

Physiological behavior of *Genipa americana* L. seeds regarding the capacity for desiccation and storage tolerance¹

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ABSTRACT - Knowledge of the capacity of desiccation and storage tolerance of seeds is essential to provide adequate conditions necessary to maintain viability after seed collection. Drying rate is one of the factors which have been described as affecting the physiological response of seeds of various species. Literature reports that *Genipa americana* seeds exhibit an intermediate behavior, quickly losing viability if stored at negative temperatures. Thus, this work aimed to verify the effect of slow drying over the capacity of desiccation and storage tolerance of *Genipa americana* seeds. Seeds with 47% of water content (recently harvested) were dried over salt solutions until reaching 30%, 20%, 15%, 10% and 5% of water content. After drying to 10% and 5% of water content a reduction in seed viability was observed after 30 days of storage without the total loss of germination after three months in these conditions. The slow drying increased the capacity for desiccation and storage tolerance of the *Genipa americana* seeds if compared to the information described in literature.

Index terms: physiological classification, brazilian forest species, genipapo plants.

Comportamento fisiológico de sementes de *Genipa americana* L. quanto à tolerância à dessecação e ao armazenamento

RESUMO – Conhecer a capacidade de tolerância à dessecação e ao armazenamento das sementes tem sido imprescindível para disponibilizar condições adequadas para a manutenção da viabilidade após a coleta. A velocidade de secagem é um dos fatores que tem influenciado na resposta fisiológica das sementes de diversas espécies. Deste modo, buscou-se verificar o efeito da secagem lenta na capacidade de tolerância à dessecação e ao armazenamento das sementes de *Genipa americana* L. Sementes com 47% de umidade (recém-colhidas) foram secas em soluções salinas até 30, 20, 15, 10 e 5% de umidade, testando-se a viabilidade. Ao atingir 10 e 5% de umidade as sementes foram armazenadas a -20 °C, avaliando-se a viabilidade a cada 30 dias durante três meses. Sementes recém-colhidas apresentaram 98% de germinação sem perda de viabilidade até 10% de umidade. Sementes secas a 10 e 5% de umidade apresentaram redução na viabilidade após 30 dias de armazenamento, porém não houve a perda total de germinação após este período. A secagem lenta aumentou a capacidade de tolerância à dessecação e ao armazenamento das sementes de *Genipa americana* se comparada às informações descritas na literatura.

Termos para indexação: classificação fisiológica, espécie florestal brasileira, jenipapeiro.

Introduction

In recent decades there has been a significant increase in research related to the physiological classification of forest seeds regarding tolerance to desiccation and storage. Davide et al. (2003) reported that such studies have been driven by the increasing need for viable seeds to meet production schedules or forest conservation.

Despite efforts to clarify such knowledge, there is still a big gap on basic information related to the technology of native

forest seeds, which have different physiological behavior due to the great ecological diversity. After harvesting, the correct management, along with knowledge of the physiological seed characteristics, are essential to be able to provide ideal conditions for the maintenance of seed viability during storage.

Several studies seek to improve the storage conditions using techniques involving drying or lowering the temperature, seeking to reduce the metabolism of seeds (Kohama et al., 2006). Additionally, Hong and Ellis (1996) emphasize that, to understand the behavior of seeds for storage, it is essential to

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check the desiccation tolerance in subzero temperatures.

The first author to propose classification categories for physiological behavior for dried and stored seeds was Roberts (1973), who established that orthodox seeds are tolerant to drying (about 5%) and storage at low temperatures for long periods of time without significant loss of viability; in contrast, recalcitrant seeds do not tolerate these conditions, which preclude their long-term storage.

Subsequently, Ellis et al. (1990) proposed a third category of behavior, introducing the intermediate term for seeds that tolerate partial desiccation (10% to 7% moisture) and rapidly lose viability when stored at low temperatures.

Genipa americana L. (Rubiaceae) is a naturally occurring tree species in Neotropical forests, from northern Argentina to Mexico (Carvalho, 1994). In Brazil, this species is known as jenipapo tree and is found mainly in the regions of hot and humid weather, as in the extreme north of the country (the Amazonian floodplains) in the states of São Paulo and Mato Grosso (gallery forests) (Gibbs and Leitão Filho, 1978; Cavalcante, 1996).

This species can be used in landscape and ornamental projects (Costa et al., 2005), in the production of wood for the manufacture of hoe cables, sickle, ax, construction and shipbuilding (Lorenzi, 1992) or in commercial orchards for the consumption of raw fruits or for the exploitation of their products (wines, liquors, dyes), drugs and popular medicines (Costa et al., 2005), and may be an alternative source of income for small farms.

