATION OF MOISTURE CONTENT AND STORAGE POTENTIAL OF GUANANDI SEEDS

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Resumo

Determinação do teor de água e do potencial de armazenamento de sementes de guanandi. O guanandi é uma espécie florestal nativa com particularidades que dificultam o armazenamento das sementes, pelo seu comportamento recalcitrante e por não haver protocolo específico e prático para determinação do teor de água da semente. O trabalho teve por objetivo testar procedimentos para determinação do teor de água e estudar diferentes condições de armazenamento das sementes de guanandi. Para a determinação do teor de água utilizou-se o método estufa a baixa temperatura 101 - 105 °C, testando-se formas de preparo da semente (íntegras, cortadas ao meio e fragmentos menores que 7,0 mm) e diferentes períodos de secagem. Para o armazenamento das sementes testaram-se quatro ambientes (câmara úmida, câmara seca, refrigerador e ambiente de laboratório), com dois tipos de embalagens, durante quatro períodos (0, 1, 2, 3 e 4 meses). Para análise estatística, um delineamento inteiramente casualizado, em esquema fatorial, foi utilizado para teor de água; de parcelas subdivididas para armazenamento. Realizou-se análise de regressão para avaliação do potencial fisiológico e teor de água durante o armazenamento, com ajuste da equação significativa pelo teste de Tukey. Pelos resultados obtidos observou-se que o método da estufa a baixa temperatura 101 - 105 °C / 17 h, utilizando-se sementes íntegras, deve ser utilizado para a determinação do teor de água das sementes de guanandi. A melhor combinação entre ambiente e embalagem para armazenamento das sementes foi condição de ambiente de laboratório (média entre 19,6 - 23,1 °C e UR 53 - 62%) em embalagem de polietileno.

Palavras-chave: *Calophyllum brasiliense*, conservação, recalcitrante, método da estufa.

Abstract

Guanandi is a native forest species with particularities that make it difficult to store of seeds, because of its recalcitrant behavior and since a specific and practical protocol to determine the moisture content of its seeds is not available yet. This work had the purpose to test procedures to determine the moisture content and to study different environmental conditions of the storage of the guanandi seeds. In order to determine the moisture content, the low-temperature oven method at 101 – 105°C was used, testing seed preparation methods (intact, cut in half and fragments smaller than 7.0 mm) and different drying periods. Four environments (wet chamber, dry chamber, cooler and laboratory environment) were tested for the storage of the seeds, with two packaging types, during four periods (0, 1, 2, 3 and 4 months). For the statistical analysis, a completely randomized design, with a factorial arrangement, was used for moisture content; a split-split plot design was used for storage. A regression analysis was performed to evaluate the physiological potential and moisture content during the storage, adjusting the significant equation by Tukey's test. From the obtained results, it was observed that the low-temperature oven method 101 - 105°C / 17 h, using intact seeds, should be used to determine the moisture content of guanandi seeds. The best combination of environment and packaging to store seeds was the laboratory environment condition (average between 19.6 - 23.1 °C and RH 53-62%) in polyethylene packaging.

Keywords: *Calophyllum brasiliense*, conservation, recalcitrant, oven method.

INTRODUCTION

The search for tools to help preserving forest species seeds is a major challenge for research, since seeds are the main way to preserve species (PERES, 2016); it also gained more importance with the need to preserve genetic resources of native species, which constantly undergo anthropic pressures.

The deterioration of seeds during storage cannot be avoided, but it must be minimized by applying techniques to maintain more appropriate environmental conditions, in order to preserve their physiological quality for as long as possible; such conditions vary for each species (FERREIRA *et al.* 2010; SOUZA *et al.*, 2011; ABUD *et al.*, 2012) and depend on characteristics of seeds themselves (such as physical, physiological and biochemical) and their tolerance to water loss and low temperature. Therefore, seeds are classified as having an orthodox, intermediate or recalcitrant behavior (MARTINS *et al.*, 2007; MATOS *et al.*, 2008; BARBEDO *et al.*, 2013; NERY *et al.*, 2014; WALTERS, 2015; MAYRINCK *et al.*, 2016).

