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Seed storage influences the seedling emergence and growth of *Tocoyena formosa,* a native species from Brazilian Savannah?

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ABSTRACT – Studies related to seed performance during storage represent a start for the sustainable use of native plant species, such as Tocoyena formosa, a native species from the Brazilian savannah with antifungal and anti-inflammatory properties. This study aimed to verify the effects of packaging, temperature, and storage period on the seeds of Tocoyena formosa. The seeds were packed in aluminum foil and glass bottles and stored at 5 °C, 15 °C and 25 ± 2 °C for 0, 14, 28, and 56 days. After, the seeds were sowed in cell trays containing substrate composed of pine bark chips, aggregating agents, vermiculite, coconut fiber, and mineral complements at 70% shade; seedlings quality was evaluated until complete stabilization of emergence. The fresh seeds had a lower percentage (42%) of emergence than the seeds stored for 56 days, which presented 57% emergence regardless of the temperature or packaging. The stored seeds in impermeable glass packaging at and temperature range of 5 to 15 °C showed higher emergence in less time and provided seedlings of high quality.

Keywords: *ex-situ* conservation, forest seeds, Rubiaceae, seedling quality

RESUMO – O armazenamento de sementes influencia a emergência e o crescimento de plantas de *Tocoyena formosa,* uma espécie nativa do Cerrado brasileiro? Os estudos relacionados ao desempenho de sementes durante o armazenamento representam o início do uso sustentável de espécies vegetais nativas, como para *Tocoyena formosa,* nativa do cerrado e com propriedades antifúngicas e anti-inflamatórias. O objetivo deste trabalho foi avaliar os efeitos de embalagens, temperaturas e períodos de armazenamento de sementes de *Tocoyena formosa*. As sementes foram embaladas em papel alumínio e recipientes de vidro e armazenadas a 5 ° C, 15 ° C e 25 ± 2 ° C por 0, 14, 28 e 56 dias. Em seguida, as sementes foram semeadas em bandejas contendo substrato composto por lascas de casca de pinheiro, agregantes, vermiculita, fibra de coco, complementos minerais e mantidas sob sombrite 70%. A qualidade das mudas foi avaliada até a completa estabilização da emergência. As sementes que não foram armazenadas apresentaram menor porcentagem (42%) de emergência do que as armazenadas por 56 dias, as quais apresentaram 57% de emergência, independentemente da temperatura ou embalagem. O armazenamento de sementes de *Tocoyena formosa* em embalagem de vidro sob temperatura de 5 a 15 °C durante 56 dias proporciona maior emergência e mudas com maior qualidade.

Palavras-chave: conservação *ex situ*, sementes florestais, Rubiaceae, qualidade de mudas

INTRODUCTION

Ex-situ germplasm is important for the conservation and propagation of plant species, and its main objective is to preserve the quality of seeds until they are sown. In this context, the efficiency of seed storage is a challenge for seed banks established for *ex-situ* germplasm conservation (Nagel & Börner 2010, Scalon *et al*. 2013). Seed banking involves collecting seeds from wild plants, drying and storing them in cool conditions. Seeds conservation provides insurance against threats to plants *in situ* including habitat loss and degradation, introduction of alien species; over-exploitation, pollution, disease and climate change (O'Donnell & Sharrock 2017, Silveira *et al*. 2018).

However, information for the adequate management of forest species is limited (Martins *et al*. 2012, Bento *et al*. 2016). In this way, studies related to seed viability during storage represent a start for the conservation and sustainable use of native plant species, since the knowledge of the seeds postharvest physiology could improve seedlings stand establishment during environment restoration, especially for those occurring in endangered biomes such as the Brazilian Savannah which has suffered devastation by both human and climatic actions.

Tocoyena formosa (Cham. & Schltdl.) K. Schum. (Rubiaceae) is a woody species of shrub-tree size, native and widely distributed in the Cerrado, and found mainly in xeromorphic formations in several states of Brazil (Gottsberger & Ehrendorfer 1992). It is popularly known as jenipapinho in Brazil, and it is used for several purposes: *Tocoyena formosa* has allelochemical compounds, called iridoids and genipins in its leaves, which gives it antifungal and anti-inflammatory properties (Coelho *et al*. 2006, Souza *et al*. 2013). In addition, this species has the potential for timber and ornamental use (Rondon Neto *et al*. 2010, Carvalho *et al*. 2012).