Its fruits have a juicy, edible and aromatic pulp (Donadio et al., 1998), making it attractive to wildlife in general; in addition, the species tolerates waterlogged soils, which is why it is recommended for use in the restoration of riparian forests (Valeri et al., 2003). According to Prado Neto et al. (2007) the jenipapo tree can be propagated in sexual (seeds) or asexual (vegetative) ways, predominantly via the first one.

For Carvalho and Nascimento (2000) and Salomão (2004), the seeds of *G. americana* are classified as intermediate, with the total loss of seed viability after dried and stored in negative temperatures.

Thus, this study aimed to verify the ability of desiccation tolerance and storage of *Genipa americana* seeds L. subjected to slow drying.

Materials and Methods

This work was developed in the Laboratório de Sementes Florestais (Laboratory of Forest Seeds) of the Departamento de Ciências Florestais (Forest Sciences department) of Universidade Federal de Lavras (UFLA) during the period from March to December 2012. The fruits were obtained from eight mother

trees (February 2012) in the municipality of Lavras, MG.

Only ripe fruits were collected (soft consistency and brown-yellow color) after natural fall. After collection, the fruits were transported to the Viveiro Florestal at UFLA, where they remained for 12 hours before cleaning.

The cleaning of the seeds consisted of manual opening and removal of the pulp from the fruits, which was washed in running water on sieves. Then, the seeds were mixed with autoclaved sand and rubbed by hand until complete removal of the mesocarp, and rinsed again in tap water. Seeds were placed in a single layer on paper towel for two hours in air conditioned room (20 °C; 60% RH), for removal of surface water.

The seed lot was formed only by mature seeds with no visible damages, being stored in plastic bag (double layer) in a refrigerator (4 °C). The time between the collection, processing and early tests was, at most, 48 hours.

Determination of water content: for all stages of the experiment, it was performed by the method of oven drying at 103 ± 3 °C/17 hours (Brasil, 2009). Four replications of five whole seeds were used, and the average of the results was expressed in percent (wet basis).

Germination test: before each germination test, seeds were subjected to a pre-hydration in order to minimize possible damage from soaking. For this, they were arranged in a single layer on a plastic screen, in Gerbox® boxes containing water in the bottom, for a period of 24 hours at 25 °C in absence of light. After pre-hydration, the seeds were sterilized in sodium hypochlorite (1% / 10 min) and put to germinate. Germination tests were carried out in Petri dishes containing two sheets of moistened filter paper, with four replications of 25 seeds in BOD at 25 °C with constant light. The germinated seeds were counted daily using radicle protrusion (≥ 2.0 mm) as a criterion for germination. The germination test lasted 80 days when normal seedlings were counted (Brasil, 2009).

Classification of seeds regarding desiccation tolerance and storage: after determining the water content of the initial lot, the seeds were slowly dried until the following water contents: 30%, 20%, 15%, 10% and 5%. To estimate the moisture content of the seeds during drying was used the formula proposed by Hong and Ellis (1996):

$$M = \frac{(100-CA_i)}{(100-CA_d)} \times M_i$$

Where:

- M: mass of the seeds (g) in the desired water content;
- M_i: mass of the seeds (g) in the initial water content;
- CA_i: initial water content (% wet basis);
- CA_d: desired water content (% wet basis).

To ensure that the samples reached the desired water content, control samples were taken for determination of water content by the oven drying method. The drying curve was established after the determination of the periods necessary to achieve the desired water content, desiccation being held in a room heated to 20 °C.

Seeds were placed in an airtight container containing

saline solutions able to maintain stable the relative humidity (Table 1). The saline solutions remained in the bottom of the container and the seeds were accommodated in a single uniform layer on a plastic screen, without direct contact with the solutions. At the end of the drying process, a sealed container containing silica gel was used, so that the seeds reached 5% of water content.

Table 1. Saline solutions used in slow drying of *Genipa americana* seeds.

Saline solution	Concentration in H ₂ O	Equilibrium Relative Humidity	Exposure time
LiCl	5g/100 mL	95%	96 hours
MgSO ₄ .7H ₂ O	Saturated solution	89%	96 hours
NaCl	Saturated solution	75%	96 hours
Mg(NO ₃) ₂	Saturated solution	53%	744 hours
Silica gel	–	10%	48 hours

Source: SUN, 2002.

Upon reaching 10% and 5% of water content, the seeds were accommodated in airtight containers and stored at -20 °C, according to the protocol proposed by Hong and Ellis (1996) for the physiological classification of seeds regarding desiccation tolerance and storage, viability assays were performed every 30 days for three months.

