

CONSERVATION OF SEEDS OF *Myrcianthes pungens* (Berg) Legr. IN DIFFERENT PACKAGING IN A CONTROLLED ENVIRONMENT¹

Patrícia Gibbert^{2*}, Marlene de Matos Malavasi³, Ubirajara Contro Malavasi³ and João Alexandre Lopes Dranski⁴

¹ Received on 21.08.2018 accepted for publication on 29.03.2019.

² Universidade Estadual do Oeste do Paraná, Programa de Pós-Graduação em Agronomia, Toledo - PR- Brasil. E-mail: <patriciagibbert@gmail.com>.

³ Universidade Estadual do Oeste do Paraná, Departamento de Ciências Agrárias, Marechal Candido Rondon, PR- Brasil. E-mail: <marlenemalavasi@yahoo.com.br> and <biramalavasi@yahoo.com.br>.

⁴ Universidade Estadual do Oeste do Paraná, Marechal Candido Rondon, PR - Brasil. E-mail: <joaodranski@yahoo.com.br>

*Corresponding author.

ABSTRACT – Studying the physiological and biochemical behavior of *Myrcianthes pungens* seeds stored in different packages for up to ten months was the objective of this work. Seeds were collected in Toledo, Pato Bragado, and Marechal Cândido Rondon and stored in a cold and dry chamber (11 °C and 6,3% RH) for ten months. The germination test and the germination speed index (GSI), the seedling length (SL), the seedling dry matter mass (DMS), and the tetrazolium (ZT) test were performed and, for each evaluation, the water content was determined. Non-parametric analysis was used. During storage, the water content of the seeds in the plastic and glass containers varied little, but decreased in the paper packaging. The germination was maintained for ten months when the seeds were stored in plastic containers and for two months in the glass and paper ones. The variables GSI, SL, and DMS presented upwards and downwards variations during the storage period. The ZT revealed that, in the plastic packaging, the seeds remained viable for up to ten months, in glass, up to two months, and in paper, up to four months. Therefore, seeds of *Myrcianthes pungens* with high initial quality can be stored in a cold and dry chamber in plastic bags, maintaining viability and vigor for a period of ten months

Keywords: Guabiju; Physiological quality; Storage.

CONSERVAÇÃO DE SEMENTES DE *Myrcianthes pungens* (Berg) Legr. EM DIFERENTES EMBALAGENS EM AMBIENTE CONTROLADO

RESUMO – Estudar o comportamento fisiológico e bioquímico de sementes de *Myrcianthes pungens* armazenadas em diferentes embalagens por até dez meses foi o objetivo deste trabalho. Sementes foram coletadas em Toledo, Pato Bragado e Marechal Cândido Rondon e armazenadas em câmara fria e seca (11 °C e 6,3% UR) por dez meses. A cada avaliação além da determinação do teor de água, foram realizados teste de germinação (TG) e vigor índice de velocidade de germinação (IVG), comprimento de plântulas (CP) e massa de matéria seca de plântulas (MSP) e teste de tetrazólio (TZ). Utilizou-se a análise não paramétrica. Durante o armazenamento, o teor de água das sementes nas embalagens de plástico e vidro variou pouco, no entanto decresceu na embalagem de papel. A germinação manteve-se até os dez meses quando as sementes foram armazenadas em embalagens de plástico e por dois meses nas de vidro e papel. As variáveis IVG, CP e MSP apresentaram acréscimos e decréscimos ao longo do armazenamento. O TZ revelou que na embalagem de as sementes permaneceram viáveis até os dez meses, no vidro até dois meses e no papel até quatro meses. Sementes de *Myrcianthes pungens* com elevada qualidade inicial podem ser armazenadas em câmara fria e seca em sacos plásticos, mantendo a viabilidade e vigor pelo período de dez meses.

Palavras-chave: Guabiju; Qualidade fisiológica; Armazenamento.



1. INTRODUCTION

Myrcianthes pungens (Berg) Legr., popularly known as guabiju, belongs to the Myrtaceae family, and is now at risk of extinction (IUCN, 2018). The species is native and appears from the state of São Paulo to the north of Uruguay, reaching Bolivia, Paraguay, and Argentina (Raseira et al., 2004). The fruit of the species are globose berry type, containing one to two reniform seeds, which measure around 6 to 7 mm (Souza et al., 2011).