The seeds behavior of *Tocoyena formosa* during storage could influence the seedling establishment for restoration purposes and, consequently is an important criterion for storage management in seed banks.

The water content of seeds is an important aspect because it can affect the success of long-term storage with implications for *ex situ* conservation (Rodrigues-Junior *et al*. 2015). For seeds to remain viable for a specific period by reducing the rate of seed deterioration, other factors need to be considered, such as the temperature at which the seeds remain exposed (Sano *et al*. 2016) and type of packaging, which can have a great influence on seed conservation and may affect biochemical reactions that regulate the metabolism involved in this process (Martins & Lago 2008).

The packaging used for storage must reduce the rate of deterioration, improving seed conservation and maintaining seed viability (Walters 2007, Nagel & Börner 2010). Different types of packaging have different responses to the water content of seeds, which is influenced by the permeability characteristics of each type of packaging (Cardoso *et al*. 2012) and may help to maintain the viability and vigor of seeds.

Evidence related to seeds' behavior during storage includes physical characteristics related to seed size and ecological aspects of the environment of species occurrence (Lamarca & Barbedo 2015); dormancy alleviation (Basbouss-Serhal *et al*. 2016) and taxonomic groups, such as families and genera (Dussert *et al*. 2000, Delgado & Barbedo 2012).

In this context, the reduced seeds survival during storage of some species that belong to the family Rubiaceae in the Brazilian Savannah have been reported: seeds of *Psychotria vellosiana* (Benth) showed a reduction in viability during storage with 12% water content (Araújo & Cardoso 2006); seeds of *Genipa americana* (L.) tolerated slow drying up to 10% water content, but did not survive storage at − 20°C for 90 days (Magistralli *et al*. 2013); and seeds of *Alibertia edulis* (Rich.) A. Rich. ex DC. tolerated drying up to 10% water content but did not survive freezing for 60 days (Bento *et al*. 2016). Because of the particularities of each species, factors related to the storage conditions of the seeds need to be elucidated to increase the chance of ensuring the establishment of quality seedlings.

Despite its importance, conservation methods for the seeds are still scarce to allow the management and sustainable exploitation of the species. To increase the success of *Tocoyena formosa* propagation, the objective of this study was to evaluate different packaging and temperatures for seed storage and verify their influence on seedling quality.

MATERIAL AND METHODS

Seeds collection and processing

The ripe fruits of *Tocoyena formosa* were collected from 18 matrices in remnant areas of Cerrado on the BR 267 highway (21° 39′ 11.8″ S and 56° 36′ 1.6″ W), which is a locality at Bonito to Jardim in the state of Mato Grosso do Sul, Brazil. The fruits were manually pulped for seed removal in running water and surface drying for 5 min with paper towels in a laboratory environment (25 ± 1 °C/ 55% RH). Subsequently, all poorly developed and broken seeds were discarded, and the lot was composed of mature seeds with uniform color and size.

Seeds storage and seedlings growth

The water content of the seeds was determined using the oven method at 105 ± 3 °C for 24 h with two samples of 3 g seeds, and the results were expressed in percentages based on the fresh weight basis of the seeds (Brasil 2009).