Data analysis: the experiment was conducted in a completely randomized design, with treatments arranged in a plots split in time, the water content being the main plot and the three storage periods being the secondary plots. The data were tested for Shapiro-Wilk normality and Bartlett's test of homogeneity, and when these conditions were not fulfilled the data were transformed to $\arcsin(x/100)^{0.5}$. The unfolding of the interactions, when necessary, was performed by comparing the averages by Scott-Knott and/or polynomial regression with 5% probability. In case of significant effects of the quadratic equations, was determined the critical point (CP). For all statistical analyzes the SISVAR software was used.

Results and Discussion

The water content of fresh seeds (47%) progressively decreases during drying (Figure 1), reaching 10% after 1007 hours (42 days) and 5% of humidity at 1081 hours (45 days). The root protrusion of fresh seeds was observed from day 8 reaching 98% of germination and normal seedlings (Table 2).

Studies undertaken by Oliveira et al. (2011) and Queiroz et al. (2012) reported that newly harvested jenipapo seeds showed a high percentage of germination; however, Carvalho and Nascimento (2000) and Salomão (2004) reported that its seeds tend to lose their germination ability to the extent that reduction occurs in the water content.

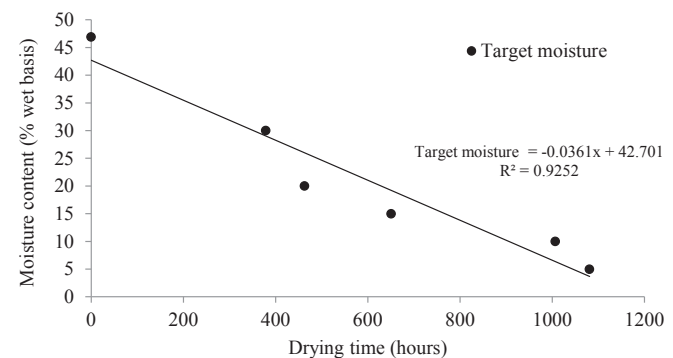


Figure 1. Water content of the *G. americana* L. seeds due do slow drying.

Table 2. Effect of moisture loss in germination behavior of seeds of *Genipa americana* subjected to slow drying.

Moisture content (%)	Germination (%)	Normal seedlings (%)
47 ¹	98 a	98 a
30	91 a	89 a
20	95 a	93 a
15	91 a	87 a
10	91 a	84 a
5	49 b	46 b
CV (%)	12.07	16.43

*Means followed by the same letter in the columns do not differ significantly by the Scott-Knott test ($p \leq 0.05$). ¹Values for fresh seeds (control).

In our study, this behavior was not observed; during drying, the percentage of germination and normal seedlings did not differ statistically up to 10% of moisture level. The loss of physiological quality was only reported when the seeds reached 5% of water content, and root necrosis was observed

in the presence of exudates in the endosperm.

According to Pammenter and Berjak (1999), the loss of seed germination after drying may be associated with lack of efficient mechanisms linked to desiccation tolerance or repair of damage mechanisms at the time of soaking. The ability to tolerate desiccation is one of the most important features that a seed may exhibit (Medeiros and Eira, 2006) and numerous studies have sought to elucidate the mechanisms linked to this behavior. Characteristics such as reducing the degree of vacuolation; the accumulation of insoluble reserves; the reactions of the cytoskeleton; the nuclear DNA conformation; intracellular differentiations; metabolic shutdown, presence and efficiency of antioxidant system; accumulation of protective molecules; deposition of amphipathic molecules; presence of a peripheral layer of oleosins, effective surrounding lipid bodies; as well as the synthesis of ABA, have already been correlated with the ability to tolerate desiccation in orthodox seeds after maturation drying (Oliver et al., 2000; Berjak and Pammenter, 2001; Pammenter and Berjak, 1999; Tweddle et al., 2003; Marcos-Filho, 2005; Leprince et al., 1993).

The activation of genes involved in these mechanisms may arise from external stimuli (Kranter et al., 2010); in this sense, the intensity and duration of dehydration are factors that can influence the capacity of tolerance to seed desiccation.

The drying method used provided higher capacity for desiccation tolerance of the seeds of *G. americana* if compared to the results found by Salomão (2004) and Oliveira et al. (2011) for this same species in similar water contents. The benefits of slow drying were also reported for other species with seeds of intermediate behavior, such as *Magnolia ovata* (José et al., 2011) and *Coffea canephora* (Rosa et al., 2005), which exhibited lower sensitivity to desiccation at lower water contents.

