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Effect of storage in overcoming seed dormancy of *Annona coriacea* Mart. seeds

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ABSTRACT

The aim of this study was to evaluate the effect of pre-treatments on overcoming dormancy of *A. coriacea* seeds. Seeds were processed and stored in polyethylene bags at temperatures of at -18°C (42% RH), 5°C (34% RH), 15°C (60% RH) and 25°C (34% RH), during 0, 30, 60, 90, 120 and 150 days. After storage, seeds were immersed in 350 mg.L⁻¹ gibberellic acid for 144 hours. Sowing was carried out in plastic bags containing Red Latosol + Bioplant[®]. Moisture content, emergence percentage, emergence speed index, length and dry mass of seedlings, were evaluated. The experimental design was completely randomized in a factorial with four replications of 50 seeds each. The seed storage at 5°C and subsequent immersion in gibberellic acid was efficient to reach high percentage, emergence speed and plant growth. *A. coriacea* seeds showed non-deep simple morphophysiological dormancy wherein the physiological component can be overcome after the seeds are stored at 5°C for a maximum period of 53 days and subsequently immersed in exogenous GA (350 mg.L⁻¹ for 144 hours).

Key words: Annonaceae, gibberellins, Brazilian Savanna, temperatures.

INTRODUCTION

The genus *Annona* (Annonaceae) comprises approximately 250 species in Brazil. *Annona coriacea* Mart., commonly known as “araticum”, “marôlo”, “araticum-liso”, “araticum-dos-grandes” and “araticum-do-campo” is found in areas of Cerrado (Brazilian savannah) vegetation in the states of Bahia, São Paulo, Minas Gerais, Goiás, Mato Grosso, and Mato Grosso do Sul (Lorenzi 2000, Lorenzi

and Souza 2008). Its leaves are used in popular folk medicine as stomachic, anti-rheumatic and anthelmintic remedies through oral ingestion, as external compresses, by gargling in treating stomatitis, nerve disorders and chronic headaches, and as a cataplasm to treat boils and ulcers by inducing suppuration (Lorenzi and Matos 2002).

Many forest species, including *A. coriacea*, produce seeds that do not germinate easily even under favorable environmental conditions, indicating the presence of dormancy – which can make seedling production and development under greenhouse

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conditions both expensive and time consuming (Da Silva et al. 2007). A number of authors have reported long germination times for seeds of the genus *Annona*, including Rizzini (1973), who noted that *Annona crassiflora* Mart. seeds required between 240 and 260 days, while Silva (1998) reported a germination time of 230 - 300 days for the same species, and De Melo et al. (1998) observed a time between 240 - 300 days. These long germination periods can be reduced by applying GA₃, as was demonstrated by Pereira et al. (2004) and Cavalcante et al. (2007, 2008).

Dormancy is an intrinsic phenomenon among seeds that can provide natural resistance to adverse factors in the environment by impeding germination for a certain period of time even under what might otherwise constitute favorable physical and environmental conditions (of temperature, light/dark, etc) (Bewley and Black 1994, Baskin and Baskin 2004a). Dormancy classification in seeds is based on the permeability of their teguments (or fruits) to water (impermeable or permeable), on embryo morphology (seeds may have underdeveloped or fully-developed embryos), and on numerous physiological responses to temperature (or sequences of temperatures) (Baskin and Baskin 1998, 2004a, b).

Gibberellins such as GA₁, GA₃, GA₄ and GA₇ are biologically active compounds that can reduce the germination times of dormant seeds, and therefore the time required to produce seedlings. Cold stratification can simulate natural conditions in temperate climates and has been efficiently used as a pretreatment for overcoming dormancy and for promoting germination and seedlings establishment in species whose seeds are dispersed during winter months. Cold stratification can not only increase germination percentages, but also the thermal amplitude under which seeds will germinate (Baskin and Baskin 1998, Chien et al. 2011).

Seed dormancy in some cases manifests itself by means of embryo immaturity (classified as morphological dormancy) when the meristematic tissues of the embryo are not fully differentiated at

dispersal. According to Rizzini (1973), when the seeds of *A. crassiflora* are dispersed at the end of the summer their embryos are not yet totally developed and will require a subsequent rest or storage period in order to complete their growth and it is possible that a critical storage period would also be required to overcome dormancy in *A. coriacea*.