The seeds were stored in aluminum foil packaging and transparent glass bottles with a screw cap and maintained under the following environmental conditions: chamber adjusted to 5 ± 2 °C/ 50% RH, laboratory environment (25 ± 1 °C/ 65% RH), and cold and dry chamber (15 ± 2 °C/ 45% RH). After 14, 28, and 56 days of storage under each condition, the water content (Brasil 2009) was determined, and part of the seeds was sown in styrofoam trays of 128 cells (three seeds per cell) containing the moistened substrate Bioplant® (Bioplant, Minas Gerais, Brazil) and maintained in a greenhouse with 70% shading at 25 ± 3 °C, daily irrigated and maintaining field capacity 60%, approximately. According to the manufacturer, the Bioplant® substrate is composed of bio-stabilized pine bark, vegetable peat, expanded vermiculite, coconut fiber, acid correctives, and simple superphosphate fertilizer powder + NPK + micronutrients. The following characteristics were evaluated:

Emergence: Seedlings that emerged above the soil and showed expansion of the first pair of leaves were considered to have criteria for emergence; the results were expressed as a percentage of seedlings that met the criteria for emergence. The mean time of emergence (MTE) was calculated according to Edmond & Drapalla (1958). The evaluations were carried out until the stabilization of the seedling emergence.

Plant growth: It was determined by the lengths of the shoot and root, measured with a digital caliper, of 10 plants selected at random from each replication. To determine the length of the primary root, the root tip was measured until the insertion in the hypocotyl. For the shoot, the measurement from the insertion portion of the hypocotyl to the apical bud was performed; the results were expressed in centimeters. Ten plants from each replication were used to determine the root dry mass and shoot dry mass. After the dimensions of the plants were recorded, they were separated with scissors and dried in an oven at 60 °C for 72 h. The root dry mass (RDM) and shoot dry mass (SDR) were determined on a precision scale (0.0001 g), and the results were expressed in grams.

The number of leaves (NL) was calculated by counting the total number of leaves per plant. The stem diameter was obtained with a digital caliper. To estimate the quality of seedlings formed by seeds submitted to storage under different conditions, the Dickson quality index (DQI) was determined according to the method proposed by Dickson *et al*. (1960). The leaf area (LA) was determined using a leaf area integrator (model LI-COR 3000) in cm^2. Ten plants were used from each replicate for the analyses.

Experimental design and data analysis

The experimental design was completely randomized 2 × 3 × 4 (packaging × temperature × storage period) factorial design with four replicates of 50 seeds for each treatment. The results were submitted for analysis of variance, and significant results of the packaging and temperature treatments were compared with the Tukey test at 5% probability. The storage period results and their interaction with the other factors were adjusted using regression analysis, at 5% significance, with SISVAR software (Ferreira 2011).

RESULTS

The initial water content of the seeds was 26.3%, and no variations were observed after 28 days. However, after 56 days of storage, the seeds stored in all packaging and temperatures showed a reduction in water content, which was more pronounced at 25 °C (Tab. 1).

There was the effect of storage period on emergence percentage and meantime of emergence ($p < 0.05$) (Fig. 1), with the highest emergence percentage found in seeds stored for 6 days (57%; Fig. 1A). However, seeds of *T. formosa* that were not stored showed a minimum of 199 days for plant emergence (Fig. 1B), indicating the positive results of the seeds storage for the establishment of *T. formosa* seedlings and suggesting that the seeds have a dormancy period.

The effects of seed storage periods on shoot length, number of leaves, and stem diameter were significant ($p < 0.05$) (Fig. 2). The minimum value was observed for nom stored seeds: shoot length was 2.1 cm (Fig. 2A), number of leaves was 3.76 (Fig. 2B) and stem diameter of 1.26 mm (Fig. 2C). The maximum value for seeds 56-days stored shoot length (3.53 cm), number of leaves (7) and stem diameter of 1.67 mm (Figs. 2A, 2B, and 2C, respectively). These results show the positive effects of *T. formosa* seed storage on seedling growth and indicate that the stored seeds were germinated in a shorter time and presented larger shoot length, number of leaves, and stem diameter.

The effects of the storage period on root length and root dry mass were significant ($p < 0.05$), with maximum values of 12.9 cm and 0.095 g for seeds stored for 56 days, respectively (Fig. 3A and 3B, respectively). A significant interaction between temperatures × storage

Table 1. Water content (%) of *Tocoyena formosa* (Cham & Schltdl.) K. Schum. seeds after storage in different packaging and temperatures.