Recalcitrant seeds, as *Calophyllum brasiliense* Cambess. (guanandi) (NERY *et al.*, 2017) ones, are less favored by conventional storage techniques, which are based on water removal from seeds until very low limits, together with low temperature and relative air humidity; these conditions are not tolerated by recalcitrant seeds (BARBEDO *et al.*, 2013).

Storage can be divided into short, medium and long term; the first ones are used for the conservation of seeds for days, months and years respectively, in large volumes, to meet commercial demands, whereas in long term storage (over 10 years), smaller amounts are typically used, normally in germplasm banks for the conservation of genetic material, which implies the use of ultra-low temperatures (-80 °C) or cryopreservation in liquid nitrogen (SILVA; FERRAZ, 2015).

Studies addressing the storage of native species seeds have revealed a generally short conservation period (months), at warm temperatures and high relative air humidity (RH) using packages that minimize seed water loss, as for forest species *Geoffroea spinosa* (19,2 - 23,5 °C; 85% RH), packed in plastic bag up to 60 days (SOUZA *et al.*, 2011); *Heteropterys tomentosa* (26,6 °C; 79,9% RH), packed in plastic bag up to 60 days (ARRUDA *et al.*, 2011); and *Talisia esculenta* (A. St. Hil.) Radlk (28 ± 5 °C; 65% RH), packed in polythene bag up to 25 days (SENA *et al.*, 2016).

Guanandi seeds present difficulties in being stored for long periods, since there is no protocol to indicate the best way for short-term conservation, minimum time often necessary to transport seeds, development of laboratory research and seedling production scheduling, thus improving the logistics of the nursery.

On the other hand, determining the moisture content of seeds is fundamental to keep track of the physiological quality during the storage period. Thus, methodologies that guarantee reliability and practicality in this determination are pertinent demands for forest seeds.

Among the most reliable methods is the low-temperature oven at 101-105 °C/17 h, indicated in the Rules for Seed Testing (RST) since it is a basic method for the introduction of new species (BRASIL, 2009); it was also adopted by the International Rules for Seed Testing (ISTA, 2015). In the case of guanandi seeds, which are considered large and high in moisture content, the recommendation of the method involves cutting the seeds into fragments that are smaller than 7.0 mm, in a short exposure time (four minutes) of the material to the environment, considering that guanandi seeds have a rigid endocarp (SILVA *et al.*, 2014; SILVA *et al.*, 2018), which hinders the rapid fragmentation and exposure of the material to the indicated time limit.

The purpose of this work was to test seed preparation methods to determine the moisture content and to evaluate different environmental conditions and packaging for the storage of guanandi seeds.

MATERIAL AND METHODS

Ripe guanandi fruits were collected in May 2015 from 10 distinct trees, located in natural areas of the Atlantic Forest, in the municipality of Pontal do Paraná (25° 35' S and 48° 33' W). The climate of the region is classified as Cfa according to Köppen (VANHONI; MENDONÇA, 2008), that is, subtropical climate with mean temperatures of 18°C and 22°C, respectively, in the coldest and hottest month, with hot summers and infrequent frosts, rainfalls concentrated in the summer, and no defined dry season.

As soon as they were collected, fruits were taken to the Seed Analysis Laboratory of the Federal University of Paraná, where they were hand pulped, and the seeds washed under running water and placed for drying on paper towel, in an environment with a temperature of 20 °C.

In order to study the determination of the seed moisture content, three different preparation types were used: intact seeds, seeds cut in half and seeds cut in fragments that are smaller than 7.0 mm (BRASIL, 2009). Four replications of five seeds, or a weight equivalent to five fragmented seeds, were placed in aluminum capsules and oven-dried at 103 °C ± 2 (BRASIL, 2009). The seed moisture content was determined by weighing on an analytical scale with 0.001 g precision every three hours (until 15 hours of evaluation) and, after that, every two hours until the end of weighing - at 25 hours, one more hour than the maximum recommended period for the determination of seed moisture content (BRASIL, 2009). Results were expressed in percentages, on a wet basis.