The fruit of guabiju is very appreciated by the native fauna and also by humans. The species can be used for decoration, urban afforestation (Fior et al., 2010), and can be a source of income for families of farmers, considering that the fruit can be used in sweets, yogurts, juice, ice cream, etc. (Hossel et al., 2016). The wood is suitable for luxury joinery, the flowers are melliferous, and the species is recommended for mixed plantings destined to the restoration of degraded areas of permanent preservation (Lorenzi, 1992).

Since its main means of propagation are the seeds, ex situ conservation should be a priority in seedling production programs for the various purposes of cultivation. However, guabiju presents limitations when it comes to storage (Hossel et al., 2016), because its seeds are classified as recalcitrant, and seeds with this physiological behavior have high water content due to fruit ripeness and short longevity, even in conditions with high air humidity and low temperature (Fior et al., 2010).

Preservation of seed quality during storage requires the integration of several factors, such as temperature, relative humidity, type of packaging, and moisture content of the seeds, as well as previous knowledge of the physiological behavior of the seeds during storage, since some seeds require specific conditions (Gomes et al., 2013).

Seed conservation under given conditions has the basic function of maintaining the physical, physiological, and sanitary qualities, preserving the viability and vigor of the seeds (Marcos Filho, 2015). Therefore, seed quality assessment is very important for successful storage and seedling production. Vigor is one of the most important aspects of seed quality analysis, considering that the deterioration process is directly related to loss of vigor (Santos, 2018).

The execution of vigor tests tries to detect significant differences in the physiological potential providing additional information to germination tests. Simultaneously, the results of these tests are expected to distinguish between high and low vigor lots. Also, the differences found can be related to seed behavior during storage and/or after sowing. The feasibility tests, in turn, aim at verifying if the seed is or is not able to germinate, by direct or indirect means (Marcos Filho, 2015).

The germination test has some limitations on its accomplishment, such as delays and possible modifications of the results due to the presence of fungi. Thus, it is necessary to use fast and reliable tests to estimate the viability of storage seeds (Cripa et al., 2014), such as the tetrazolium test, which is based on the activity of the dehydrogenase enzymes involved in the respiration process (Sousa et al., 2017).

Considering the risk of extinction of the species and its economic and ecological importance, in addition to its use for the purpose of forests restoration, it is important to store the seeds in order to conserve them for future use. Thus, the objective of the present study is to study the physiological and biochemical behavior of *Myrcianthes pungens* seeds stored in different packages for a period of up to ten months.

2. MATERIALS AND METHODS

Pieces of fruit of *M. pungens* were collected from eight trees located in forest fragments of the Semideciduous Seasonal Forest. The harvests were carried out in the municipalities of Toledo (24°38'15.85"S, 53°57'24.70"O, altitude 354 m), Pato Bragado (24°37'22.02"S, 24°13'17.11"O, altitude 270 m), and Marechal Cândido Rondon (24°33'04.50"S, 54°02'46.14"O, altitude 420 m).

The harvesting was performed manually when the fruit pieces were dark purple and started falling spontaneously (Lorenzi, 1992). The globose berry fruits were manually sifted in a sieve under running water, separating the seed from the pericarp. To eliminate excess water, the seeds were dried in the shade for 4 days.

The seeds from the three collection sites were stored in a cold and dry chamber (11 °C and 6.3% HR), separately in trifoliate kraft paper bags (600 mL), clear glass (600 mL preserved type), and

clear polyethylene plastic bags (approximately 8 mm thick and 600 mL), with initial moisture, for 0, 2, 4, 6, 8, and 10 months. The seed germination rate (SGR), seedling length (SL), and vigor were determined for each storage period and type of packaging, seedling dry matter mass (DMS), and tetrazolium test, so that if seeds remained in the package they were discarded, not influencing the seeds that remained stored.

The germination test was done with 4 replicates of 25 seeds, seeded in vermiculite, moistened until saturated. The test was carried out in a germination chamber (BOD) at 25 °C (Santos et al., 2004) with photoperiod of 12 hours. Evaluations were performed daily until stabilization and the results were expressed as the percentage of normal seedlings. The normal seedling criterion was the presence of epicotyl, hypocotyl, and primary root development and the first pair of definitive leaves. After stabilization, the germination rate (Maguire, 1962) was calculated and the seedling length and dry matter mass of the normal seedling were evaluated.