Temperature	Packaging	Storage (days)			
		0	14	28	56
5 °C	Aluminum	26.3	24.3	26.0	19.7
	Glass	26.3	26.1	26.3	21.1
15 °C	Aluminum	26.3	25.9	26.7	19.6
	Glass	26.3	24.1	24.7	17.2
25 °C	Aluminum	26,3	26.8	25.1	15.0
	Glass	26.3	26.2	25.1	14.0

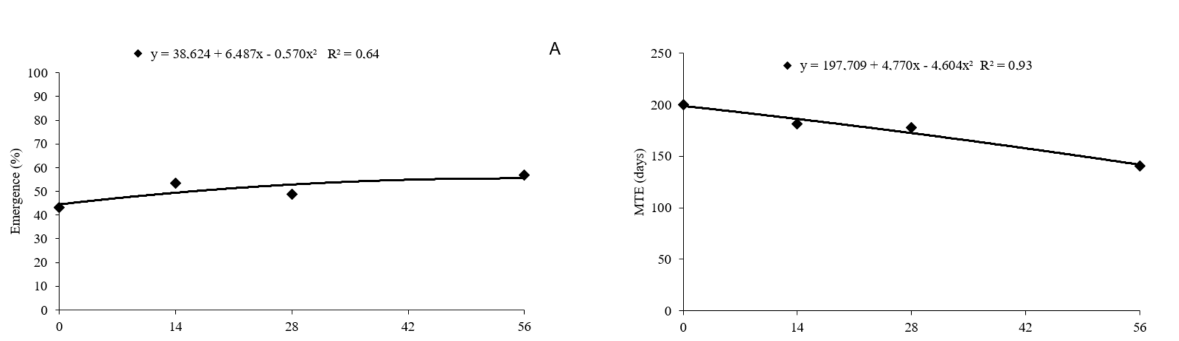

Figure 1. Emergence and seed vigor of *Tocoyena formosa* seeds stored under different conditions. **A.** Emergence (%); **B.** Mean time of emergence (MTE).

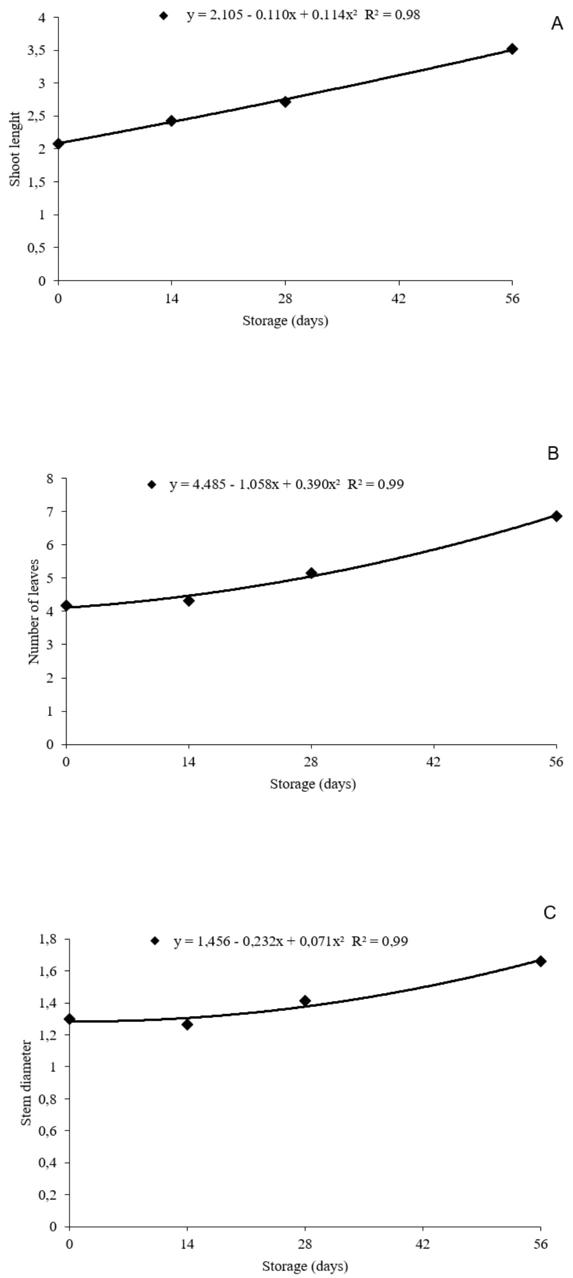

Figure 2. Seedling's growth of *Tocoyena formosa* from seeds stored under different conditions. **A.** Shoot length (cm); **B.** Number of leaves; **C.** Stem diameter (mm).