In order to evaluate the storage potential of seeds, four environmental conditions (wet chamber, dry chamber, refrigerator and laboratory environment conditions) and two packaging types, permeable (Kraft paper) and semipermeable (polyethylene 0.15 mm) were tested during five periods (0, 1, 2, 3 and 4 months).

The physiological potential of seeds during the storage period was evaluated monthly by the following determinations:

Germination test: five replications with 10 seeds each were used, distributed in rolls of paper towel moistened with water at a ratio of 2.5 times the mass value of the substrate (SILVA *et al.*, 2014), kept in a Mangelsdorf-type germinator, at 30 °C and without light, with weekly evaluations until germination stabilization, that is, until being able to observe the possibility of obtaining normal seedlings.

Germination rate index (GRI): conducted together with the germination test, performing weekly evaluations until observing germination failure.

Moisture content: determined according to the best method obtained in the study conducted specifically for this purpose, previously mentioned.

For the statistical analysis, a completely randomized design, with a factorial arrangement, was used for moisture content (drying periods x seed preparation); a split-split plot design was used for seed storage, where plots received combinations between storage environments and packaging, and subplots received the storage periods. A regression analysis was performed to evaluate the physiological potential and moisture content of seeds during the storage period, adjusting the significant equation by Tukey's test ($p < 0.05$). All the analyses and graphs were generated in the free software R.

RESULTS

Data obtained by determining the moisture content of guanandi seed show that in the preparation of seeds cut in half and in fragments smaller than 7.0 mm, the extraction of water after three hours of drying were, respectively, 6.9 and 2.7%, in relation to the total extracted water (25 hours), whereas for intact seeds this value was 25.2% (Table 1).

Table 1. Moisture content of guanandi seeds, determined by low-temperature oven method at 101 - 105 °C, testing different types of seed preparation and various drying periods.

Tabela 1. Teor de água das sementes de guanandi, determinado pelo método de estufa a baixa temperatura 101-105 °C, testando-se diferentes formas de preparo da semente e vários períodos de secagem.

Drying periods (h)	Moisture content (101 – 105 °C)		
	Seed preparation		
	Intact	Cut in half	Fragments < 7.0 mm
%.....		
3	8.8 Ac	2.4 Bb	0.9 Bb
6	26.4 Bb	32.1 Aa	33.0 Aa
9	32.6 Aa	34.0 Aa	33.6 Aa
12	34.2 Aa	34.4 Aa	33.8 Aa
15	34.2 Aa	34.4 Aa	33.8 Aa
17	34.7 Aa	34.5 Aa	33.8 Aa
19	34.9 Aa	34.6 Aa	33.8 Aa
21	34.9 Aa	34.6 Aa	33.9 Aa
23	34.9 Aa	34.6 Aa	33.9 Aa
25	34.9 Aa	34.6 Aa	33.8 Aa
C.V. (%)	3.93		

Averages followed by the same lowercase letter in the column and the same capital letter on the line do not differ among themselves by Tukey's test at 5% probability

Médias seguidas de mesma letra, maiúscula na linha e minúscula na coluna, não diferem entre si pelo teste de Tukey ($p < 0,05$).

After six hours of drying, it was possible to observe a water loss stabilization in both seeds cut in half and in fragments smaller than 7.0 mm, since at this stage seeds had already lost 92.8 and 97.4% of the total

water, respectively; as for intact seeds, stabilization may only be reached three hours later (9 drying hours), presenting a 93.4% water loss (Table 1). Thus, for treatments cut in half and fragments, it was observed not statistically difference after six hours, only for intact treatment was observed after nine hours; the three ways of seed preparation were stable as for water extraction up to 25 hours of drying (Table 1).

Figures from 1A to 1D (Figure 1) depicts the averages of relative air humidity and the averages of maximum, minimum and average temperatures, obtained during the storage period in each environment. The laboratory environment conditions showed the greatest variation of both mean temperature (19.6 - 23.1 °C) and average relative humidity (53-62%) (Figure 1A). The wet chamber showed the lowest temperature variation (5.6 - 5.8 °C), followed by the dry chamber (17.4 - 17.7 °C) and the cooler (7.3 - 8.5 °C). As for the mean relative humidity, the lowest variation was obtained in the dry chamber (56 – 57%), followed by the cooler (49-52%) and wet chamber (69-73%) (Figures 1B to 1D).