Successful seed storage is related to the inherent (or selected) capacity of species to maintain their viability under appropriate environmental conditions; storage under unfavorable conditions, however, will result in precocious germination or deterioration (Schmidt 2007). Many different techniques have been tested to determine the ideal storage conditions for different seeds, and therefore would require involve reducing seed metabolism usually by reducing seed water contents or lowering their storage temperatures (Kohoma et al. 2006). The storage of "orthodox" seeds (such as *A. coriaceae*) at -18°C has been adopted by most seed banks, and this technique can prolong seed longevity for decades (Chmielarz 2010, Coelho et al. 2012).

A better understanding of the environmental conditions that promote germination is crucial for comprehending the process as well as for commercial considerations, and it is important to obtain information about germination times in the field and at what stage the seeds and the seedlings are best adapted to any given habitat (Baskin and Baskin 1998).

There is currently a significant market for native Cerrado fruits, although most are now harvested only from wild plants using aggressive extractivist and predatory techniques that increase the risk of several species becoming endangered (Aquino et al. 2007).

In light of this situation, more information is needed regarding the methods for overcoming seed dormancy, storage, emergence and increasing the production and establishment of *A. coriacea* seedling. The present work was therefore designed to evaluate the effects of different pretreatments on overcoming the dormancy of *A. coriacea* seeds.

MATERIALS AND METHODS

FRUIT COLLECTION AND SEED PROCESSING

Annona coriacea fruits were collected in December/2010 from six mature specimens growing in an area of Cerrado vegetation on the Santa Madalena Farm (22°8'16"S and 55°8'18"W) in Dourados, state Mato Grosso do Sul (MS), Brazil. The fruit pulp was removed manually and the seeds washed in running water and dried for 12 hours on paper towels in a laboratory environment ($25 \pm 1^\circ\text{C}$ and 60% RH). The experiments were undertaken between December/2010 and September/2011 in the Plant Metabolism and Nutrition Laboratory and in an open-air plant nursery (covered by 70% light reduction screening) at the Agrarian Sciences Faculty of the Grande Dourados Federal University, MS, Brazil.

SEEDS STORAGE AND GERMINATION

After processing, the loose seeds were stored in polyethylene bags (0.25 mm) at -18, 5, 15 and 25°C , during 0, 30, 60, 90, 120 and 150 days. After each storage period, the seeds were sterilized in 3% sodium hypochlorite for 5 minutes, washed in running water for 3 min, and subsequently immersed in a solution of 350 mg L^{-1} gibberellic acid (GA_3) for 144 hours (dose of gibberellic acid and immersion time determined through pre-tests). The seeds were subsequently sown, four centimeters deep into plastic planting sacks containing a mixture of dystrophic red clay Latossol + Bioplant® (1:1, v:v). Germination and seedling development were accompanied for 120 days after sowing. The Bioplant® substrate was composed of a mixture of bio-stabilized *Pinus* bark, peat, expanded vermiculite, and coconut fibers corrected for acidity and supplemented with phosphate fertilizer powder + NPK + micronutrients.

The effects of the different pretreatments on seed germination were evaluated based on following variables: **Moisture content** – the

seeds were dried at $105 \pm 3^\circ\text{C}$ for 24 hours, with four replicates of five seeds each, and the results expressed in percentages based on their fresh weights; **Emergence Percentage** – the evaluations calculated the percentages of emergent seedlings; **Emergence Seed Index (ESI)** – calculated using the numbers of seedlings that emerged each day divided by the numbers of days between sowing and emergence (Maguire 1962); **Lengths of the Aerial and Root Portions** – 10 plants were measured (using a millimeter ruler) and the results expressed in cm.seedling^{-1} ; **Numbers of leaves** – obtained by counting the total number of leaves per plant; **Leaf area** – measured leaf area with an integrator LI-COR Model (LI 3000); **Chlorophyll index (SPAD units)** – determined using a Konica-Minolta SPAD 502 chlorophyll meter); **Dry Mass** – obtained in relation to the aerial and root portions of the seedlings, dried to a constant weight at 60°C for 72 hours (precision 0.0001 g) and expressed as g.seedling^{-1} .