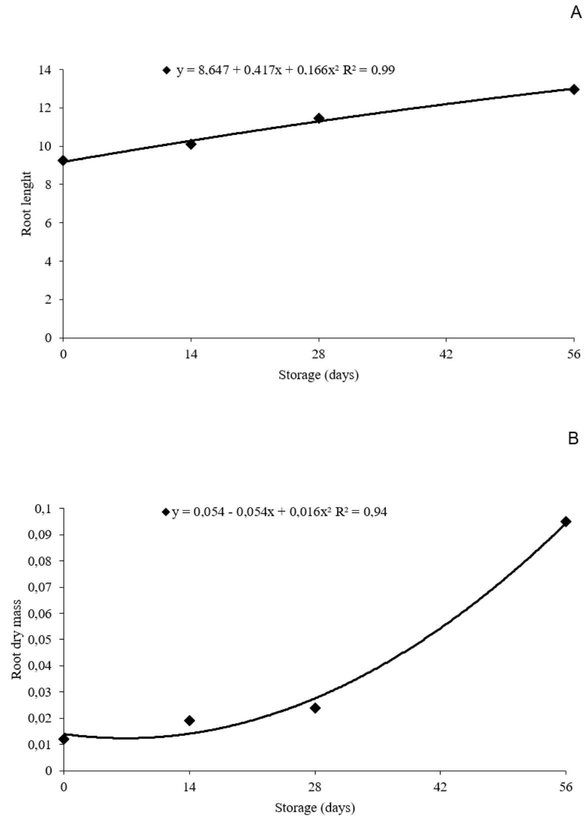

Figure 3. Root growth of *Tocoyena formosa* from seeds stored under different conditions. **A.** Root length (cm); **B.** Root dry mass (g).

periods was observed for shoot dry mass and leaf area. For shoot dry mass, a maximum value of 0.129 g and 0,126 g was observed for seeds stored at 5 and 15 °C, respectively, and 0.062 g for seeds stored at 25 °C for 56 days (Fig. 4A).

A maximum value of leaf area of 23.45 cm^2, 20.25 cm^2, and 10.70 cm^2 was found for seeds stored for 56 days at 5 °C, 15 °C, and 25 °C, respectively (Fig. 4B).

A significant effect ($p < 0.05$) of storage period and a significant interaction ($p < 0.05$) between packaging × temperatures were observed for the Dickson quality index (Fig. 5), with a maximum value result of 0.038 for plants from seeds stored for 56 days (Fig. 5A). The quality of the seedlings from seeds kept in aluminum foil packaging at 5 °C were found to be lower than those produced by seeds in glass packaging at the same temperature (Fig. 5B), indicating that the impermeable glass packaging, composed of thicker and non-porous material was efficient in maintaining the physiological potential of the seeds during storage under low temperatures.

DISCUSSION

If ongoing metabolic activity is an underlying cause of viability loss in fresh and stored seeds, an approach to increasing the seedling's growth of *T. formosa* would be to store the seeds before sowing. This approach is not feasible in many instances because of damage that can be envisaged as occurring when seeds are submitted to storage (Berjak & Pammenter 2013). There appear to be differences of opinion concerning the importance of this phenomenon in desiccation-tolerant vegetative tissue, as we demonstrated