The tetrazolium test was performed with 4 replicates of 25 seeds. The seeds were conditioned between paper moistened for 24 hours in BOD at 30 °C, without tegument. Subsequently they were placed in a 0.75% tetrazolium solution for 24 hours at 30 °C in the absence of light. The seeds were evaluated individually, and the results expressed as the percentage of viable seeds, as predetermined by the authors for the species.

The design was completely randomized. Non-parametric analysis was used, since the data did not present normality and homogeneity. First, each factor (storage period, packaging, and collection site) was analyzed separately using the Friedman test and then

the comparison of averages, comparing the packages in each storage period and the storage periods for each package to a 5% probability of error. The program used is Sigmaplot 14.0.

3. RESULTS

During storage, it was observed that the highest percentages of water content were found for seeds stored in plastic and glass, differing significantly from the paper bag storage. All three types of packages were used in all collection sites (Table 1). When analyzing the behavior of the containers throughout the storage packages, it was verified that, for the plastic bag, there was reduction of the water content, behavior similar to the observed for glass. The paper bag, in turn, showed significant decrease in water content. The variation of the water content throughout the storage units for seeds in plastic bags and glass varied by up to 6%. In contrast, the seeds in paper bags presented an average variation of 29.6% reduction.

Enter Table 1. The percentage of germination was maintained until the end of the study only in the seeds stored in plastic bags. The seeds stored in glass or paper bags germinated by the first two months (Table 2). In the packages throughout the storage, it was observed that there was variation in germination, with increases and decreases, being the highest percentages 90% for Toledo and 91% for Pato Bragado and Marechal Cândido Rondon. For seeds stored in glass in Toledo and Marechal Cândido Rondon, at two months, the percentages were lower (16 and 3% respectively), and in Pato Bragado, the germination was 80%. In paper bags, the percentages were low in the three collection sites and, at two months, it was 7, 16, and 8% for Toledo, Pato Bragado, and Marechal Cândido Rondon, respectively.

Table 1 – Average water content (%) of seeds of *M. pungens* submitted to storage in different packages.

Tabela 1 – Teores médios de água (%) de sementes de *M. pungens* submetidas ao armazenamento em diferentes embalagens.

Months	Toledo			Pato Bragado			Marechal Cândido Rondon		
	Plastic	Glass	Paper	Plastic	Glass	Paper	Plastic	Glass	Paper
0	39,9aB	39,9aD	39,9aA	40,4aC	40,4aD	40,4aA	39,3aD	39,3aC	39,3aA
2	40,7bB	43,2aAB	19,6cB	42,1aAB	43,1aB	26,7bB	42,7bB	44,7aA	23,2cB
4	40,7aB	40,4aCD	12,1bD	42,1aAB	42,1aC	13,3bD	42,7aB	43aBC	13,5bC
6	41,3aAB	41,9aBC	10,2bE	39,8bBC	42,7aBC	9,3cE	41,4bC	43,3aB	10,2cE
8	42,9aA	43,9aA	10,2bE	43,1aA	43,9aA	9,3bE	45,2aA	44,3bA	10,2cE
10	42aAB	43,6aA	13,1bC	42,9bA	44,1aA	14,5cC	44,9aA	45,1aA	12,7bD

Lowercase letters in the row at each collection site and uppercase letter in the column in each package and place of collection do not differ according to the Friedman's test ($p > 0,05$).

Table 2 – Mean values of germination (%) and germination speed index of *M. pungens* seeds submitted to storage in different packages.
Tabela 2 – Valores médios de germinação (%) e índice de velocidade de germinação de sementes de *M. pungens* submetidas ao armazenamento em diferentes embalagens