The experiments were performed using all possible combinations of temperatures and storage times, with four replicates of 50 seeds each. The results were submitted to analysis of variance and, when significant, the averages at each temperature and each storage time (adjusted using regression equations) were compared using the Tukey test, considering a 5% level possibility, using the SISVAR software.

RESULTS

Recently processed seeds of *Annona coriacea* demonstrated 9.8% moisture content levels, although when stored at -18°C they demonstrated gradual increases in their moisture contents, reaching 16.01% humidity by 150 days (Fig. 1). Seeds maintained at 25°C did not demonstrate alterations in their moisture contents during storage. Small reductions were noted after 150 days (to 7.3% and 7.2%) among seeds maintained at 5 and 15°C respectively.

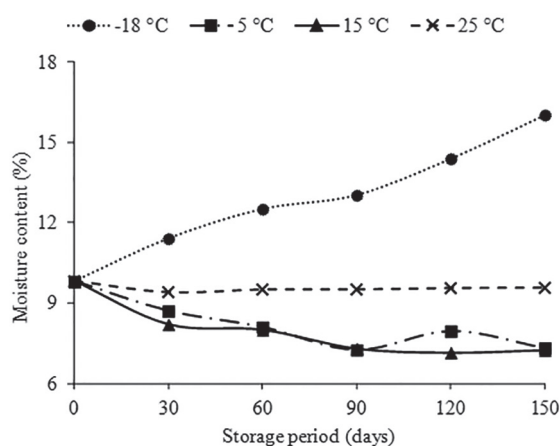


Figure 1 - Seeds moisture content (fresh weight basis %) of *Annona coriacea* Mart. after storage at different temperatures and periods.

The seeds that were not stored demonstrated the lowest seedling emergence percentages (44%), indicating the presence of dormancy in *A. coriacea* seeds. Based on regression equation, seeds stored for about 53 days at 5°C demonstrated the greatest emergence percentages (69.4%) (Fig. 2A). Storing seeds at low temperatures (5 and 15°C) resulted in the greatest emergence velocity indices (0.6260 and 0.6141 respectively) (Fig. 2B), with storage at 5°C showing the greatest emergence speed (39 days).

Storage at -18°C resulted in the complete loss of viability of *A. coriacea* seeds in terms of all of the characteristics evaluated (Figs. 1, 2, 3, 4 and 5). Both emergence and the emergence speed indices demonstrated reductions after 60 days of storage at 5, 15 and 25°C, with total losses of germination ability after 120 and 150 days (Fig. 2A and B).

In terms of the lengths of the aerial portions of the seedlings, storage at 5 and 25°C allowed the greatest growth (9.23 and 9.48 cm, respectively) after the shortest storage times (2.93 and 2.61 days, respectively) (Fig. 3A). In terms of the lengths of the primary roots, storage at 5 and 15°C allowed the greatest growth (16.42 and 16.66, cm respectively) after storage for 36 and 38 days respectively (Fig. 3B).

Regarding the number of leaves and leaf area, different storage temperatures and periods negatively affected leaf growth of seedlings (Figure 4A and B). For chlorophyll indexes quadratic adjustments in all storage temperatures, were observed and the highest indexes estimated at temperatures of 5, 15 and 25°C (41.15, 38.37 and 41.26 SPAD units, respectively) after 1, 28 and 15 days of storage, respectively. However, positive effect of storage at 5°C were observed, such as the measurements of the dry masses of the