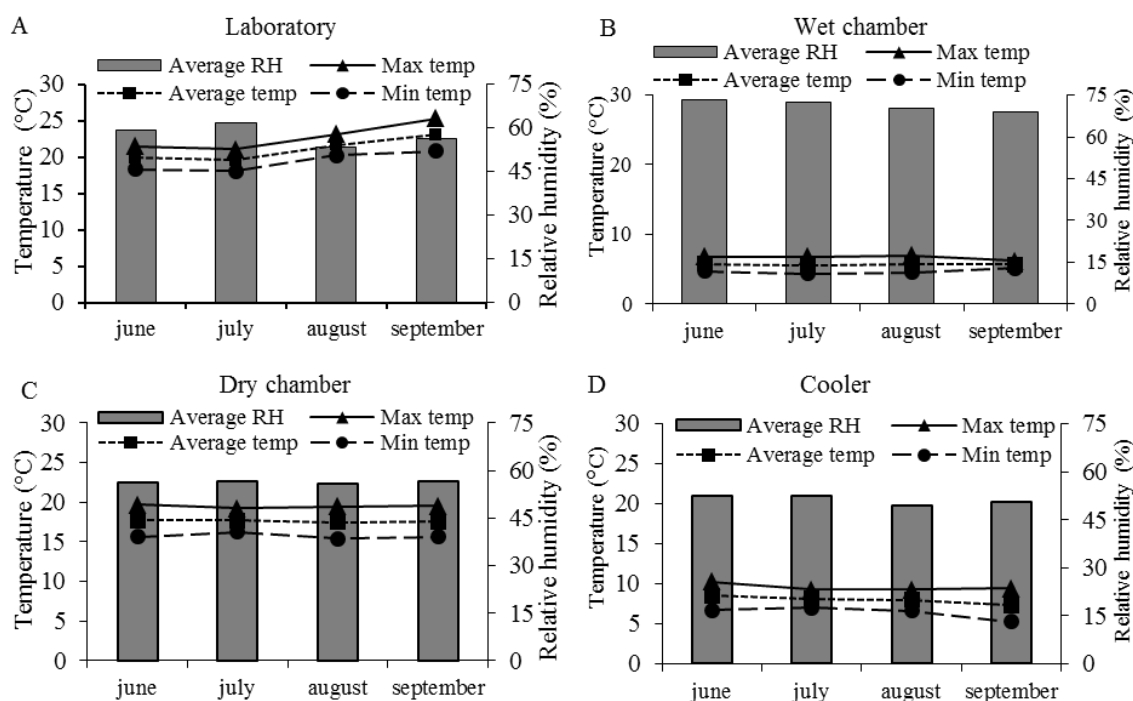


Figure 1. Data about temperature and relative air humidity in the different tested environments, during the storage period of guanandi seeds. A - laboratory (Curitiba, Paraná state). B - wet chamber. C - dry chamber. D - cooler.

Figura 1. Dados de temperatura e umidade relativa do ar nos diferentes ambientes testados, durante o período de armazenamento das sementes de guanandi. A – laboratório (Curitiba, PR). B – câmara úmida. C – câmara seca. D – refrigerador.

The germination of seeds, when stored in a laboratory environment and in polyethylene packaging, remained stable until the third storage month, that is, without significant losses of their germination power, which remained between 85 and 88% (Figure 2A); however, in the fourth month, the germination power decreased to 68%. The germination of guanandi seeds in placed in polyethylene and maintained in the other environments, except for the cooler, was also not affected until the second storage month (Figure 2B to 2D).