%G	Toledo			Pato Bragado			Marechal Cândido Rondon		
	Plastic	Glass	Paper	Plastic	Glass	Paper	Plastic	Glass	Paper
0 month	74aC	74aA	74aA	86aB	86aA	86aA	85aAB	85aA	85aA
2 months	78aC	16bB	7bB	93aA	80bA	16cB	83aAB	3bB	8bB
4 months	79aBC	0bC	0bC	87aAB	0bB	0bC	74aB	0bC	0bC
6 months	90aA	0bC	0bC	91aAB	0bB	0bC	91aA	0bC	0bC
8 months	88aAB	0bC	0bC	90aAB	0bB	0bC	83aAB	0bC	0bC
10 months	87aAB	0bC	0bC	86aAB	0bB	0bC	91aA	0bC	0bC
SGR	Plastic	Glass	Paper	Plastic	Glass	Paper	Plastic	Glass	Paper
0 month	0,33aC	0,33aA	0,33aA	0,46aC	0,46aA	0,46aA	0,43aE	0,43aA	0,43aA
2 months	0,64aAB	0,09bB	0,03cB	0,52aB	0,41bB	0,02cB	0,48aDE	0,01bB	0,03bB
4 months	0,71aA	0bC	0bC	0,60aA	0bC	0bC	0,64aBC	0bC	0bC
6 months	0,74aA	0bC	0bC	0,50aB	0bC	0bC	0,74aAB	0bC	0bC
8 months	0,50aBC	0bC	0bC	0,54aAB	0bC	0bC	0,54aCD	0bC	0bC
10 months	0,55aAB	0bC	0bC	0,43aC	0bC	0bC	0,84aA	0bC	0bC

Lowercase letters in the row at each collection site and uppercase letter in the column in each package and place of collection do not differ according to the Friedman's test ($p>0,05$). %G: germination (%); SGR: seed germination rate.

The rate of germination (SGR) in the seeds stored in different packages was higher for seeds in plastic bags, differing significantly from the other packages, a fact observed at all collection sites (Table 2). For seeds in plastic bags, a constant variation was observed throughout the storage. For the seeds in glass and paper, there was a decrease of the SGR with the reduction of germination, a relation that was not observed in plastic bags.

From all the collection sites, only seeds in plastic bags presented normal seedlings until the end of the storage period (Table 3). In the other packages, the

normal seedlings occurred by the second month. Comparing the effects of the packages over time, there was variation in the formation of seedlings in plastic bags. In glass, there was an increase in the length of seedlings whereas, in paper, a decrease could be observed for all the collection sites.

For the average dry matter mass of normal seedlings, it was observed that in the period of two months of storage in glass, the highest average value occurred in the seeds at Toledo (150 mg). In Pato Bragado, the highest average occurred in seeds stored in paper bags (103 mg). And, in the seeds collected in

Table 3 – Mean value of length (cm) and dry matter mass of normal seedlings (mg) of *M. pungens* submitted to storage in different packages.

Tabela 3 – Valores médios do comprimento (cm) e massa de matéria seca de plântulas normais (mg) de *M. pungens* submetidas ao armazenamento em diferentes embalagens.

SL	Toledo			Pato Bragado			Marechal Cândido Rondon		
	Plastic	Glass	Paper	Plastic	Glass	Paper	Plastic	Glass	Paper
0 month	14,6aA	14,6aB	14,6aA	11,6aB	11,6aB	11,6aA	14,2aAB	14,2aA	14,2aA
2 months	14,4aA	15,7aA	8,4bB	13aAB	13,4aA	11,4bA	13,6aBC	13,7aA	7aB
4 months	15,1aA	0bC	0bC	12aAB	0bC	0bB	12,2aC	0bB	0bC
6 months	15,1aA	0bC	0bC	12,3aAB	0bC	0bB	12,1aC	0bB	0bC
8 months	14,3aAB	0bC	0bC	13,3aA	0bC	0bB	14,5aAB	0bB	0bC
10 months	12,9aB	0bC	0bC	12,6aAB	0bC	0bB	14,6aA	0bB	0bC
DMS	Plastic	Glass	Paper	Plastic	Glass	Paper	Plastic	Glass	Paper
0 month	132aA	132aA	132aA	101aA	101aA	101aB	172aA	172aA	172aA
2 months	80aBC	150aA	121aA	70cB	78bB	103aA	68aC	102aB	79aB
4 months	72aC	0bB	0bB	118aAB	0bC	0bC	128aAB	0bC	0bC
6 months	84aB	0bB	0bB	85aAB	0bC	0bC	90aBC	0bC	0bC
8 months	73aC	0bB	0bB	77aB	0bC	0bC	72aC	0bC	0bC
10 months	92,6aAB	0bB	0bB	92aAB	0bC	0bC	102aAB	0bC	0bC

Lowercase letters in the row at each collection site and uppercase letter in the column in each package and place of collection do not differ according to the Friedman's test ($p>0,05$). SL: seedling length; DMS: dry matter mass.