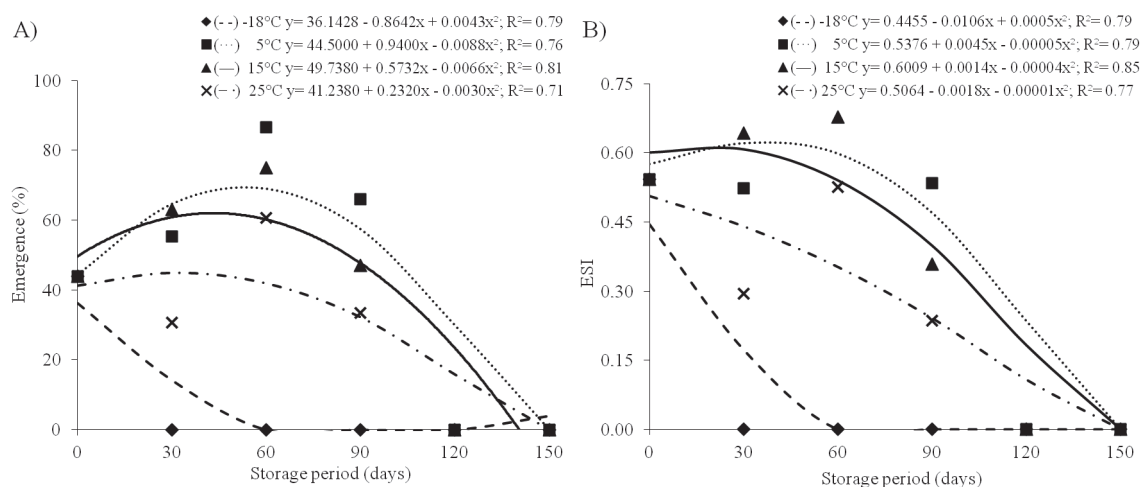


Figure 2 - Percentage emergence (%) (A) and emergence speed index (ESI) (B) of *Annona coriacea* Mart. after storage at different temperatures and periods.

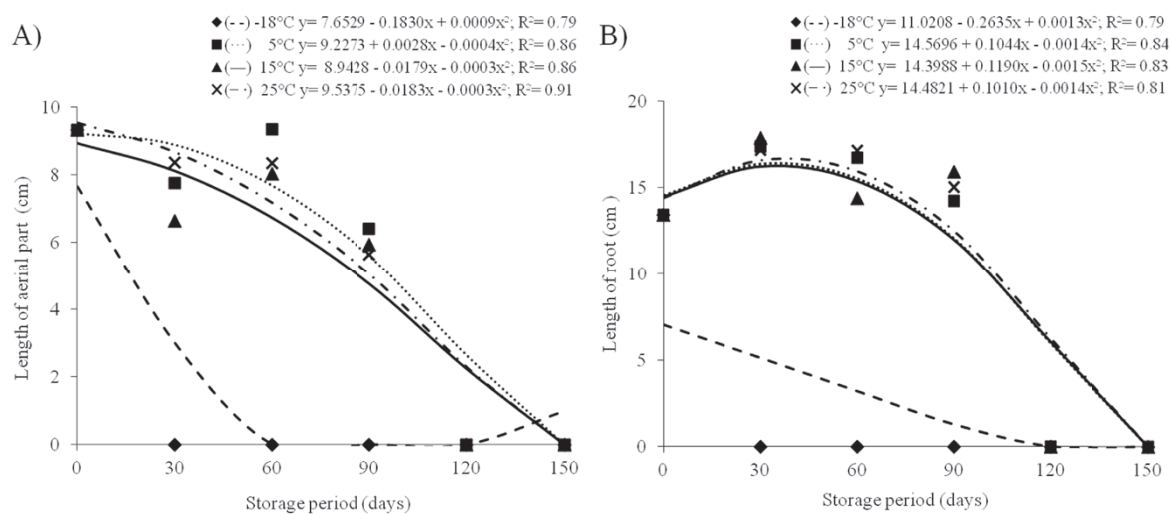


Figure 3 - Length of aerial part (cm seedlings⁻¹) (A) and length of root (cm seedlings⁻¹) (B) of *Annona coriacea* Mart. after storage at different temperatures and periods.