Seeds dried to 10% of moisture showed high germination, similar to freshly harvested seeds; however, after 30 days at $-20\text{ }^{\circ}\text{C}$, they tended to lose viability (Figure 2). This trend was also evident for seeds dried to 5% of moisture; however, to a lesser extent, compared to the dry seeds at 10% of moisture. Despite the foregoing, in no one of the two aforementioned moisture contents was observed total loss of germination after three months of storage.

The formation of normal seedlings (Figure 3) was also affected after 30 days of storage for seeds dried at 10% and 5% of moisture, and there wasn't a greater tendency to loss of viability for seeds dried at the water content of 5%. This trend can probably be explained by the intensification of drying damage to the embryo, previously reported for this water content before storage; however, the seeds did not show complete loss of viability after 90 days at $-20\text{ }^{\circ}\text{C}$.

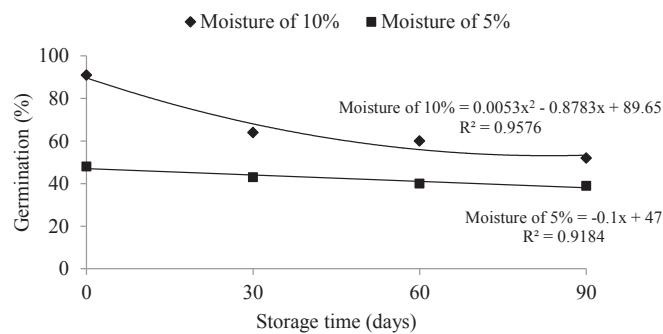


Figure 2. Dry *Genipa americana* seeds germination (10% and 5% of moisture) and stored in different periods.

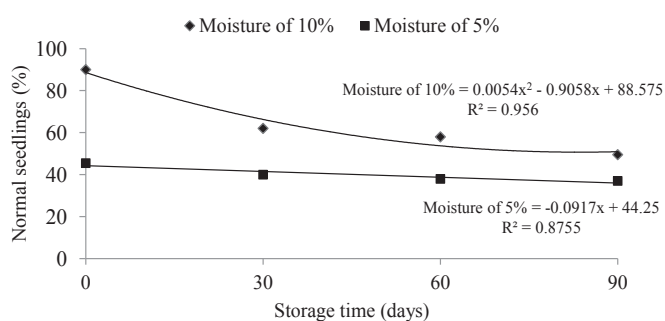


Figure 3. Dry *Genipa americana* seeds normal seedlings at 10% and 5% of moisture and stored in different periods.

Carvalho and Nascimento (2000) reported that the deleterious effects of freezing ($-18\text{ }^{\circ}\text{C}$) on the viability of jenipapo seeds is fundamentally linked to a greater or lesser degree of sensitivity that seeds manifest when exposed to different humidity levels.

In tropical species which have seeds with intermediate behavior, loss of longevity after desiccation (7% -10% of moisture) and storage in temperatures below $10\text{ }^{\circ}\text{C}$ was already reported (Ellis et al., 1990, 1991a, 1991c; Hong and Ellis, 1992).

Salomão (2004) when studying the longevity of *G. americana* seeds in three storage temperatures (5, 10 and $15\text{ }^{\circ}\text{C}$) during 12 months found that seeds stored with high water contents (38 and 42%) almost completely lost viability after six months of storage. In contrast, dry seeds at 11% of moisture remained able to germinate after one year of storage under all conditions evaluated.

Thus, it is evident the importance of reducing the water content along with storage conditions favorable to the preservation of the physiological quality of *G. americana* seeds. In seeds sensitive to desiccation and storage, maintaining the viability is still one of the biggest challenges faced by researchers for conservation in germplasm banks. According to Viggiano et al. (2000), the water content,

temperature, relative humidity of air and type of packaging used are the main factors that can affect seed quality during storage.

According to Hong and Ellis (1996) seeds reserved for long periods of storage must be dried and stand low temperatures during storage, especially sub-zero temperatures. Apparently slow drying to 10% of moisture met this assumption during storage, but the maximum period in which the seeds of *G. americana* can tolerate these conditions must be investigated.

The loss of viability of dry seeds during storage slowly resembles the physiological behavior of coffee, papaya and oil palm trees (Ellis et al., 1990; 1991a; 1991b) in terms of sensitivity to desiccation and storage, confirming the intermediate behavior of *G. americana* seeds.

Conclusions

The method of drying used allowed drying the *G. americana* seeds to 10% of moisture without loss of viability; however, it was not sufficient to maintain seed viability when stored at -20 °C.

It was confirmed that jenipapo seeds exhibit intermediate characteristics with respect to drying and storage and moisture content below 10% cause major losses in germination.

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