Marechal Cândido Rondon, there was no difference between the packages in this period, which were 68, 102, and 79 mg for plastic bags, glass, and paper bags, respectively. In the period of four to ten months, only the seeds stored in plastic bags germinated and generated normal seedlings and therefore reached the highest averages when compared to the seeds stored in glass and paper bags, which resulted in null values (Table 3).

As for the effects of packaging along the storage, it was verified that seeds in the glass and paper packages at Toledo did not differ significantly at zero and two months. However, seeds in glass at Pato Bragado and Marechal Cândido Rondon, and in paper bags at Marechal Cândido Rondon had a decrease in the mass of dry matter. Also, seedlings in paper packages at Pato Bragado presented increase of the mass of dry matter. Seedlings in plastic bags varied.

Regarding the feasibility of the tetrazolium test, it can be concluded that in the range of two to ten months in plastic bags, the number of viable seeds decreased and differed significantly from those in other packages, with the highest percentages for all collection sites.

The seeds collected from Toledo and Pato Bragado and stored in glass remained viable until two months. Those of Marechal Cândido Rondon remained viable until four months, although with smaller percentages. Seeds collected at Toledo and Marechal Cândido Rondon and stored in paper bags remained viable until four months and those collected from Pato Bragado, up to six months (5% at 6 months, table 4). The seeds in glass and paper packages presented reduced percentages of viability, differing significantly, whereas in plastic bags, variations occurred over time.

Table 4 – Average viability results (%) of seeds of *M. pungens* stored in different packages according to the tetrazolium test.

Tabela 4 – Viabilidade (%) média das sementes de *M. pungens* armazenadas em diferentes embalagens, pelo teste de tetrazólio.

Month	Toledo			Pato Bragado			Marechal Cândido Rondon		
	Plastic	Glass	Paper	Plastic	Glass	Paper	Plastic	Glass	Paper
0	85aB	85aA	85aA	94aCD	94aA	94aA	97aAB	97aA	97aA
2	84aB	37bB	16cC	95aBC	82bB	20cB	88aC	5cB	20bB
4	83aB	0cC	17bB	90aD	0cC	7bC	70aC	2cC	6bC
6	93aA	0cC	2bD	95aBC	0cC	5bD	98aA	0bD	0bD
8	95aA	0bC	0bE	98aAB	0bC	0bE	90aBC	0bD	0bD
10	93aA	0bC	0bE	100aA	0bC	0bE	90aBC	0bD	0bD

Lowercase letters in the row at each collection site and uppercase letter in the column in each package and place of collection do not differ according to the Friedman's test ($p>0.05$).

4. DISCUSSION

The water content of the seeds was similar to that found by Santos et al. (2004) (around 40%) and by Fior et al. (2010) (between 41.1 and 43.6%), except for seeds stored in paper bags. These authors have reported that high levels of water at the maturation stage are a characteristic of recalcitrant species.

In the storage of *Myrcia glabra* (O. Berg) and *Myrcia palustris* DC. in polyethylene at 5 °C for 150 days, it has been verified that there was no significant change in water content (Leonhardt et al., 2010). In turn, Hossel et al. (2017), when storing seeds of *Eugenia pyriformis* (Cambess) in paper bags at 5 °C, have verified loss of water content, as observed in the present study and by Hossel et al. (2016) with seeds of *M. pungens*. On other hand, seeds of *Cordia trichotoma* (Vellozo) Arrabida ex Steudel stored in glass have had little variation of the water content during twelve months (Gusatto, 2015).

A major factor in storage is packaging (Hossel et al., 2016). The packaging used in storage should help to slow down the deterioration process by maintaining the initial water content of the seeds stored in order to reduce respiration (Amaro et al., 2015). Also, the deterioration of seeds is directly linked to the characteristics of the containers where they are stored, depending on their influence on the water vapor exchange between the seeds and the atmosphere and the conditions of the storage environment (Cardoso et al., 2012).

The modifications of the water content of the seeds in the present work can be explained by the fact that the cold chamber was opened frequently during some periods, causing oscillations of temperature and humidity of the environment. Thus, the seeds stored in paper bags quickly responded to the change in their

microclimate. On the other hand, the semipermeable and impermeable packages did not change as much, since they did not exchange the water content with the environment or in, the case of plastic, it happened in a less efficient way.