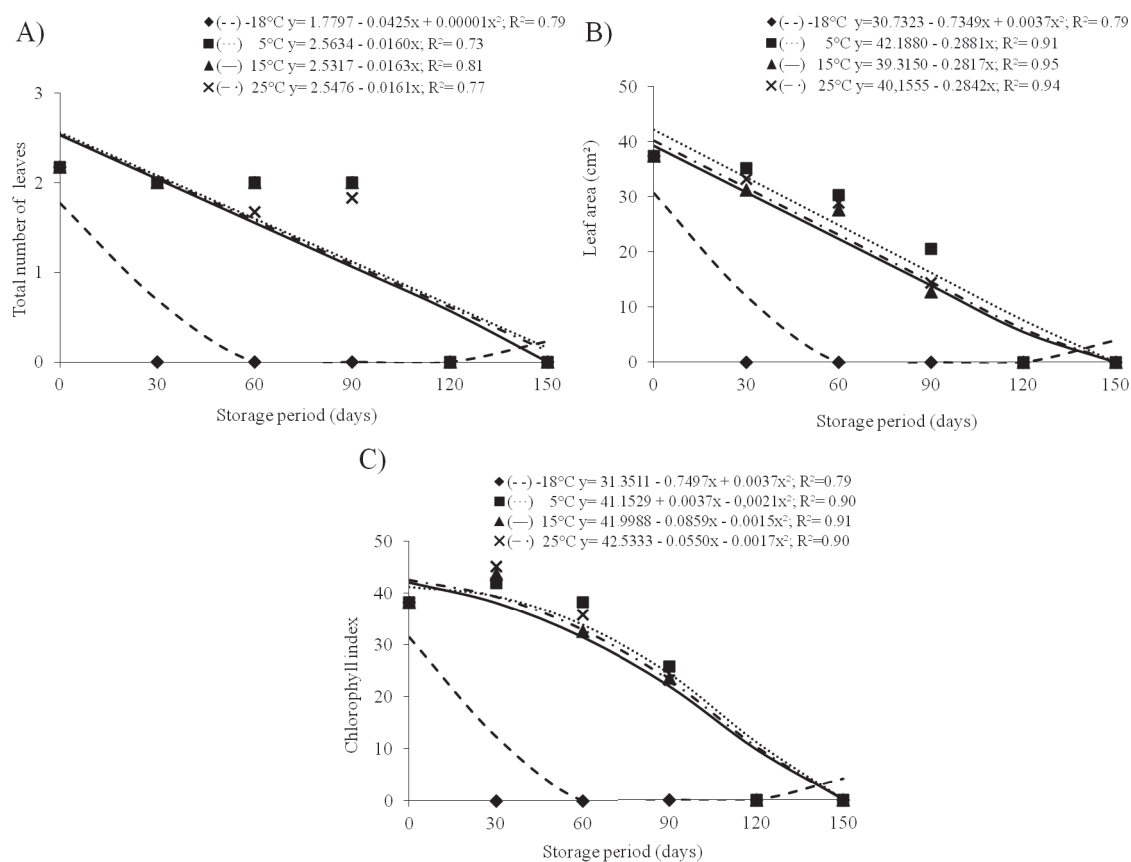


Figure 4 - Numbers of leaves (A), leaf area (B) and chlorophyll index (SPAD units) (C) of *Annona coriacea* Mart. after storage at different temperatures and periods.

aerial portions (0.1494 g) and the roots (0.6402 g) as estimates by the regression equation after 35 and 3 days of storage, respectively (Fig. 5A and B). Seeds stored

at 15°C and 25°C demonstrated smaller seedlings and, consequently, smaller dry mass accumulations in their aerial and root portions (Fig. 5A and B).