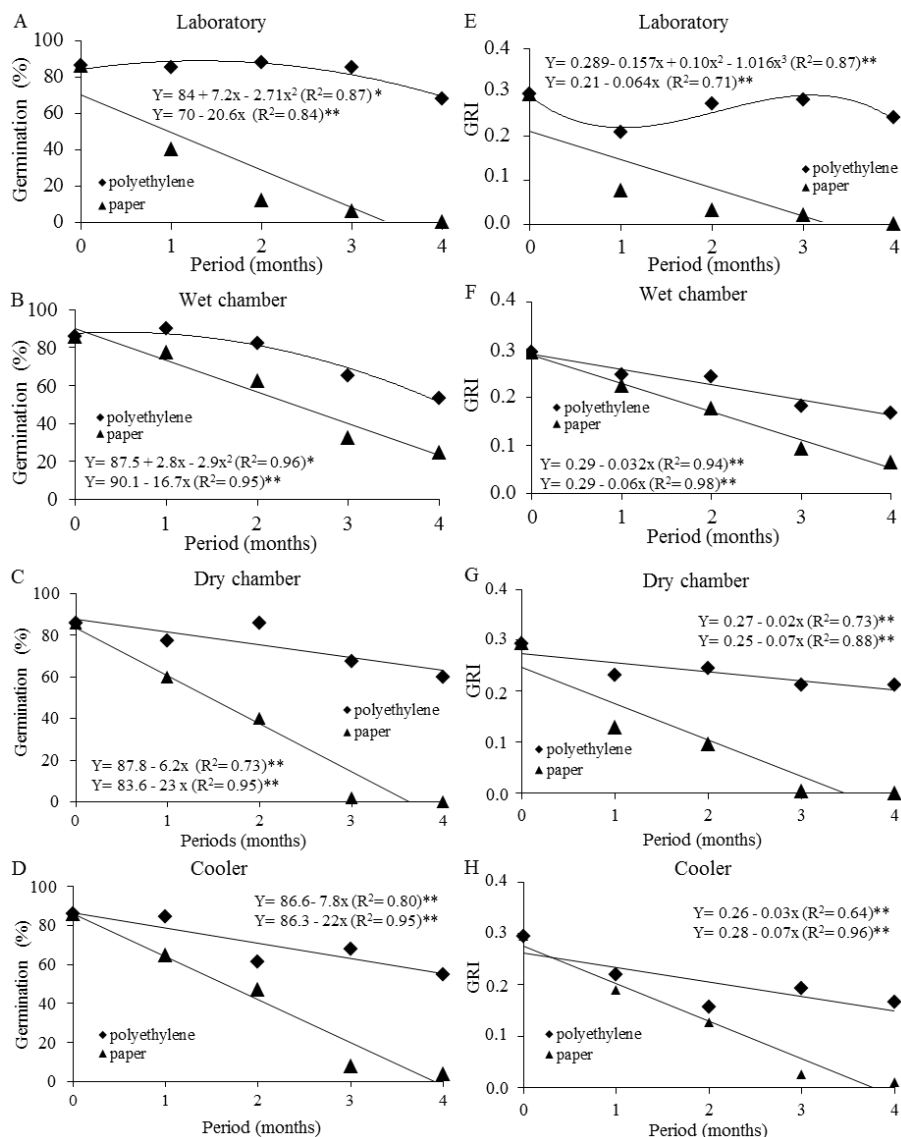


Figure 2. Germination and germination rate index (GRI) of guanandi seeds stored in different environments and packaging. A to D - germination. E to H - germination rate index. A and E - laboratory environment conditions. B and F - wet chamber. C and G - dry chamber. D and H - cooler. (*) and (**) significance $p < 0.5$ and $p < 0.01$, respectively.

Figura 2. Germinação e índice de velocidade de germinação (GRI) de sementes de guanandi armazenadas em diferentes ambientes e embalagens. A a D – germinação. E a H – índice de velocidade de germinação. A e E – condições de ambiente de laboratório. B e F - câmara úmida. C e G - câmara seca. D e H - refrigerador. (*) e (**) significância $p < 0,5$ e $p < 0,01$, respectivamente.

It is worth highlighting that the vigor of seeds decreased linearly in all environments and packaging during storage, except for the laboratory environment and polyethylene packaging, where the decrease was more attenuated, following a third order regression (Figures 2E to 2H).