In the case of germination, Fior et al. (2010) have verified that seeds of *M. pungens* stored for up to eight months in polyethylene maintained percentages above 80% up to five months and subsequently declined by more than 50%. Some batches have lost practically all viability; however, germination has been maintained until the end of storage. In the present study, germination percentages were higher in most evaluations, and at ten months the percentage was above 91% (Table 2) for seeds stored in plastic bags. It was also verified that, during the storage, the seeds showed different performances in the germination test, varying between increases and decreases in the percentages. That could be related to the viability of the seeds or even errors at the time of the test execution, factors that can affect the measurement of the percentage of germination, as observed in the work.

In contrast, seeds of *Eugenia pyriformis* Cambess stored in paper bags have had a reduction in germination power and a rate of germination as water content is reduced (Hossel et al., 2017). A similar result was observed in the present study, where the reduction of the water content of the seeds was harmful, since seeds with water content between 12.1 and 13.5% (Table 1) are not able to germinate (Table 2). On the other hand, the seeds stored in glass maintained the water content. Nevertheless, they did not germinate from four months onwards.

Seeds of *Myrcia palustris* DC. stored in polyethylene bags have had a reduction of SGR at 150 days storage (Leonhardt et al., 2010). In the present study, evaluating the three collection sites, each one presented reduction of the SGR at different moments. According to Gusatto (2015), recalcitrant seeds do not support storage, presenting a decrease in germination and vigor, which is represented by SGR.

According to Amaro et al. (2015), the reduction of seedling emergence may be a result of the deterioration of the seed when packaged in a permeable package, since the greater permeability of this package does not reduce the metabolism of the seeds to the desired levels for the storage, causing the greater consumption

of reserves and, thus, maintaining vigor. In addition to reducing the germination speed, the developmental unevenness between seedlings is another symptom of decline in physiological quality.

The germination of seedlings of *Punica granatum* L. stored in polyethylene at 5 °C, at 30 days, have shown a reduction in the average length of both shoot and root (Monteiro, 2017). The same author verified that, when storing the seeds in polyethylene, paper bags, and glass, there has been a significant increase of the mass of dry matter at 30 days when compared to the control group. In the present work, when evaluating at two months, there was a significant reduction of dry matter mass of seedlings. The author also verified that, up to 120 days of storage, there has been a variation of the mass of dry matter, alternating increases and decreases of the value, as it was observed in the present work.

Seeds stored in a controlled environment tended to be conserved for a longer period, since with the use of reduced temperature there may be less water loss to the environment. Thus, one of the ways to maintain the germination power for short periods of storage is to use packages that maintain the initial water content at temperatures of 4 and 6 °C (Hossel et al., 2016).

Packaging and storage conditions should contribute to the maintenance of uniformity of the seed water content. When these are stored in permeable packages, the water content changes as the relative humidity changes, but if they are semipermeable, there is resistance to gaseous exchanges, but nothing that completely prevents the exchange of moisture. In impermeable packages, in turn, there is no influence of the external air humidity on the seeds (Oliveira-Bento et al., 2015).

For the conservation of the germinative power of the seeds, it is necessary to keep them in a dry and cold environment. The drier and colder, within certain limits, the greater the chances of prolonging the conservation of the seeds. In environments without humidity and temperature control, the moisture present in the air may be sufficient to promote the resumption of embryo activities if oxygen and temperature are sufficient. Breathing, together with the action of microorganisms, provoke the heating of stored seeds, which can drastically reduce their viability (Silva, 2015).

By verifying the results of viable seeds through the tetrazolium test (Table 4) and percentage of germination (Table 2), it was observed that the percentage of viability was always higher than the germination. In addition, the seeds stored in paper bags and collected in Toledo and Pato Bragado presented viability up to four months, but the germination occurred at two months or before. According to Grunennvaldt et al. (2014), this difference between the tests is due to the fact that the tetrazolium test is an indirect and rapid analysis, informing the seed quality in less than 24 hours, depending on the species, but it is not possible to obtain more information on the percentage of dormant seeds and on the contamination by pathogens.

The differences between the values of the tetrazolium test and other vigor tests may occur due to differences in sampling, techniques unsuitable for tests, presence of hard seeds, and mechanical damages in seeds (Pinto Junior, 2010). The author, when studying the storage of seeds of *Jatropha curcas* L. in permeable, semipermeable, and impermeable packages, also has found differences when comparing the values of the tetrazolium test with those obtained from the other tests.