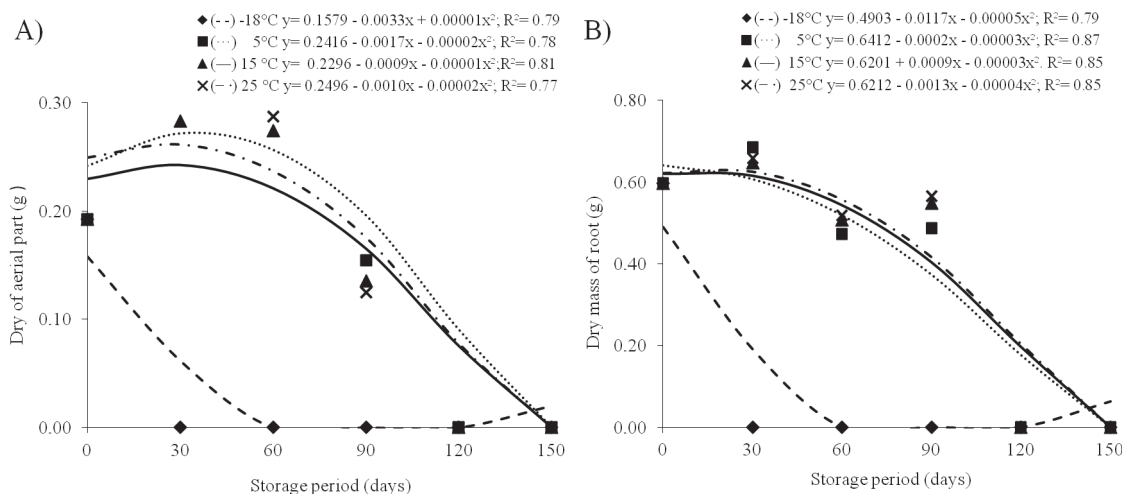


Figure 5 - Dry mass of aerial part (g seedlings⁻¹) (A) and dry mass of root (g seedlings⁻¹) (B) of *Annona coriacea* Mart. after storage at different temperatures and periods.

DISCUSSION

The increases in the moisture content of *Annona coriacea* seeds observed after storage at -18°C may have been associated with the formation of ice crystals. In any way, the high germination percentages and germination velocity indices of seeds stored at 5°C indicated that this low storage temperature, with the subsequent addition of gibberellic acid, was sufficient to overcome seed dormancy in *A. coriacea*, indicating the breaking of physiological dormancy by temperature reductions. According to the seed dormancy classification system proposed by Baskin and Baskin (2004a), the type of dormancy of *A. coriacea* seeds can be considered morpho-physiological (MPD). Seeds demonstrating this type of dormancy have poorly developed embryos as the physiological component of dormancy, requiring a pretreatment to overcome dormancy before germination can proceed.

Seeds demonstrating MPD require considerably longer periods of time for embryo growth/

root emergence than the seeds demonstrating morphological dormancy (Baskin and Baskin 2004a). According to the protocol proposed by Baskin and Baskin (1998) and Walck et al. (1999), eight levels of morpho-physiological dormancy can be identified based on the effects of combined (or not) treatments of hot/cold temperatures and the addition (or not) of GA₃.

According to our results, *A. coriacea* seeds demonstrated simple, non-profound, morpho-physiological dormancy as dormancy was overcome by storing them at 5°C (for 53 days), by the posterior addition of gibberellin, and by embryo growth at higher temperatures during their presence in the greenhouse (25 ± 3°C). According to Chien et al. (2011), simple, non-profound, morpho-physiological dormancy can be broken by stratification (either hot or cold), depending on the species, although the best embryo growth occurs at higher temperatures than those favorable to cold stratification (Baskin and Baskin 1998). The requirement for environmental temperatures above zero for cotyledon emergence

represents an adaptation for awarding seedling lesions during rigorous winters (Masuda and Washitani 1990).

Similar results were reported by Baskin and Baskin (1998) and Da Silva et al. (2007), who identified the dormancy of *A. crassiflora* as being of the simple, non-profound, morpho-physiological type, with dormancy being overcome only by the combined effects of cold stratification and the application of GA₃.

Chien et al. (2011) observed that *Ilex maximowicziana* Loes. seeds had germination levels above 90% when incubated at 25°C, 20/10°C, or 25/15°C, and did not require cold stratification to break dormancy thus demonstrating simple, non-profound, morpho-physiological dormancy. Martins et al. (2012) reported that *Picconia azorica* (Tutin) Knobl. seeds demonstrated germination and embryonic characteristics typical of simple, non-profound, morpho-physiological dormancy, as well as the occurrence of a possible chemical inhibition mechanism. These authors also reported that epicotyl development required low temperatures, and that up to three months were needed for cotyledon leaf expansion, and they recommended incubating seed without coating at a temperature of 10/5°C or 15/10°C.

It is important to note that *A. coriacea* seeds are dispersed under high temperature conditions (summer) in the natural environment in Cerrado vegetation and are subsequently exposed to a low temperature period (fall/winter) which breaks physiological dormancy through that thermal transition (warm/cold); as a result, root protrusion and seedling emergence occur only in the Spring when temperatures are more suitable. Da Silva et al. (2007) reported that the physiological component of the morpho-physiological dormancy of *Annona. crassiflora* Mart. seeds is broken by low temperatures and/or the temperature fluctuations that precede the rainy season (October), and by the application of exogenous GA.