The polyethylene packaging enabled the humidity of guanandi seeds not to be drastically lost during the storage period; there was a significant linear decrease only in the cooler environment (Figure 3A); on the other hand, seeds placed on paper (Figure 3B) lost their moisture significantly in all the environments; the wet chamber environment combined to this packaging was the one presenting the lowest reduction after four months (7.5%), and the laboratory environment had the highest reduction (23.9%).

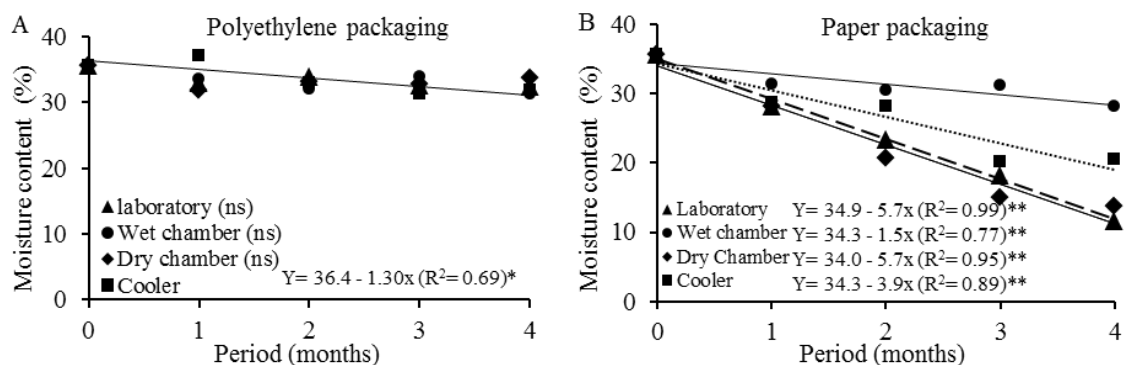


Figure 3. Moisture content of guanandi seeds, stored in two types of packaging and four environments. A - polyethylene packaging. B - paper packaging. (*) and (**) significance $p < 0.5$ and $p < 0.01$, respectively. (ns) not significant.

Figura 3. Teor de água das sementes de guanandi, armazenadas em dois tipos de embalagem e em quatro ambientes. A – embalagem polietileno. B – embalagem papel. (*) e (**) significância $p < 0,5$ e $p < 0,01$, respectivamente. (ns) não significativo.

The moisture condition of seeds (28.1% moisture content) (Figure 3B), maintained in the combination between wet chamber environment and paper packaging, provided an average germination of 25% at the end of the storage period, whereas in the other environments, the germination mean varied between 0 and 4% for the same packaging (Figures 2A to 2D). The same behavior pattern was observed for vigor (Figures 2E and 2F).

DISCUSSION

Seed drying involves two processes: the transference of water from the surface of the seed to the air, and the movement of the water from the inside to the surface of the seed (GARCIA et al., 2004). Seeds cut in fragments had a slower initial process of water loss than those intact seeds (Table 1); this difference may be related to the bigger compaction of the sample inside the aluminum capsules, because when seeds are fragmented they form particles with varied formats that allow their rearrangement, reducing the total exposure of the sample to the air action. The intact guanandi seeds are spherical in shape, provide a wider space between them inside the aluminum capsule, not being compacted, thus allowing the more porous surface (the endocarp) to be more exposed to the drying process, facilitating the initial water loss.

From nine hours of drying, no differences were observed among the treatments; the three ways of seed preparation were stable as for water extraction up to 25 hours of drying (Table 1), demonstrating that the determination of the moisture content by the low-temperature oven method at 101 – 105 °C / 17 h can be efficient even with the use of intact seeds. It is easier and more practical to use in a laboratory, since guanandi seeds have a resistant endocarp, which makes it difficult to apply the methodology indicated in the Rules for Seed Testing (BRASIL, 2009) for fragments smaller than 7.0 mm, in a maximum four-minute period of exposure to the environment.