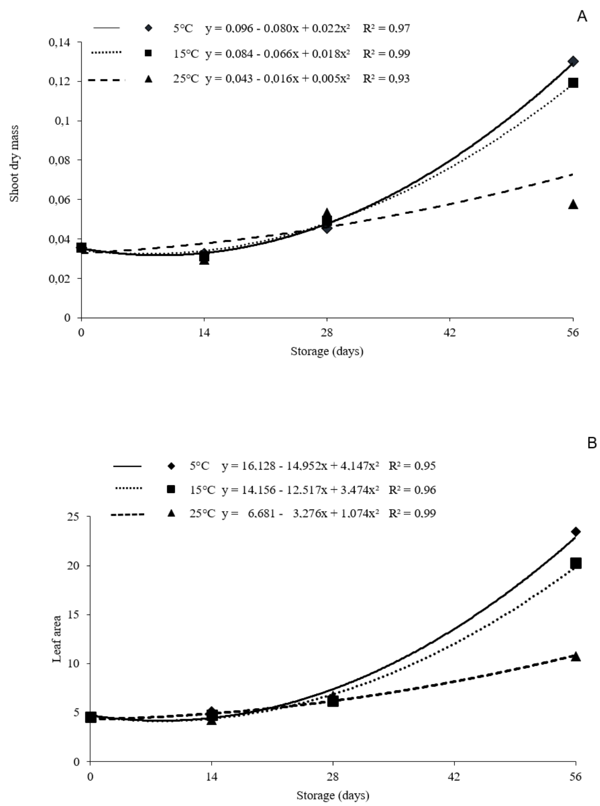

Figure 4. Seedling's growth of *Tocoyena formosa* from seeds stored under different conditions. **A.** Shoot dry mass (g); **B.** Leaf area (cm²).

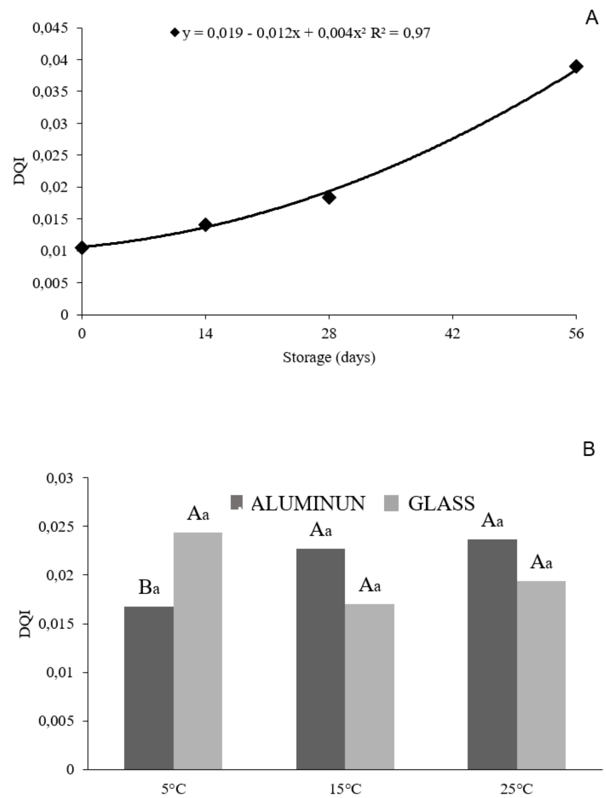

Figure 5. Dickson quality index (DQI) of *Tocoyena formosa* seedlings. **A.** DQI of seedlings from different storage periods; **B.** DQI of seedlings from different storage environments. Uppercase letters compare different packaging for the same temperature, and lowercase letters compare temperatures for the same packaging according to the Tukey test at 5% probability.

for *T. formosa* seeds (Fig. 1 and Fig. 2). Similar results were observed for seeds of *Nephelium lappaceum* L. (Sapindaceae), for which storage at temperatures between 20 and 25 °C was efficient to maintain the physiological potential of the seeds after fruit extraction for up to 6 days (Andrade *et al*. 2017).