In terms of *A. coriacea* seeds, storage at 5°C followed by the application of gibberellic acid

(GA₃) for 144 hours overcome seed dormancy, with the highest emergence rate (69.4%) estimated by the regression equation after 53 days of storage. *A. crassiflora* seeds that were stored for 180 days required 59 days to emerge (approximately 25% emergence) after immersion in a 4000 mg L⁻¹ gibberellic acid solution for 73 hours (Cavalcante et al. 2007). According to Baskin and Baskin (2004a), the quantities of GA necessary to initiate the germination of mature seeds is controlled by their exposure to different abscisic acid concentrations during seed development, where seeds exposed to low levels of abscisic acid during development ("low-level dormancy"), require only low levels of GA to germinate, while seeds exposed to high concentrations of abscisic acid during development ("profound dormancy") require high GA concentrations to germinate. Our results showed that the use of 350 mg L⁻¹ of gibberellic acid (GA₃) for an extended period of time (144 hours) was able to improve *A. coriacea* seedlings production under plant nursery conditions. These pretreatments resulted in the high emergence percentages (69.4%) required for commercial nursery activities, which aim for rapid seedling emergence, with low energy and resource consumption, and high emergence totals.

The viability losses observed in seeds stored at -18°C were probably the result of the formation of ice crystals in the cell interiors, indicating intolerance to freezing. Low emergence percentages and emergence velocities of seedlings after 60 days of storage indicated there was deterioration. Similar results in terms of the percentages and velocities of emergence were seen in measurements of the growth and development of the aerial, root portions of the seedlings and chlorophyll index after 60 days of storage, regardless of their storage temperatures. This behavior reflected seed deterioration during storage, which probably negatively impacted reserve translocation and therefore the formation of those expanding plant structures, as observed in the lower leaf growth of seedlings through variable number of leaves and leaf

area. According to Terskikh et al. (2008), among the main processes observed during seed deterioration are the exhaustion and chemical alteration of embryo reserves (including lipid oxidation and partial degradation of proteins), alterations of cell membrane properties (their reduced integrity and organization, and increases in permeability), and enzymatic and nucleotide alterations.

The seed storage temperature of 5°C contributed to the higher accumulation of biomass in the aerial part and roots. According to Haase (2008), seedlings that demonstrated high root biomasses tended to grow more and show better survival rates than seedlings with smaller root biomasses with strict correlations between the dry masses of the roots and the heights of the aerial portions.

Storing *A. coriacea* seeds at 5°C and their subsequent immersion in gibberellic acid (350 mg L⁻¹ for 144 hours) was sufficient for obtaining high germination percentages and high seedling emergence and growth velocities. *A. coriacea* Mart. seeds demonstrated simple, non-profound, morpho-physiological dormancy that could be overcome by storage at 5°C for no more than 53 days and the subsequent immersion in exogenous GA.

RESUMO

O objetivo desse trabalho foi avaliar o efeito dos pré-tratamentos na superação de dormência de sementes de *Annona coriaceae*. As sementes foram processadas e armazenadas em embalagens de polietileno nas temperaturas de -18°C (42% UR), 5°C (34% UR), 15°C (60% UR) e 25°C (34% UR), durante 0, 30, 60, 90, 120 e 150 dias. Após o armazenamento, as sementes foram imersas em 350 mg.L⁻¹ de ácido giberélico por 144 horas. A semeadura foi realizada em sacos plásticos contendo Latossolo Vermelho + Bioplant®. O teor de água, porcentagem de emergência, índice de velocidade de emergência, comprimento e massa seca das plântulas foram avaliados. O delineamento foi inteiramente casualizado em esquema fatorial com quatro repetições de 50 sementes.

Para as sementes de *A. coriacea*, o armazenamento das sementes a 5°C e posterior imersão em ácido giberélico é eficiente para obter elevada porcentagem, velocidade de emergência e crescimento de plantas. As sementes de *A. coriacea* apresentam dormência morfofisiológica não-profunda simples, sendo que componente fisiológico pode ser superado com o armazenamento a 5°C por um período máximo de 53 dias e posterior imersão em GA exógeno (350 mg.L⁻¹ por 144 horas).

Palavras-chaves: Annonaceae, giberelina, Cerrado, temperaturas.

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