As highlighted by Barbedo and Lamarca (2015), the most suitable method to determine the moisture content of forest species seeds may vary according to the characteristics of each species, such as size and chemical composition; of the purpose, such as ripening follow-up on the field or storage conditions; and also of the need for precision and/or rapid determination. Moreover, according to the authors, the low-temperature oven method at 101 – 105 °C is among the most reliable ones.

According to the obtained data (Table 1), in nine hours of drying, the greatest difference in moisture content, in relation to the highest humidity obtained in 25 hours of drying, was 2.3 % in all preparations, and this value was within the tolerance limit between samples (2.5%) indicated in the Rules for Seed Testing (BRASIL, 2009) for seeds with moisture contents above 25.0%. Thus, if there is a need for a faster moisture content determination of guanandi seeds, tests can be conducted with nine drying hours and any kind of preparation, or with six drying hours using seeds cut in half or fragments smaller than 7.0 mm.

As for guanandi seed storage, generally speaking, in all the tested environments the polyethylene packaging was the one that best preserved the physiological potential of seeds throughout the storage period (Figure 2). Nery et al. (2017) also observed that the use of polyethylene packaging (resistant to water vapor exchanges) was the most suitable for preserving moisture content of guanandi seeds.

The relative air humidity in the environments, dry chamber, laboratory environment conditions and wet chamber, was not determinant to change seed moisture when the polyethylene packaging was used, because even

with the humidity variation within these environments (56, 58 and 71%, respectively) (Figures 1A through 1C), there was no significant change, indicating that the polyethylene packaging was efficient in these environments, preventing the excessive exchange of water vapor between seeds and environment (Figure 3), a fundamental condition to maintain the physiological quality of recalcitrant seeds.

Other works on the storage of forest species have reported similar results in maintaining moisture when this type of packaging was used, as recalcitrant seeds of *Euterpe espirosantensis* for 30 weeks (MARTINS *et al.*, 2007), *Talisia esculenta* for 100 days (SENA *et al.*, 2016) and *Euterpe oleracea* for 180 days (LIMA *et al.*, 2018). In recalcitrant species, the polyethylene packaging has been efficient in maintaining the viability of seeds, since the fact of being semipermeable makes it difficult to exchange water vapor between seeds and environment; however, it allows the exchange of essential gases to maintain their viability, such as oxygen, which is necessary because the metabolic activities of recalcitrant seeds remain active during the storage period (PUPIM *et al.*, 2009; NASCIMENTO *et al.*, 2010; SOUZA *et al.*, 2011; SILVA; FERRAZ, 2015).

The results of Figure 2A demonstrate that maintaining the moisture content of guanandi seeds is fundamental to preserve their physiological potential; it is necessary to adapt the best combination of packaging and environment to maintain maximum seed quality.

Guanandi seeds, for being recalcitrant (NERY *et al.*, 2017), do not tolerate intensive desiccation or the exposure to low temperature. In this study, it was observed that by maintaining a proper seed moisture content, the determining factor to maintain viability started to be the temperature, since when seeds were stored in polyethylene packaging, they presented no significant humidity reduction in three different environments; however, they maintained a higher quality during the entire storage period only under laboratory environment conditions, which presented in the period an average temperature variation between 19.6 and 23.1 °C. The two other best environments (wet chamber and dry chamber) had average temperatures between 5.6 - 5.8 and 17.4 - 17.7 °C, respectively (Figures 1A to 1C).

As highlighted by Silva and Ferraz (2015), among the general recommendations for recalcitrant seed storage there is reducing the storage temperature to values close to the minimum required for germination. In a work about guanandi germination, Nery *et al.* (2007) observed a high percentage of seed germination (88%) at the constant temperature of 20 °C, and no germination when the seeds were kept at 15°C, which reinforces the idea that the temperature conditions maintained in the laboratory environment may be close to the ideal for the storage of the species.

CONCLUSIONS

- The moisture content of seeds is determined more easily by the low-temperature oven method at 101 - 105°C / 17 h, using intact seeds, without prejudice to the outcome.
- The best combination between environment and packaging to store guanandi seeds, during the tested period, is the laboratory environment condition, with a mean temperature of 19.6 - 23.1 °C and a mean RH of 53-62 %, in polyethylene packaging.

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