In the tetrazolium test, when the seed is immersed in the tetrazolium solution, it diffuses through the tissues, causing a reduction reaction in the living cells, which results in a red, non-diffusible compound, triphenylformazan, indicating that there is respiratory activity in the mitochondria and, consequently, the viability of the tissue (live) (Oliveira et al., 2018). There is a clear separation of living tissue, which breathes, from dead tissue that does not stain, and consequently does not breathe (Carvalho, 2016).

From two months of storage on, the appearance of brown colored seeds after the application of the tetrazolium salt began. When investigating a possible presence of fungi, it was verified that the seed had the *Aspergillus niger* and the *Penicillium* sp. fungi. This was mainly observed in seeds stored in paper bags and glass, even though it could be detected, in a lower incidence, in seeds stored in plastic. However, it is not possible to attribute this fact (brown coloration in the tetrazolium test) to the presence of fungi and that should be further investigated in new research. Therefore, the seeds that presented this coloring were classified as non-viable.

As reported by Aguiar et al. (2012), fungi of the species *Aspergillus* and *Penicillium* can cause direct damage to the seeds and in germination, with increase of the rate of fatty acids and production of toxins. The action of these microorganisms, provided there are conditions of humidity and temperature, accelerates the rate of decay of the seeds during storage (Carvalho and Nakagawa, 2000). The authors have added that this is a major problem for recalcitrant seeds, since they need to keep high water content, which is a favorable condition for the development of these fungi. Thus, the authors suggested that a fungicide treatment of the seeds should be carried out prior to storage, after partial drying, followed by storage at the lowest possible temperature (tolerable by the species).

Another factor that could explain the darkening of the seeds is that, during deterioration, there is a decrease in soluble sugars and in the total sugar content, and an increase of the reducing sugar levels. Consequently, there is loss in the ability to use carbohydrates, affecting the mobilization, from the reserve tissues to the embryonic axis. The presence of reducing sugars may induce deterioration of protein components through Amadori and Maillard reactions, especially in dry seeds. Maillard's reactions comprise a series of non-enzyme-driven reactions. These Maillard complexes undergo modifications for secondary ketoamines known as Amadori molecular arrangements, which become more stable but chemically reversible (Marcos Filho, 2015).

It can be verified in the results of the tests applied to the seeds collected in different places that there are differences, even on seeds of the same species. According to Teles (2017), it is important to work with fruit and seeds from different localities because it is possible to verify the phenotypic differences determined by the environmental variations, even for the same species. In each locality, the seeds are subject to variations in temperature, light hours per day, rainfall indexes, and other characteristics that end up emphasizing certain aspects of its genetic composition, that is, one environment may be suitable for the expression of certain characteristics that may not manifest in another.

5. CONCLUSIONS

Seeds of *Myrcianthes pungens* with high initial quality can be stored in a cold room and dried in

plastic bags, maintaining viability and vigor for a period of ten months.