During the experiment, emergence was observed to occur slowly and unevenly, even in the treatments in which the meantime of emergence was lower. Besides, as long as the *T. formosa* seeds remained stored, the mean time of emergence reduced, indicating the time was a benefit to embryo growth and seedling establishment. This lack of uniformity in the establishment of seedlings is found in species with seeds that have a dormancy period, which is a mechanism for survival because germination is delayed until conditions for the establishment of seedlings are more favorable (Basbouss-Serhal *et al*. 2016). In addition, dormancy allows the distribution of seed germination over time, which benefits survival. It includes nom-deep physiological dormancy which can be broken by high (≥ 15 °C) or low (0–10° C) temperatures depending on the species, and it is the most common kind of seed dormancy (Baskin & Baskin 2014). In general, in non-cultivated species, such as *T. formosa*, germination is almost always slower and not uniform, unlike cultivated species that undergo a selection process to overcome seed dormancy.

It is worth mentioning that, according to Huang *et al*. (2015), seed dormancy cannot be easily defined because it is dynamic even among the same species and subjected to variations in adaptation to different environments.

Under natural conditions, the germination time may vary from days to months, and this wide variation indicates that the species tends to establish persistent seed banks (Baskin & Baskin 2014). This behavior is common in savannas, deserts, and regions characterized by environmental stresses, such as the Brazilian Cerrado in dry seasons and/or fire seasons (Daibes *et al*. 2017). *Tocoyena formosa* is a fruit tree found in the Cerrado, Restinga, and Caatinga (Gottsberger & Ehrendorfer 1992), which are possibly included in the category of environments characterized by stress; this suggests that the presence of dormancy may be associated with the formation of persistent seed banks in the soil.

Although our results show the positive effects of *T. formosa* storage-seeds on the formation of high-growth seedlings, they also indicate that the temperature of 25 °C during storage produces plants with lower shoot growth than those from seeds stored at lower temperatures. The deleterious effects may be associated with the reduction in water content of the seeds, which was approximately 14.5% in both types of packaging. However, the effects of water content and temperature are interdependent, and the critical water content of the seeds increases as the storage temperature decreases (Eira *et al*. 2006).

In spite of *T. formosa* seeds apparently have dormancy, which was slightly overcome after storage, the higher temperatures during storage induced seedlings with poorly growth that those originated from the storage at low temperatures, especially after 28 days-storage (Fig. 4). Seed deterioration during storage is due to cellular disorders and several physiological changes that culminate in the delay of germination and seedling growth, susceptibility to stress and changes in the speed of synthesis of compounds. Because of these factors, the loss of vigor in the stored seeds is inevitable, which can be faster depending on the species (Walters 2015, Sano *et al*. 2016). Although tighter control of water vapour permeance may not always improve efficiency or seed longevity (Walters 2007), the progress of deterioration becomes more evident when the *T. formosa* seeds were not inside packages nearly impermeable to water (Fig. 5).

However, a complete loss of viability was not observed even when the water content of the seeds was reduced to 14%. In addition, plant growth from the stored seeds was higher than that one from newly harvested seeds, suggesting that seed dormancy was overcome. Moreover, a comparison by Tukey ($p < 0.05$) test between the emergence values of nor-stored seeds and those 56 days stored showed significant results in relation to the benefit of storage (data not shown).

There are many reports about sensitivity to low temperatures in several seeds of tropical species from the Rubiaceae family (Dussert *et al*. 2000, Magistralli *et al*. 2013, Bento *et al*. 2016). The results indicate that the storage of *T. formosa* seeds at 5 and 15 °C produced a positive effect on seedling growth. However, systematic investigation of the factors involved in the storage of *T. formosa* seeds for longer periods is recommended considering the deterioration phenomena during prolonged storage, especially for undomesticated tropical species. Nevertheless, from our experimental study, we can state that the results allow us to infer that regardless of the packaging, the storage potential of *T. formosa* seeds depends on the environmental temperature. What is new about our research is that for the first time, we document the storage behavior of *T. formosa* seeds; in this sense for 56 days in impermeable glass packaging and temperature range of 5 to 15 °C is efficient for achieving higher emergence in lesser time and obtaining seedlings of high quality.

CONCLUSIONS

The storage of *Tocoyena formosa* seeds in glass packaging at 5 to 15 °C for 56 days generates high emergence and quality seedlings.

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