6. REFERENCES

- Aguiar RWS, Brito DR, Ootani MA, Fidelis RR, Peluzio JN. Effect of carbon dioxide temperature and storage on soybean seeds and associated mycoflora. *Revista Ciência Agrônômica*. 2012;43(3):554-60.
- Amaro HTR, David AMSS, Assis MO, Rodrigues BRA, Cangussú LVS, Oliveira MB. Physiological potential of crambe (*Crambe abssynica* Hoechst) seeds during the storage packaging according. *Magistra*. 2015;27(1):138-44.
- Cardoso RB, Binotti FFS, Cardoso ED. Physiological potential of crambe seeds according to packaging and storage. *Pesquisa Agropecuária Tropical*. 2012;42(3):272-8.
- Carvalho NM, Nakagawa J. Sementes: ciência, tecnologia e produção. 4^a.ed. Jaboticabal: FUNEP; 2000.
- Carvalho SMC. (Viability of *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea*) seeds by tetrazolium test [dissertação] Mossoró: Universidade Federal do Semi-Árido; 2016.
- Cripa FB, Freitas LCN, Grings AC, Bortolini MF. Tetrazolium test for viability estimation of *Eugenia involucrata* DC. and *Eugenia pyriformis* Cambess. seeds. *Journal of Seed Science*. 2014;36(3):305-11.
- Fior CS, Rodrigues LR, Calil AC, Leonhardt C, Souza LS, Silva V.S. Physiological quality of guabijuzeiro (*Myrcianthes pungens* (Berg) Legrand – Myrtaceae) seeds under storage. *Revista Árvore*. 2010;34(3):435-42.
- Gomes JP, Oliveira LM, Saldanha AP, Manfredi S, Ferreira PI. Drying and physiological classification o *Acca selloiana* (O. Berg) Burret seeds regarding desiccation tolerance and storage. *Floresta e Ambiente*. 2013;20(2):207-15.
- Grunenvaldt RL, Cantarelli EB, Salamoni AT. Storage and viability of *Cabrlea canjerana* (Vell.) Mart. seeds. *Comunicata Scientiae*. 2014;5(1):98-105.
- Gusatto FC. Drying and storage of *Cordia trichotoma* (Vellozo) Arrabida ex Steudel [tese]. Marechal Cândido Rondon: Universidade Estadual do Oeste do Paraná; 2015.
- Hossel JSAO, Hossel C, Wagner Júnior A, Fabiane KC, Citadin I. Seed viability in storage guabijuzeiro. *Applied Research & Agrotechnology*. 2016;9(2):79-85.
- Hossel JSAO, Hossel C, Wagner Júnior A, Fabiane KC, Mazzaro SM. Moisture content lost on the feasibility of seeds of uvaieira under storage *Revista Brasileira de Tecnologia Agropecuária*. 2017;1(2):113-7.
- IUCN. *Myrcianthes pungens*. [acesso em: 19 jun. 2018]. Disponível em: <http://www.iucnredlist.org/details/full/38739/0>.
- Leonhardt C, Calil AC, Fior CS. *Myrcia glabra* (O. Berg) D. Legrand and *Myrcia palustris* DC. – Myrtaceae seed germination in cold chamber storage. *Iheringia*. 2010;65(1):25-33.
- Lorenzi H. Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil. Nova Odessa: Instituto Plantarum; 1992.
- Maguire JD. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Science*. 1962;1(1):176-7.
- Marcos Filho J. Fisiologia de sementes de plantas cultivadas. 2^a.ed. Londrina: Abrates; 2015.
- Monteiro LNH. Storage and pre-germinating treatments in seeds of *Punica granatum* L. [dissertação] Ilha Solteira: Faculdade de Engenharia – UNESP; 2017.
- Oliveira GP, Camili EC, Morais OM. Methodology or the tetrazolium test in cowpea seeds. *Investigación Agraria*. 2018;20(2):110-117.
- Oliveira-Bento SRS, Torres SB, Bento DAV, Silva BKA, Dantas FJC, Melo VC. Storage of silk flower seeds [*Calotropis procera* (Aiton) W.T. Aiton]. *Revista Caatinga*. 2015;28(1):39-47.
- Pinto Junior AS. Physiological quality of seeds of *Jatropha curcas* L. effects of containers environment and storage period [dissertação]. Marechal Cândido Rondon: Universidade Estadual do Oeste do Paraná; 2010.
- Raseira MCB, Antnes LEC, Trevisan R, Gonçalves ED. Espécies frutíferas nativas do sul do Brasil.

Pelotas: Embrapa Clima Temperado; 2004. (Documento, 129)

Santos CMR, Ferreira AG, Áquila MEA. Fruits characteristics and germination of seeds of ix natives myrtaceae species from Rio Grande do Sul. *Ciência Florestal*. 2004;14(2):13-20.

Santos WC. Germination and seed vigor of *Genipa americana* L. as a function of water stress at different temperatures (monografia). Areia: Universidade Federal da Paraíba; 2018.

Silva GS. Storage of angico seed in different packs (monografia). Cáceres: Universidade do Estado de Mato Grosso; 2015.

Sousa DMM, Bruno RLA, Silva KRG, Torres SB, Andrade AP. Viability and vigour in seeds of *Poincianella pyramidalis* (Tul.) L. Queiroz by tetrazolium test. *Revista Ciência Agronômica*. 2017;48(2):381-8.

Souza LS, Fior CS, Souza PVD, Schwarz SF. Desinfection of seeds and *in vitro* multiplication o guabijuzeiro from apical segments juveniles (*Myrcianthes pungens* O. Berg) D. Legrand. *Revista Brasileira de Fruticultura*. 2011;33(3):691-7.

Teles TAS. Diagnostic of the production chain of seeds of native forest species of closure in the metropolitan region of Goiânia. [dissertação]. Goiânia: Universidade Federal de Goiás; 2017.